**Edaphic factors trigger diverse AM fungal communities associated to exotic camellias in closely located Lake Maggiore (Italy) sites**

Borriello R; Berruti A; Lumini E; Della Beffa MT; Scariot V; Bianciotto V. Edaphic factors trigger diverse AM fungal communities associated to exotic camellias in closely located Lake Maggiore (Italy) sites. MYCORRHIZA. 25 (4) pp: 253-265.
DOI: 10.1007/s00572-014-0605-4

**Edaphic factors trigger diverse AMF communities associated to exotic camellias in closely located Lake Maggiore (Italy) sites**

Roberto Borriello[1Ω], Andrea Berruti*[1Ω], Erica Lumini[1], Maria Teresa Della Beffa[1], Valentina Scariot[2], Valeria Bianciotto[1]

[Ω]These authors contributed equally to the work.

[1]Institute for Sustainable Plant Protection – Torino UOS, National Research Council (CNR) – 25, viale Mattioli, 10125 Torino, Italy;

[2]Department of Agricultural, Forest and Food Sciences – University of Torino – 44, via Leonardo da Vinci, 10095 Grugliasco (TO), Italy.

*Corresponding author:

Dr Andrea Berruti

Address: Viale Mattioli 25, 10125, Torino (Italy)

E-mail: andrea.berruti@unito.it

Tel. (+39) 011 6705951 - Fax (+39) 011 6705962

**Abstract**

*Camellia japonica* L. is an acidophilic ornamental shrub of great economic value that has its center of origin in Japan and has been introduced in several European environmental niches. This exotic species is known to form root arbuscular mycorrhizae (AM), known for their ability to affect positively plant growth. However, AM fungal communities associated to *C. japonica* in field have never been characterized. For the first time, the AM fungal community naturally selected by *C. japonica* was screened in three sites located on the shores of the Lake Maggiore (Italy), where specimens of this plant were introduced in the 19$^{th}$ century. Mycorrhizal levels were assessed and the AM fungal communities associated to roots and soil were molecularly characterized based on the SSU rDNA region. The occurrence of mycorrhizal colonized root fragments was high in all sampled root systems (>90%). Overall, 39 Operational Taxonomic Units (OTUs; 22 Glomerales, 9 Paraglomerales, 7 Archaeosporales, and 1 Diversisporales) were found in the root and soil samples. OTU richness did not significantly differ between the root and the soil niche (2-8 and 4-15 observed OTUs per sample, respectively) and the three neighboring sites analyzed (6-10, 2-9, and 5-15 observed OTUs per sample in the three sites, respectively). The AM fungal community composition significantly differed between the root-colonizing and the soil-dwelling communities (15 shared OTUs) and among the three sites under study (7 shared OTUs). A major involvement of edaphic factors, such as available N sources, Mg content and soil porosity, in the structuring of AMF communities is suggested.

**Keywords:** arbuscular mycorrhizal fungi, AMF biodiversity, SSU rDNA, soil physicochemical properties, exotic plant

## Introduction

Mutualistic symbioses between plant roots and arbuscular mycorrhizal fungi (AMF or AM fungi) have been recognized as the most widespread (Smith and Read 2008). Over the years, the particular ecological role played by these obligate symbiotic fungi drew the attention of growers willing to assess their potential use in sustainable agriculture as biofertilizers (Fitter et al. 2011; Gianinazzi et al. 2010; Gianinazzi and Schüepp 1994; Jeffries et al. 2003). Although plant-AMF associations are considered the least specific symbiotic partnership known so far, recent data support the presence of a certain degree of host/niche-specificity (Helgason et al. 2007; Öpik et al. 2009; Verbruggen et al. 2013). Large-scale data on the distribution and abundance of AMF have shown that although the composition of geographically and edaphically distant AMF communities can vary remarkably, some taxa are ubiquitously found (Dumbrell et al. 2010; Fitter et al. 2005; Öpik et al. 2010). While less common AMF tend to associate with host plant species that occupy particular unique ecological niches (Öpik et al. 2010; Öpik et al. 2009), the widely distributed taxa interact symbiotically with a wide range of host plants and are predominant in association with introduced alien plant species (Moora et al. 2011). This could be the case of *Camellia japonica* L. (Theaceae Mirb. Theales) (Tianlu and Bartholomew 2007), an exotic Magnoliophyta whose center of origin is in Japan and that comprises more than 3,000 named ornamental cultivars (Mondal 2011). In the first half of the 19$^{th}$ century, camellias conquered the European taste and were imported and planted in gardens and parks, in the many pedoclimatically suitable environmental niches, with particular regard to some Italian areas. This species was successfully introduced on the shores of the Lake Maggiore (Piedmont, Italy) and there now resides a large pool of centennial and decennial specimens. *Camellia* is a mycorrhizal plant genus (Berruti et al. 2013a; Karthikeyan et al. 2005; Mejstrik 1974; Singh et al. 2010). Two recent greenhouse studies showed that inoculation of *C. japonica* with some generalist isolates of AMF (*Funneliformis mosseae* BEG12, *Rhizophagus* and Claroideoglomeraceae) resulted in very poor colonization (Berruti et al. 2013b) while

inoculation with AMF collected in camellia's introduced range gave high levels of mycorrhization (Berruti et al. 2013a). However, AM fungal communities associated to *C. japonica* in field have never been described.

In the present study, for the first time, the AMF diversity occurring in association with *C. japonica* in field was molecularly characterized. Three long-lived (ca 100-150 years) camellia specimens were selected and their AMF distribution was assessed in the root-colonizing and the soil-dwelling communities and in response to edaphic factors.

## Materials and methods

*Experimental sites*

The research focused on three close sites (maximum 2 km far from each other) of the Lake Maggiore district near Verbania (Piedmont, Italy; Fig. S1, supplementary data), each representative of relatively different soil properties but essentially identical climatic conditions. These included the abandoned Rovelli nursery garden (abbreviated for ease of labeling as "RO", 45° 55' 0.95" N, 8° 33' 37.13" E), the public garden of Villa San Remigio (abbr. "RE", 45° 55' 23.38" N, 8° 33' 43.50" E) and the garden island of Isola Madre (abbr. "IS", 45° 54' 43.46" N, 8° 32' 21.35" E). In general, the area considered is characterized by a mean annual temperature of 12.3 °C and a mean annual precipitation of 1,564 mm. All three sites stand ca 200 m above the sea level. These are characterized by three different management regimes. The first one is an abandoned garden of camellias and rhododendrons, infested by bamboos and brambles. The second one is a public garden of shrubs and old trees, with occasional grass cover. The third one is a garden island in which the target camellia plant was kept free of understory vegetation and was in proximity of other specimens of the same species.

*Soil and root sampling*

One centennial specimen (ca 100-150 years old) of *C. japonica* 'Alba Plena' was selected in each of the three sites. Samples were collected on May the 22nd 2009, during camellia late flowering period. Sampling operations consisted in digging to the firsts 5-20 cm and collecting fine feeder roots (Niranjana and Viswanath 2008) belonging to *C. japonica* 'Alba Plena' and, separately, a portion of bulk soil (ca 1.5 Kg) surrounding the roots. During the digging, the main root branches were carefully followed and young camellia roots were visually recognized and collected. Three replicate samples were collected at the bottom of each plant, for a total of 18 samples (9 root samples and 9 bulk soil samples). Bulk soil and root samples were taken to the lab, gently homogenized, and partly frozen in nitrogen liquid and separately stored at -80°C for further molecular analyses. The remaining roots from each replicate plant sample were washed free of soil, air-dried at room temperature, and immediately used for a morphological assessment of mycorrhizal levels. The remaining bulk soil sample replicates were pooled, homogenized and divided in three replicate portions that were submitted to physicochemical analyses (9 samples in total, 3 for each site).

*Physicochemical analyses*

The physicochemical analyses were carried out according to the European Standard methods (EN). Electrical conductivity, salinity, and pH were measured with a pH-meter on 10 g of soil in aqueous extract (soil:water 1:5) according to the standard protocols EN13037 and EN13038. Bulk density, particle density, and porosity were measured according to the standard protocol EN13041. Total Carbon and total Nitrogen were measured on 0.03 g of soil through the complete oxidation method with the element analyzer NA2100 (CE INSTRUMENTS). Total P and Nitric Nitrogen were measured by ultraviolet-VIS spectrophotometry on 0.5 g of soil after acid digestion ($H_2SO_4$, $H_2O_2$, and HF). Ammonia was measured on 10 g of soil by Kjeldahl digestion and ultraviolet-VIS

126 spectrophotometry. Ca, Mg, and K were measured on 0.5 g of soil through atomic absorption
127 spectrophotometry (AAnalyst 400; Perkin Elmer) after digestion with aqua regia ($HCl:HNO_3$ 3:1).
128
129 *Assessment of root colonization by AM fungi*
130 Camellia roots were stained with 0.1% cotton blue in 80% lactic acid for about 18 h and
131 then de-stained six times with 80% lactic acid washes. Roots were cut into 1 cm fragments and
132 placed onto microscope slides. Forty fragments were observed for each replicate, for a total of 360
133 root fragments. Mycorrhiza frequency (F%), AMF colonization intensity in the mycorrhizal roots
134 (m%) and in the whole root system (M%), and presence of arbuscules and coils in the mycorrhizal
135 roots (a%) and in the whole root system (A%) were determined and calculated as described by
136 Trouvelot et al. (Trouvelot et al. 1986).
137
138 *DNA extractions from soil and roots*
139 Two different extraction kits were used according to the different nature of the sample, soil
140 or roots. DNA extractions (0.5 g of soil each, without roots) from the 9 soil samples were
141 performed using a FastDNA Kit (MP BIOMEDICALS), according to the manufacturer's
142 recommendations. An additional step, consisting of 6 washes with guanidine thiocyanate (6M), was
143 introduced after the addition of the binding matrix suspension, in order to remove PCR inhibitors
144 (humic acids).
145 DNA extractions from the 9 root samples were performed using a DNeasy Plant Mini Kit
146 (QIAGEN, Crawley, UK) on 0.1 g of sonicated roots, according to the protocol for frozen samples.
147 The subsequent microsatellite DNA amplification with specific STMS primers (Caser et al. 2010)
148 confirmed the species origin of each root sample (data not shown).
149
150 *Nested PCR, cloning and sequencing of the fungal ribosomal (rRNA) gene*

Two sets of primers were used to amplify a region of the small subunit (SSU) of the *Glomeromycota* ribosomal DNA. The nested PCR approach used consisted in a first amplification with the universal eukaryotic primers NS1 and NS4 (White et al. 1990) and a following amplification round with Glomeromycota-specific primers AML1 and AML2 (Lee et al. 2008). PCR was carried out using 0.2 mM dNTPs, 3.5 mM of $MgCl_2$, 0.5 µM of each primer and the supplied reaction buffer, with 2 units of GoTaq® (Promega) to obtain a final volume of 20 µl. Amplifications were carried out in 0.2 ml PCR tubes using a Biometra T Gradient thermocycler according to the following steps: 5 min initial denaturation at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C and 58°C for the two nested PCR rounds, respectively, 1 min at 72°C; and a final elongation of 10 min at 72°C. A negative control was included in the PCR to check for contamination. All PCR products were checked using 1.5% agarose gel stained with ethidium bromide (Sigma-Aldrich). The PCR products were purified using QIAquick (Qiagen, Hilden, Germany), cloned in a pGEM-T Easy vector (Promega, Madison, Wisconsin, USA) and transformed into *Escherichia coli* (Xl1 blue). After colony PCR, 480 inserts (24-32 per sample replicate) of approximately the correct size were Sanger sequenced in each resulting rDNA gene library, using T7 vector primers by Macrogen sequencing services (Macrogen, Seoul, Korea).

*Sequence analyses and phylogenetic inference*

Sequence editing was done using SEQUENCHER V4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Potential chimera sequences were identified using the Chimera UCHIME algorithm implemented in MOTHUR v1.33.3 for Mac (Schloss et al. 2009). All the sequences were aligned using the multiple sequence comparison alignment tool by MAFFT v6 (Katoh and Toh 2008). Distance matrices were constructed using dist.seqs() function implemented in MOTHUR. These pairwise distances were used as input in order to cluster the sequences into Operational Taxonomic Units (OTUs) of a defined sequence identity. A threshold of 97% identity,

176 corresponding to 0.03 dissimilarity, has been used to define OTUs. Although this distance cut-off is
177 arbitrary and can be considered controversial, it was chosen on the basis of previous studies on
178 AMF biodiversity (Borriello et al. 2012; Lumini et al. 2010). A search for similar sequences was
179 conducted with the BLAST tool (Zhang et al. 2000) on the GenBank database and the MaarjAM
180 AMF specific Virtual Taxa database (Öpik et al. 2010). Recently, two reorganizations of the
181 Glomeromycota classification were published (Krüger et al. 2012; Oehl et al. 2011). In this study,
182 for ease of data handling, the nomenclature suggested by Krüger et al. (Redecker et al. 2013) was
183 adopted. The Virtual Taxa that had max ID ≥97% (18) and an *Endogone pisiformis*
184 (Mucoromycotina) sequence as outgroup (gb|NG017181.1) were used as reference for one
185 representative sequence per sample of each OTU. A new alignment was run with MAFFT, refined
186 with MEGA5 (Tamura et al. 2011), and a Maximum Likelihood phylogenetic analysis (500
187 bootstrap replicates) based on the General Time Reversible model was carried out. A discrete
188 Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G,
189 parameter = 0.3901)]. The 123 OTU representative sequences used to build the phylogenetic tree
190 were registered in GeneBank under the following accession number string: KM114311-KM114433.
191
192 *Statistical data analyses*
193 The physicochemical data were subjected to a one-way ANOVA or Kruskal-Wallis non-
194 parametric ANOVA and post-hoc tested using the Ryan-Einot-Gabriel-Welsch F (REGW-F) test or
195 the Mann-Whitney U test with Bonferroni correction, respectively. In order to assess their
196 involvement in the AMF community structuring, statistically significant physicochemical variables
197 were used to compute Gower distance matrices among samples and were tested for correlation with
198 the OTU presence/absence Jaccard distance matrix through Mantel tests (5000 permutations).
199 The mycorrhizal colonization data (%) were arccosine transformed and subjected to a one-
200 way ANOVA to determine the effect of the site. Transformed data were post-hoc tested using the

REGW-F test.

The Shannon's diversity index (a biodiversity index), the individual sample rarefaction curves (a sampling-effort graphical assessment), and the estimated sample coverage (a sampling-effort percentage descriptor that range from 0 to 1, with 1 representing the complete coverage) were calculated. A two-way ANOVA was performed on both squared root-transformed Shannon's diversity index and OTU richness in order to determine the effect of both site and matrix (soil or roots) and their interaction on the AMF diversity. A Venn diagram was constructed to show the number of OTUs shared among the three sites and the two matrices. OTU abundances per sample were transformed into occurrence classes (class 0, <1% absent; class 1, 1%-5% rare; class 2, 5%-30% common; class 3, >30% dominant) to create a heat-map describing the sample communities.

A Non-metric Multi-Dimensional Scaling (NMDS) based on the Jaccard distance (presence/absence data) was constructed to graphically assess the differences in the community composition among the three sites and the two matrices. Significant physicochemical variables were plotted as vectors in the NMDS and their Pearson's correlation with NMDS axes was calculated to assess their potential role in the community structuring. Each OTU was tested for Spearman's correlation with NMDS axes for the same reason. A two-way PERMANOVA (9999 permutations) based on the Jaccard distance was carried out to determine the effect of both site and matrix (soil or roots) and their interaction on the community composition. A METASTATS analysis (White et al. 2009) was carried out for detecting differentially abundant OTUs among the three sites and the two matrices according to a p-value and a corrected false discovery rate q-value.

This set of analyses was carried out using the SPSS statistical package (v16; SPSS Inc., Chicago), the PAST multivariate statistics software package v3 (Hammer et al. 2011), and MOTHUR.

## Results

*Physicochemical analysis of the soil*

Soil physical analysis (Table 1) showed that the soil sampled in the three sites differed in the salinity level and consequently in their electrical conductivity (EC). The RO soil was significantly higher in EC and salinity. Porosity was the lowest in IS (9%). Chemical analysis described the macro-element soil concentration of the three sites (Table 2). The RO soil was significantly higher in the levels of total C, total N, nitric N and Ca. The soil of RE was significantly richer in ammonia, while IS soil was significantly higher in Mg.

*Mycotrophic status of the roots*

All camellia root samples exhibited AM colonization. Mean values for frequency and intensity of colonization in the root system, and abundance of arbuscules or coils in the mycorrhizal roots are shown in Table 3. No significant differences were highlighted among the mycorrhizal levels in the three sites. Typical AMF structures encountered in camellia roots are shown in Fig. S2 (supplementary data).

*SSU rDNA library construction, sequence assemblage and sample coverage*

A total number of 480 clones were screened and sequenced. MOTHUR analyses revealed that, out of the 429 SSU good quality sequences obtained, 386 sequences (90% of total) featured high similarity to sequences from taxa belonging to the phylum Glomeromycota. The remaining sequences (18) were related to other organisms (10 to plants, 6 to fungi, 2 to nematodes) or recognized as the result of a chimeric amplification (25 sequences). After discarding all the non-target fragments, the sequences were clustered, using MOTHUR, into OTUs. Considering a similarity threshold of 97%, the programme identified a total of 39 OTUs in the three sites, including 6 doubletons and 14 singletons (Table 4). OTUs were assigned to Virtual Taxa present in the MaarjAM database through a manual BLAST search when max ID and query coverage of the

representative sequences were both ≥97% (Table 4). The sequencing depth per sample (sample size) ranged from 13 to 31 (Table 5). The value of the sampling effort was described by the rarefaction curves (Fig. S3, supplementary data) and the estimated sample coverage which ranged from 0.58 to 1.00 (Table 5).

*Phylogenetic analyses of the AMF sequences*

The tree built with the SSU rDNA sequences with the highest log likelihood (-4003.3610) is shown in Fig. 1. To construct the phylogenetic tree, 123 sequences representative of the 39 OTUs were used. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Regardless of the sample origin, the most abundant and diverse AMF order was the Glomerales, with 249 sequences grouped in 22 OTUs. A high number of sequences (105) belonged to the order of Paraglomerales (9 OTUs). Archaeosporales were represented by 30 sequences distributed over 7 OTUs, whereas Diversisporales occurrence was limited (2 sequences, 1 OTU).

*Effect of site and matrix on the differences in AMF community composition*

The number of observed OTUs (richness) did not significantly differ between the root and the soil niche (2-8 and 4-15 observed OTUs per sample, respectively) and the three neighboring sites analyzed (6-10 for IS, 2-9 for RE, and 5-15 for RO), and so did the Shannon's diversity index. No interaction was shown between these factors. The Venn diagram in Fig. 2 indicates that 15 OTUs were shared between the root and the soil niche, whereas 7 OTUs were shared by the three sites. The NMDS shown in Fig. 3 was based on the Jaccard distance matrix (Stress: 0.18, 3D: NMDS1, NMDS2 and NMDS3 with $R^2$ of 0.414, 0.137 and 0.04, respectively). Fig. 3 plots the samples according to the only first two axes. The two-way PERMANOVA (Table 6) shows that the AMF community composition was significantly affected by the site factor (p-value=0.0001) and the matrix type (p-value=0.0190). No interaction was shown between these two factors. Eight OTUs

276 represented by more than 5 sequences correlated with NMDS axes and/or were significantly
277 differentially distributed according to the METASTATS analysis (Table 7).
278
279 *Edaphic factors involved in the AMF community composition*
280 The soil physicochemical variables significantly different among the sites were plotted as
281 vectors in the NMDS biplot (Fig. 3). Ammonia and porosity were positively correlated with
282 NMDS1, while nitric N and Mg were negatively correlated with the same NMDS axis (Table 8).
283 Total C, ammonia, nitric N, Mg, and porosity-based distance matrices computed at the sample level
284 correlated with the OTU-based Jaccard distance matrix.
285
286
287 **Discussion**
288 This study, for the first time, compared the composition of the community of root-
289 colonizing and soil-dwelling arbuscular mycorrhizal fungi (AMF) from the exotic flowering plant
290 species *C. japonica* in three closely located sites in the Lake Maggiore area (Piedmont, Italy).
291 Although the first report dates back to 1974 (Mejstrik 1974), literature regarding the
292 interaction between *C. japonica* and AMF colonization is still limited. It is only recently that two
293 works have been dedicated to the interaction between this plant species and AMF (Berruti et al.
294 2013a; Berruti et al. 2013b). More information is present on the best-known *C. sinensis* (L.) O.
295 Kuntze, i.e. the tea plant (Aliasgharzad et al. 2011; Balasuriya et al. 2000; Gupta and Sharma 2010;
296 Karthikeyan et al. 2005; Singh et al. 2010; Singh et al. 2008b; Singh et al. 2008a; Wu et al. 2009).
297 With the present work, the mycorrhizal status of *C. japonica* in field has been confirmed and
298 studied in deep.
299 The staining of camellia root fragments made us able to recognize the presence of several
300 structures typical of AMF, reported in Fig. S2 (supplementary data). The mycorrhizal roots show an

301 extensive colonization, characterized by a moderate intensity of colonization and a frequent
302 arbuscule and coil formation in the mycorrhizal parts. The mycorrhizal frequency detected in this
303 study (>90%) was higher than those found in association with *C. japonica* trees and shrubs
304 occurring in natural, semi-natural and arable soils in New Zealand (20-50%) (Mejstrik 1974) and in
305 an inoculation study of containerized small-size camellias in Italy (14-37%) (Berruti et al. 2013b).
306 A parallel study, in which containerized small-sized camellias were inoculated with a field-
307 collected and concentrated mix of AMF from one of the sampling sites considered in the present
308 study (RE), showed comparable mycorrhizal frequency but higher intensity and arbuscule
309 production (data not shown). In other works describing natural and cultivated sites in India, *C.*
310 *sinensis* showed comparable AM colonization frequencies and arbuscule production (Singh et al.
311 2010; Singh et al. 2008a).

312 The amplification with AML1-AML2 primers pair, as previously assessed (Lee et al. 2008),
313 was capable of achieving a wide coverage of the Glomeromycota phylum, highlighting 39 OTUs
314 members of Glomerales, Paraglomerales, Archaeosporales and Diversisporales (Fig. 1). Overall, the
315 two most abundant and diverse taxonomical ranks found were the Glomerales (22 OTUs, 249
316 sequences) and the Paraglomerales (9 OTUs, 105 sequences). Twenty-three OTUs were detected in
317 the intra-radical communities (18 Glomerales, 2 Paraglomerales, 2 Archaeosporales, and 1
318 Diversisporales). Each root sample community was dominated (see black squares in Table 4) by a
319 low number (1-2) of different OTUs (OTU01, VTX00239; OTU02, VTX00223; OTU03,
320 VTX00074; OTU04, VTX00412; OTU05, VTX00219; OTU06, VTX00122), matching previous
321 findings (Dumbrell et al. 2010). Besides sequences showing high similarity with AMF, sequences
322 corresponding to non-target organisms were also obtained, as already reported by other authors
323 (Alguacil et al. 2008; Douhan et al. 2005; Toljander et al. 2008).

324 As expected, the root-colonizing and the soil-dwelling AMF communities were significantly
325 different. This is normal and has been found in other studies (Hart and Reader 2004; Hempel et al.

326 2007; Torrecillas et al. 2012). One reason is that not all soil-dwelling AMF can enter the specific
327 target plant root system, but instead may be present in the soil because they can colonize other host-
328 plants in the nearest proximity. Another is that some root-inhabiting AMF may extend short
329 distance in soil (Parniske 2008). As shown by the Venn diagram (Fig. 2), 16 OTUs were retrieved
330 exclusively in soil samples while only 8 were present in the only root samples. In accordance with
331 the METASTATS analysis (Table 7), only one of the most represented OTUs (>5 total sequences),
332 OTU01 (p-value=0.0030, q-value=0.0101) and OTU11 (p-value=0.0440, q-value=0.0507),
333 affiliated to the Virtual Taxa VTX00239 (Paraglomerales) and VTX00004 (Archaeosporales),
334 respectively, were differentially abundant between root and soil samples. In particular, OTU01 was
335 the most abundant OTU found (81 sequences), was positively correlated with NMDS2 axis
336 (R=0.54, p-value=0.0214) and was dominant in IS soil, RO roots, and RO soil. This OTU was
337 almost ubiquitously detected (11 samples). The only sample type that excluded the presence of this
338 OTU was the intra-radical community in the IS site. The fact that this Paraglomerales member was
339 highly abundant in soil samples but absent in roots is consistent with the results of Hempel et al.
340 (Hempel et al. 2007) and leads to hypothesize that other host-plants were readily available in the
341 nearest proximity. This hypothesis is weakened by the fact that the only surrounding vegetation in
342 the IS site consisted of other camellia cultivars with no understory vegetation (Fig. S1,
343 supplementary data). Other two hypothesis could support a) the possibility that the IS soil harbored
344 a high amount of spores with no extra-radical mycelium of OTU01 and therefore this taxon was
345 symbiotically non-active or b) a very high patchiness of the root colonization due to co-existence
346 with other over-dominant taxa that jeopardized OTU01 detection.

347 Unexpectedly, the three sites harbored significantly different AMF communities. This is
348 surprising since the AMF community composition was not expected to be highly variable, being the
349 sampling sites located within 2 km. However, the three site soils presented different
350 physicochemical profiles that showed to be involved in the community shaping. Among the most

represented OTUs (>5 total sequences), OTU02 (VTX00223, Glomerales), OTU03 (VTX00074, Glomerales), OTU04 (VTX00412, Glomerales), OTU06 (VTX00122, Glomerales), OTU07 (VTX00219, Glomerales), and OTU08 (VTX00005, Archaeosporales) were differentially abundant among the three sites and/or correlated with the NMDS1 axis (Table 7), which was the major axis ($R^2$=0.414) of the NMDS. According to the METASTATS analysis, OTU02 and OTU03 had a highly significant (q-value<0.05) differential distribution. OTU02 was differentially distributed between RE and the other two sites. This OTU was dominant in the RE site and, together with soil porosity and ammonia content, was highly positively correlated with the NMDS1 axis (R=0.88, p-value=0.0000). This may suggest an implication of these physicochemical parameters in the distribution of this OTU. OTU03 was differentially distributed between IS and RO, dominant in RO root samples and positively correlated with NMDS1 (R=0.59, p-value=0.0106). For this OTU, high porosity seemed to be a favorable condition.

In line with several other studies (Martínez-García et al. 2011; Schechter and Bruns 2012; Schechter and Bruns 2008; Zarei et al. 2010), the edaphic variables were an important factor involved in OTU distribution among the different sites. In the present study, porosity, ammonia, nitric N, and Mg soil content were correlated with the NMDS1 axis. Moreover, the Gower sample distance matrices based on these four physicochemical variables were correlated, sometimes highly, with the OTU-based Jaccard sample distance matrix. These four soil properties have been previously reported to affect the growth and distribution of AMF. N addition to soil has previously been described as a detrimental factor for AMF diversity (Avio et al. 2013; Borriello et al. 2012; Lin et al. 2012; Sheng et al. 2013; Tian et al. 2013) that could favor the establishment of more aggressive AMF, such as members of the Glomerales. This could be the case of OTU02 in RE, in which the soil featured a higher ammonia content of possible anthropogenic origin. Low porosity (soil compaction) has been reported to limit the hyphal development of certain AMF isolates, including generalist fungi such as *Funneliformis mosseae*, *Rhizophagus intraradices*, and

376 *Gigaspora margarita* (Entry et al. 2002), reducing the competition and encouraging the occurrence
377 of other better performing isolates. Several studies concerning AMF development in high Mg soils
378 have demonstrated that different AMF isolates can respond positively (Gryndler et al. 1992) or
379 negatively (Jarstfer et al. 1998) to the addition of $MgSO_4$ into the substrate. It has also been
380 demonstrated that specific AMF isolates can be found in considerable amounts in high Mg
381 serpentine soils (Schechter and Bruns 2012). OTU06, that was dominant in IS roots and common in
382 2/3 of IS soils but absent in all other samples, was potentially favored by low porosity and high Mg
383 content. These conditions might have been unfavorable for the occurrence of OTU02 and OTU03.
384 In contrast with data on AMF global distribution (Moora et al. 2011; Öpik et al. 2010; Öpik
385 et al. 2006), the entire order Diversisporales was almost absent in both soil and root samples of all
386 sites. This is not in line with Singh et al. (Singh et al. 2008a), who found that Diversisporales were
387 abundant in the rhizospheric soil of the close species *C. sinensis* from acidic Indian soils. However,
388 in a recent study, inoculated Diversisporales propagules have been demonstrated to marginally
389 colonize the roots of *C. japonica* in greenhouse, without producing arbuscules (Berruti et al.
390 2013a).
391 In conclusion, this study proves the highly mycorrhizal status of *C. japonica* in field and
392 describes 39 OTUs, 23 of which were found also inside the root system, that occur in the
393 rhizospheric soil and roots of long-lived specimens of this exotic plant species in its introduced
394 range on the shores of the Lake Maggiore (Piedmont, Italy). A strong difference in the community
395 composition, but not in the OTU richness, was detected between root-colonizing and the soil-
396 dwelling communities and among the three closely located sites analyzed. The data suggest that
397 different combinations of edaphic properties have a pivotal role in shaping the AMF communities.
398 In order to describe a more comprehensive picture of the symbiotic interaction between AMF and
399 *C. japonica*, future studies should focus on the characterization of the root and soil AMF
400 community in several other areas, including the native range of this exotic plant species.

401

402

403 **Acknowledgements**

404 This study was financially supported by the Regione Piemonte. The authors would like to thank the
405 anonymous reviewers for their contribution to the improvement of the manuscript. RB was funded
406 by Lagrange Project. AB was funded by ECOFLOR and PROLACTE projects (Regione Piemonte).

407

408 **References**

409 Alguacil MM, Lumini E, Roldán A, Salinas-García JR, Bonfante P, Bianciotto V (2008) The
410 impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. Ecol
411 Appl Publ Ecol Soc Am 18:527–536.
412 Aliasgharzad N, Hajiboland R, Olsson PA (2011) Lack of arbuscular mycorrhizal colonisation in
413 tea (*Camellia sinensis* L.) plants cultivated in Northern Iran. Symbiosis 55:91–95. doi:
414 10.1007/s13199-011-0149-8
415 Avio L, Castaldini M, Fabiani A, Bedini S, Sbrana C, Turrini A, Giovannetti M (2013) Impact of
416 nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a
417 Mediterranean agroecosystem. Soil Biol Biochem 67:285–294. doi: 10.1016/j.soilbio.2013.09.005
418 Balasuriya A, Wimaladasa GD, Ratnayake RMA (2000) Effect of application of NPK fertilizers on
419 the natural incidence of vesicular arbuscular mycorrhiza in the rhizosphere soils and feeder roots of
420 tea (*Camellia sinensis*. Sri Lanka J Tea Sci 66:61–68.
421 Berruti A, Borriello R, Lumini E, Scariot V, Bianciotto V, Balestrini R (2013a) Application of laser
422 microdissection to identify the mycorrhizal fungi that establish arbuscules inside root cells. Front
423 Plant Sci 4:135. doi: 10.3389/fpls.2013.00135
424 Berruti A, Borriello R, Della Beffa MT, Scariot V, Bianciotto V (2013b) Application of nonspecific
425 commercial AMF inocula results in poor mycorrhization in *Camellia japonica* L. Symbiosis. doi:
426 dx.doi.org/10.1007/s13199-013-0258-7
427 Borriello R, Lumini E, Girlanda M, Bonfante P, Bianciotto V (2012) Effects of different
428 management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular
429 approach. Biol Fertil Soils 48:911–922. doi: 10.1007/s00374-012-0683-4
430 Caser M, Torello Marinoni D, Scariot V (2010) Microsatellite-based genetic relationships in the
431 genus *Camellia*: potential for improving cultivars. Genome 53:384–399.
432 Douhan GW, Petersen C, Bledsoe CS, Rizzo DM (2005) Contrasting root associated fungi of three
433 common oak-woodland plant species based on molecular identification: host specificity or non-
434 specific amplification? Mycorrhiza 15:365–372.
435 Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Idiosyncrasy and
436 overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a
437 role for stochastic processes? J Ecol 98:419–428.
438 Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK (2002) Influence of adverse soil conditions on
439 the formation and function of Arbuscular mycorrhizas. Adv Environ Res 7:123–138. doi:
440 10.1016/S1093-0191(01)00109-5
441 Fitter AH, Gilligan CA, Hollingworth K, Kleczkowski A, Twyman RM, Pitchford JW, Programme
442 TM of the NSB (2005) Biodiversity and ecosystem function in soil. Funct Ecol 19:369–377. doi:

10.1111/j.0269-8463.2005.00969.x
Fitter AH, Helgason T, Hodge A (2011) Nutritional exchanges in the arbuscular mycorrhizal symbiosis: Implications for sustainable agriculture. Fungal Biol Rev 25:68–72. doi: 10.1016/j.fbr.2011.01.002
Gianinazzi S, Gollotte A, Binet M-N, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530. doi: 10.1007/s00572-010-0333-3
Gianinazzi S, Schüepp H (1994) Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhäuser
Gryndler M, Vejsadová H, Vančura V (1992) The effect of magnesium ions on the vesicular—arbuscular mycorrhizal infection of maize roots. New Phytol 122:455–460. doi: 10.1111/j.1469-8137.1992.tb00073.x
Gupta RK, Sharma C (2010) Diversity of arbuscular mycorrhizal fungi in *Camellia sinensis* in Uttarakhand State, India. Afr J Biotechnol 9:5313–5319.
Hammer Ø, Harper DAT, Ryan PD (2011) PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9.
Hart M, Reader R (2004) Do arbuscular mycorrhizal fungi recover from soil disturbance differently? Trop Ecol 45:97–111.
Helgason T, Merryweather J, Young J, Fitter A (2007) Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. J Ecol 95:623–630. doi: 10.1111/j.1365-2745.2007.01239.x
Hempel S, Renker C, Buscot F (2007) Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ Microbiol 9:1930–1938. doi: 10.1111/j.1462-2920.2007.01309.x
Jarstfer AG, Farmer-Koppenol P, Sylvia DM (1998) Tissue magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction. Mycorrhiza 7:237–242. doi: 10.1007/s005720050186
Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J-M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16. doi: 10.1007/s00374-002-0546-5
Karthikeyan A, Muthukumar T, Udaiyan K (2005) Response of Tea (Camellia sinensis (L). Kuntze) to Arbuscular Mycorrhizal Fungi under Plantation Nursery Conditions. Biol Agric Hortic 22:305–319. doi: 10.1080/01448765.2005.9755294
Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9:286–298. doi: 10.1093/bib/bbn013
Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984. doi: 10.1111/j.1469-8137.2011.03962.x
Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349. doi: 10.1111/j.1574-6941.2008.00531.x
Lin X, Feng Y, Zhang H, Chen R, Wang J, Zhang J, Chu H (2012) Long-Term Balanced Fertilization Decreases Arbuscular Mycorrhizal Fungal Diversity in an Arable Soil in North China Revealed by 454 Pyrosequencing. Environ Sci Technol 46:5764–5771. doi: 10.1021/es3001695
Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V (2010) Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environ Microbiol 12:2165–2179. doi: 10.1111/j.1462-2920.2009.02099.x
Martínez-García LB, Armas C, Miranda J de D, Padilla FM, Pugnaire FI (2011) Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. Soil Biol Biochem 43:682–689. doi: 10.1016/j.soilbio.2010.12.006

493 Mejstrik V (1974) The Frequency of Vesicular-Arbuscular Mycorrhizae in the Roots of *Camellia*
494 *japonica* L. from Different Sites in New Zealand.
495 Mondal TK (2011) *Camellia*. In: Kole C (ed) Wild Crop Relat. Genomic Breed. Resour. Springer
496 Berlin Heidelberg, Berlin, Heidelberg, pp 15–39
497 Moora M, Berger S, Davison J, Öpik M, Bommarco R, Bruelheide H, Kühn I, Kunin WE, Metsis
498 M, Rortais A, Vanatoa A, Vanatoa E, Stout JC, Truusa M, Westphal C, Zobel M, Walther G-R
499 (2011) Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa:
500 evidence from a continental-scale study using massively parallel 454 sequencing. J Biogeogr
501 38:1305–1317. doi: 10.1111/j.1365-2699.2011.02478.x
502 Niranjana KS, Viswanath S (2008) Root characteristics of tea [Camellia sinensis (L.) O. Kuntze]
503 and silver oak [*Grevillea robusta* (A. Cunn)] in a mixed tea plantation at Munnar, Kerala. J Trop
504 Agric 46:25–31.
505 Oehl F, Sieverding E, Palenzuela J, Ineichen K, da Silva GA (2011) Advances in Glomeromycota
506 taxonomy and classification. IMA FUNGUS 2:191–199. doi: 10.5598/imafungus.2011.02.02.10
507 Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M (2009) Large-scale parallel 454 sequencing
508 reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest.
509 New Phytol 184:424–437. doi: 10.1111/j.1469-8137.2009.02920.x
510 Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing arbuscular mycorrhizal
511 fungal communities in different ecosystems around the globe. J Ecol 94:778–790. doi:
512 10.1111/j.1365-2745.2006.01136.x
513 Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier ü., Zobel M (2010) The
514 online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular
515 mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241. doi: 10.1111/j.1469-
516 8137.2010.03334.x
517 Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Micro
518 6:763–775. doi: 10.1038/nrmicro1987
519 Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-
520 based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota).
521 Mycorrhiza 1–17. doi: 10.1007/s00572-013-0486-y
522 Schechter SP, Bruns TD (2012) Edaphic sorting drives arbuscular mycorrhizal fungal community
523 assembly in a serpentine/non-serpentine mosaic landscape. Ecosphere 3:art42. doi: 10.1890/ES12-
524 00059.1
525 Schechter SP, Bruns TD (2008) Serpentine and non-serpentine ecotypes of Collinsia sparsiflora
526 associate with distinct arbuscular mycorrhizal fungal assemblages. Mol Ecol 17:3198–3210. doi:
527 10.1111/j.1365-294X.2008.03828.x
528 Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley
529 BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Horn DJV, Weber CF (2009)
530 Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for
531 Describing and Comparing Microbial Communities. Appl Environ Microbiol 75:7537–7541. doi:
532 10.1128/AEM.01541-09
533 Sheng M, Lalande R, Hamel C, Ziadi N (2013) Effect of long-term tillage and mineral phosphorus
534 fertilization on arbuscular mycorrhizal fungi in a humid continental zone of Eastern Canada. Plant
535 Soil 369:599–613. doi: 10.1007/s11104-013-1585-4
536 Singh S, Pandey A, Chaurasia B, Palni L (2008a) Diversity of arbuscular mycorrhizal fungi
537 associated with the rhizosphere of tea growing in “natural” and “cultivated” ecosites. Biol Fertil
538 Soils 44:491–500. doi: 10.1007/s00374-007-0231-9
539 Singh S, Pandey A, Kumar B, Palni L (2010) Enhancement in growth and quality parameters of tea
540 [*Camellia sinensis* (L.) O. Kuntze] through inoculation with arbuscular mycorrhizal fungi in an acid
541 soil. Biol Fertil Soils 46:427–433. doi: 10.1007/s00374-010-0448-x
542 Singh S, Pandey A, Palni L (2008b) Screening of arbuscular mycorrhizal fungal consortia

543 developed from the rhizospheres of natural and cultivated tea plants for growth promotion in tea
544 [*Camellia sinensis* (L.) O. Kuntze]. Pedobiologia 52:119–125. doi: 10.1016/j.pedobi.2008.06.001
545 Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd editon. Academic London
546 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular
547 Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and
548 Maximum Parsimony Methods. Mol Biol Evol 28:2731–2739. doi: 10.1093/molbev/msr121
549 Tian H, Drijber RA, Zhang JL, Li XL (2013) Impact of long-term nitrogen fertilization and rotation
550 with soybean on the diversity and phosphorus metabolism of indigenous arbuscular mycorrhizal
551 fungi within the roots of maize (Zea mays L.). Agric Ecosyst Environ 164:53–61. doi:
552 10.1016/j.agee.2012.09.007
553 Tianlu M, Bartholomew B (2007) Theaceae (including Ternstroemiaceae). Flora China 12:366–
554 478.
555 Toljander JF, Santos-González JC, Tehler A, Finlay RD (2008) Community analysis of arbuscular
556 mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial.
557 FEMS Microbiol Ecol 65:323–338. doi: 10.1111/j.1574-6941.2008.00512.x
558 Torrecillas E, Alguacil M del M, Roldán A (2012) Differences in the AMF diversity in soil and
559 roots between two annual and perennial gramineous plants co-occurring in a Mediterranean,
560 semiarid degraded area. Plant Soil 354:97–106. doi: 10.1007/s11104-011-1047-9
561 Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un
562 système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle.
563 Physiol. Genet. Asp. Mycorrhizae Gianinazzi-Pearson V Gianinazzi S, INRA Press. Paris, pp 217–
564 221
565 Verbruggen E, van der Heijden MGA, Rillig MC, Kiers ET (2013) Mycorrhizal fungal
566 establishment in agricultural soils: factors determining inoculation success. New Phytol 197:1104–
567 1109. doi: 10.1111/j.1469-8137.2012.04348.x
568 White JR, Nagarajan N, Pop M (2009) Statistical Methods for Detecting Differentially Abundant
569 Features in Clinical Metagenomic Samples. PLoS Comput Biol 5:e1000352. doi:
570 10.1371/journal.pcbi.1000352
571 White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal
572 RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protoc.
573 Guide Methods Appl., Academic. San Diego, California, pp 315–322
574 Wu LS, Wang Y, Li M, Ding ZT, Liu RJ (2009) Arbuscular mycorrhizal fungi diversity in the
575 rhizosphere of tea plant (*Camellia sinensis*) grown in Laoshan, Shandong. Biodivers Sci 17:499–
576 505.
577 Zarei M, Hempel S, Wubet T, Schäfer T, Savaghebi G, Jouzani GS, Nekouei MK, Buscot F (2010)
578 Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and
579 heavy metal contamination. Environ Pollut 158:2757–2765. doi: 10.1016/j.envpol.2010.04.017
580 Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences.
581 J Comput Biol J Comput Mol Cell Biol 7:203–214. doi: 10.1089/10665270050081478
582

583

584

585 **Figure legends**

586

587 **Fig. 1** The phylogenetic tree constructed on a portion of the arbuscular mycorrhizal fungal SSU
588 rDNA (~650bp) using the Maximum Likelihood method. The alignment that was used to generate
589 the tree featured 123 OTU representative sequences (one for each sample where the OTU occurred),
590 18 Virtual Tax reference sequences and the Mucoromycotina sequence gb|NG017181.1 *Endogone*
591 *pisiformis* DAOM233144 as outgroup. The sample name (e.g. ISaR) is reported after the OTU code
592 and identifies the site of origin (IS, RO, or RE), the replicate (a, b, or c) and the matrix of origin (R
593 for roots and S for soil). The percentage of replicate trees in which the associated taxa clustered
594 together in the bootstrap test (500 replicates) are shown next to the branches. Branches
595 corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

596

597 **Fig. 2** Venn diagrams showing the number of shared and site or matrix-specific OTUs. Shared
598 OTUs are listed in text boxes.

599

600 **Fig. 3** The Non-metric Multi-Dimensional Scaling **(**NMDS) plot based on Jaccard distance matrix
601 of the AMF communities found in each biological replicate of each compartment (R = roots and S =
602 soil) in the three sites (RO, RE and IS). Tha sample name (e.g. ISaR) identifies the site of origin
603 (IS, RO, or RE), the replicate (a, b, or c) and the matrix of origin (R for roots and S for soil). The
604 vectors (arrows) graphically represent the correlations of the NMDS axes with each of the measured
605 soil physicochemical variables that were significantly different among the three sites: electrical
606 conductivity (EC), salinity, porosity, total C (tot C) and N (tot N), ammonia, nitric N, Ca and Mg.
607 Convex hulls are dashed for RO, dotted for RE, and solid for IS samples. Sample point symbols are
608 empty for root and filled for soil samples.

609

611 **Tables**

612 **Table 1** Physical properties of the sampled soils. Means for pH, electrical conductivity (EC),
613 salinity, bulk and particle densities and porosity are reported.

| | pH | EC (µS/cm) | Salinity (meq/100g) | Bulk Density (g/cm$^3$) | Particle Density (g/cm$^3$) | Porosity (%) |
|---|---|---|---|---|---|---|
| RO | 5.80 | 99.0b[z] | 1.55b | 1.09 | 1.53 | 29 |
| RE | 5.87 | 60.7a | 0.77a | 1.23 | 1.66 | 26 |
| IS | 5.87 | 62.7a | 0.90a | 1.15 | 1.26 | 9 |
| Sig. | ns | * | * | ns | ns | - |

614 [z]Means followed by the same letter do not differ significantly at $p<0.05$, according to REGW-F test
615 for parametric data or the Mann-Whitney U test with Bonferroni transformation for non-parametric
616 data. The statistical relevance (Sig., $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, ns = non-significant)
617 is provided.

618

**Table 2** Chemical properties of the sampled soils. Total Carbon and Nitrogen, ammonia, nitric nitrogen, total phosphorous, calcium, magnesium and potassium contents are reported.

| | Total C (%) | Total N (%) | ammonia (ppm) | Nitric N (ppm) | Total P (ppm) | Ca (ppm) | Mg (ppm) | K (ppm) |
|---|---|---|---|---|---|---|---|---|
| RO | 4.93c[z] | 0.34b | 0.80a | 3.20c | 200 | 2600c | 40400a | 4300 |
| RE | 3.52b | 0.22a | 4.81b | 2.68a | 500 | 1200a | 39800a | 4900 |
| IS | 2.75a | 0.21a | 0.99a | 3.02b | 200 | 1600b | 48600b | 4500 |
| Sig. | *** | * | *** | *** | ns | *** | *** | ns |

[z]Means followed by the same letter do not differ significantly at $p<0.05$, according to REGW-F test for parametric data or the Mann-Whitney U test with Bonferroni transformation for non-parametric data. The statistical relevance (Sig., $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, ns = non-significant) is provided.

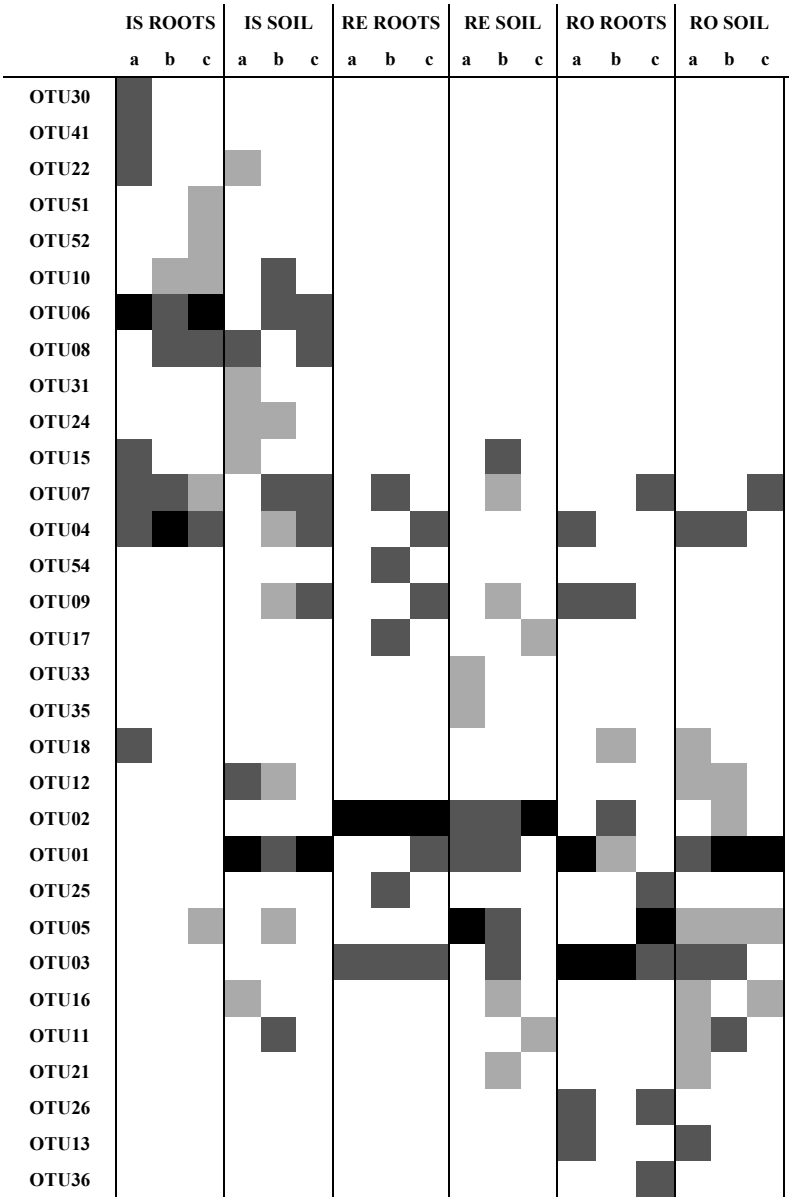

23

626 **Table 3** Mycorrhizal frequency (F%), AMF colonization intensity of the whole root system (M%)
627 and of the mycorrhizal parts (m%) and the presence of arbuscules in the mycorrhizal parts (a%) and
628 in the whole sampled root apparatus (A%) from the three sites. Values are calculated according to
629 the freeware Mycocalc, available online (http://www2.dijon.inra.fr/mychintec/Mycocalc-
630 prg/download.html). SE of means are provided for each percentage value

| site | F% | M% | m% | a% | A% |
|---|---|---|---|---|---|
| RO | 91.90±3.10 | 14.12±3.34 | 15.30±3.39 | 31.87±6.65 | 4.06±0.05 |
| RE | 90.61±0.99 | 29.37±7.17 | 32.55±8.10 | 41.61±19.30 | 13.51±7.19 |
| IS | 93.69±6.31 | 9.16±3.37 | 9.92±3.52 | 31.47±10.82 | 3.59±1.85 |
| Sig. | ns | ns | ns | ns | ns |

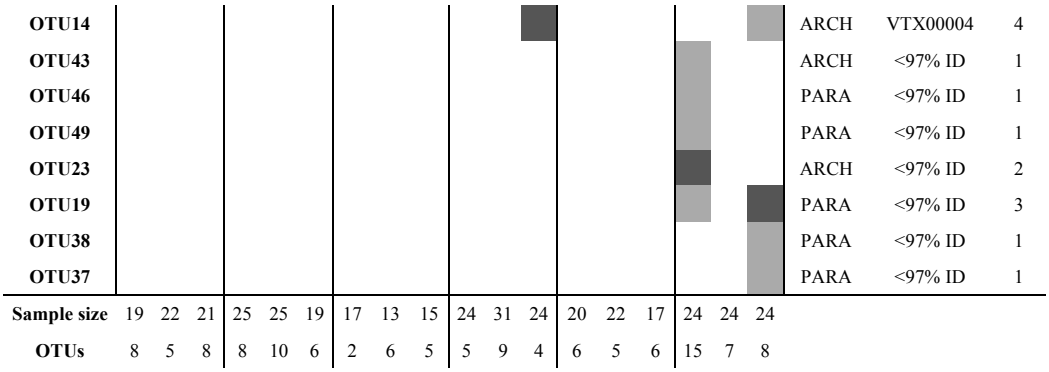
631 The statistical relevance (Sig., $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***, ns = non-significant) is
632 provided.
633

**Table 4** The ordinated heat-map based on occurrence classes (class 0, no square, <1% absent; class 1, light grey square, 1%-5% rare; class 2, grey square, 5%-30% common; class 3, black square, >30% dominant). The sample names reported identify the site and matrix of origin (e.g. IS ROOTS). Below the sample name, the replicate (a, b, or c) is reported. The order (Glomerales, GLOM; Diversisporales, DIVE; Archaeosporales, ARCH; Paraglomerales, PARA), the affiliated Virtual Taxa (VT) and the number of sequences (Seqs) are reported in columns. Rows report OTU code, sample size, and OTU richness.



| | IS ROOTS | | | IS SOIL | | | RE ROOTS | | | RE SOIL | | | RO ROOTS | | | RO SOIL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | a | b | c | a | b | c | a | b | c | a | b | c | a | b | c | Order | VT | Seqs |
| **OTU30** | | | | | | | | | | | | | | | | | | | GLOM | VTX00056 | 1 |
| **OTU41** | | | | | | | | | | | | | | | | | | | GLOM | VTX00064 | 1 |
| **OTU22** | | | | | | | | | | | | | | | | | | | DIVE | VTX00049 | 2 |
| **OTU51** | | | | | | | | | | | | | | | | | | | GLOM | VTX00199 | 1 |
| **OTU52** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 1 |
| **OTU10** | | | | | | | | | | | | | | | | | | | GLOM | VTX00199 | 8 |
| **OTU06** | | | | | | | | | | | | | | | | | | | GLOM | VTX00122 | 25 |
| **OTU08** | | | | | | | | | | | | | | | | | | | ARCH | VTX00005 | 9 |
| **OTU31** | | | | | | | | | | | | | | | | | | | GLOM | VTX00057 | 1 |
| **OTU24** | | | | | | | | | | | | | | | | | | | PARA | <97% ID | 2 |
| **OTU15** | | | | | | | | | | | | | | | | | | | GLOM | VTX00166 | 4 |
| **OTU07** | | | | | | | | | | | | | | | | | | | GLOM | VTX00219 | 17 |
| **OTU04** | | | | | | | | | | | | | | | | | | | GLOM | VTX00412 | 35 |
| **OTU54** | | | | | | | | | | | | | | | | | | | GLOM | VTX00093 | 1 |
| **OTU09** | | | | | | | | | | | | | | | | | | | PARA | <97% ID | 9 |
| **OTU17** | | | | | | | | | | | | | | | | | | | GLOM | VTX00223 | 4 |
| **OTU33** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 1 |
| **OTU35** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 1 |
| **OTU18** | | | | | | | | | | | | | | | | | | | ARCH | VTX00005 | 3 |
| **OTU12** | | | | | | | | | | | | | | | | | | | PARA | <97% ID | 6 |
| **OTU02** | | | | | | | | | | | | | | | | | | | GLOM | VTX00223 | 59 |
| **OTU01** | | | | | | | | | | | | | | | | | | | PARA | VTX00239 | 81 |
| **OTU25** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 2 |
| **OTU05** | | | | | | | | | | | | | | | | | | | GLOM | VTX00219 | 33 |
| **OTU03** | | | | | | | | | | | | | | | | | | | GLOM | VTX00074 | 44 |
| **OTU16** | | | | | | | | | | | | | | | | | | | ARCH | <97% ID | 4 |
| **OTU11** | | | | | | | | | | | | | | | | | | | ARCH | VTX00004 | 7 |
| **OTU21** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 2 |
| **OTU26** | | | | | | | | | | | | | | | | | | | GLOM | VTX00113 | 2 |
| **OTU13** | | | | | | | | | | | | | | | | | | | GLOM | VTX00084 | 5 |
| **OTU36** | | | | | | | | | | | | | | | | | | | GLOM | <97% ID | 1 |


25



| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **OTU14** | | | | | | | | | | | | | | | | | ARCH | VTX00004 | 4 |
| **OTU43** | | | | | | | | | | | | | | | | | ARCH | <97% ID | 1 |
| **OTU46** | | | | | | | | | | | | | | | | | PARA | <97% ID | 1 |
| **OTU49** | | | | | | | | | | | | | | | | | PARA | <97% ID | 1 |
| **OTU23** | | | | | | | | | | | | | | | | | ARCH | <97% ID | 2 |
| **OTU19** | | | | | | | | | | | | | | | | | PARA | <97% ID | 3 |
| **OTU38** | | | | | | | | | | | | | | | | | PARA | <97% ID | 1 |
| **OTU37** | | | | | | | | | | | | | | | | | PARA | <97% ID | 1 |
| **Sample size** | 19 | 22 | 21 | 25 | 25 | 19 | 17 | 13 | 15 | 24 | 31 | 24 | 20 | 22 | 17 | 24 | 24 | 24 | |
| **OTUs** | 8 | 5 | 8 | 8 | 10 | 6 | 2 | 6 | 5 | 5 | 9 | 4 | 6 | 5 | 6 | 15 | 7 | 8 | |

641



26

**Table 5** The estimated sample coverage, the sample size in terms of sequences found per sample, the OTU richness, and the Shannon's diversity index for the AMF communities found in each of the sample replicates corresponding to the three sites (IS, RE, and RO) and the two matrices (roots and soil) under study. The sample name (e.g. ISaR) identifies the site of origin (IS, RO, or RE), the replicate (a, b, or c) and the matrix of origin (R for roots and S for soil). Two-way ANOVA p-values are reported for OTU richness and Shannon's diversity index.

| Sample code | Site | Matrix | Estimated sample coverage | Sample size | OTU richness | Shannon's diversity index |
|---|---|---|---|---|---|---|
| ISaR | IS | ROOTS | 0.68 | 19 | 8 | 1.645 |
| ISbR | IS | ROOTS | 0.95 | 22 | 5 | 1.271 |
| IScR | IS | ROOTS | 0.76 | 21 | 8 | 1.566 |
| ISaS | IS | SOIL | 0.80 | 25 | 8 | 1.407 |
| ISbS | IS | SOIL | 0.80 | 25 | 10 | 2.058 |
| IScS | IS | SOIL | 0.89 | 19 | 6 | 1.413 |
| REaR | RE | ROOTS | 1.00 | 17 | 2 | 0.362 |
| REbR | RE | ROOTS | 0.69 | 13 | 6 | 1.484 |
| REcR | RE | ROOTS | 0.87 | 15 | 5 | 1.287 |
| REaS | RE | SOIL | 0.92 | 24 | 5 | 1.222 |
| REbS | RE | SOIL | 0.87 | 31 | 9 | 1.859 |
| REcS | RE | SOIL | 0.92 | 24 | 4 | 0.710 |
| ROaR | RO | ROOTS | 0.95 | 20 | 6 | 1.617 |
| RObR | RO | ROOTS | 0.91 | 22 | 5 | 1.112 |
| ROcR | RO | ROOTS | 0.76 | 17 | 6 | 1.381 |
| ROaS | RO | SOIL | 0.58 | 24 | 15 | 2.571 |
| RObS | RO | SOIL | 0.88 | 24 | 7 | 1.549 |
| ROcS | RO | SOIL | 0.79 | 24 | 8 | 1.370 |
| | | **2-way ANOVA p-values** | | Site | 0.1756 | 0.2382 |
| | | | | Matrix | 0.0717 | 0.2503 |
| | | | | interaction | 0.4962 | 0.8278 |


27

650 **Table 6** Two-way PERMANOVA p-values computed using the Jaccard distance matrix are
651 reported for the site factor (IS, RE, and RO), the matrix factor (roots or soil), and their interaction.

**Two-way PERMANOVA (9999 permutations. Jaccard distance)**

| Factor | F | p-value |
|---|---|---|
| Site | 2.87 | 0.0001 |
| Matrix | 2.06 | 0.0190 |
| interaction | 0.95 | 0.5156 |

652

653

654


28

**Table 7** METASTATS analysis p and q-values and Spearman's correlation with the first two NMDS axes (NMDS1 and NMDS2) are reported for OTUs that are represented by more than 5 total sequences and that show a significant METASTATS p-value. For the METASTATS analysis, the pairwise comparison between the factor levels (e.g. RE-RO) which the p and q-values refer to is reported.

| | | **METASTATS (1000 permutations)** | | | **Correlation with NMDS axes** | | |
|---|---|---|---|---|---|---|---|
| **OTU** | **VT** | **p-value** | **q-value** | **comparison** | **Axis** | **R** | **p-value** |
| **OTU01** | **VTX00239** | 0.0030 | 0.0101 | ROOTS-SOIL | NMDS2 | 0.54 | 0.0214 |
| **OTU02** | **VTX00223** | 0.0002 | 0.0257 | RE-RO | NMDS1 | 0.88 | 0.0000 |
| | | 0.0004 | 0.0410 | IS-RE | | | |
| **OTU03** | **VTX00074** | 0.0010 | 0.0453 | IS-RO | NMDS1 | 0.59 | 0.0106 |
| | | 0.0430 | 1.0000 | RE-RO | | | |
| | | 0.0112 | 0.4353 | IS-RE | | | |
| **OTU04** | **VTX00412** | 0.0524 | 0.5584 | IS-RE | NMDS1 | -0.48 | 0.0429 |
| **OTU06** | **VTX00122** | 0.0125 | 0.1969 | IS-RO | NMDS1 | -0.71 | 0.0010 |
| | | 0.0187 | 0.4369 | IS-RE | | | |
| **OTU07** | **VTX00219** | 0.0179 | 0.4369 | IS-RE | NMDS2 | -0.65 | 0.0035 |
| **OTU08** | **VTX00005** | 0.0023 | 0.0532 | IS-RO | NMDS1 | -0.62 | 0.0064 |
| | | 0.0044 | 0.2548 | IS-RE | | | |
| **OTU11** | **VTX00004** | 0.0440 | 0.0507 | ROOTS-SOIL | NMDS2 | 0.31 | 0.2007 |


29

**Table 8** Correlation between OTU-based Jaccard distance matrix and environmental variable-based Gower distance matrix at the sample level computed by Mantel tests are reported. Pearson's correlations of the environmental variables with the first NMDS axis (there were no correlations with the second) are also shown.

| | Mantel test (5000 permutations) vs OTU distance matrix | | Correlation with NMDS1 | |
|---|---|---|---|---|
| **Environmental variable** | **R** | **p-value** | **NMDS1 R** | **p-value** |
| EC | 0.02 | 0.3667 | 0.07 | 0.7682 |
| Salinity | 0.05 | 0.2679 | -0.02 | 0.9211 |
| Total C | 0.22 | 0.0098 | 0.41 | 0.0705 |
| Total N | 0.05 | 0.2673 | 0.17 | 0.4706 |
| Ammonia | 0.17 | 0.0384 | 0.69 | 0.0005 |
| Nitric N | 0.16 | 0.0382 | -0.48 | 0.0300 |
| Ca | 0.08 | 0.1790 | -0.15 | 0.5460 |
| Mg | 0.46 | 0.0002 | -0.84 | 0.0000 |
| Porosity | 0.44 | 0.0002 | 0.77 | 0.0000 |
| All (z-scores) | 0.29 | 0.0004 | | |


30

669 **Supporting information figure legends**

670

671 **Fig. S1** Map of the Lake Maggiore sampling sites (45° 54' 57" N, 8° 33' 32" E), Verbania,
672 Piedmont (Italy). The three different sampling locations are indicated by the abbreviation RO
673 (abandoned Rovelli garden), RE (Villa Remigio public garden), and IS (Isola Madre garden island)

674

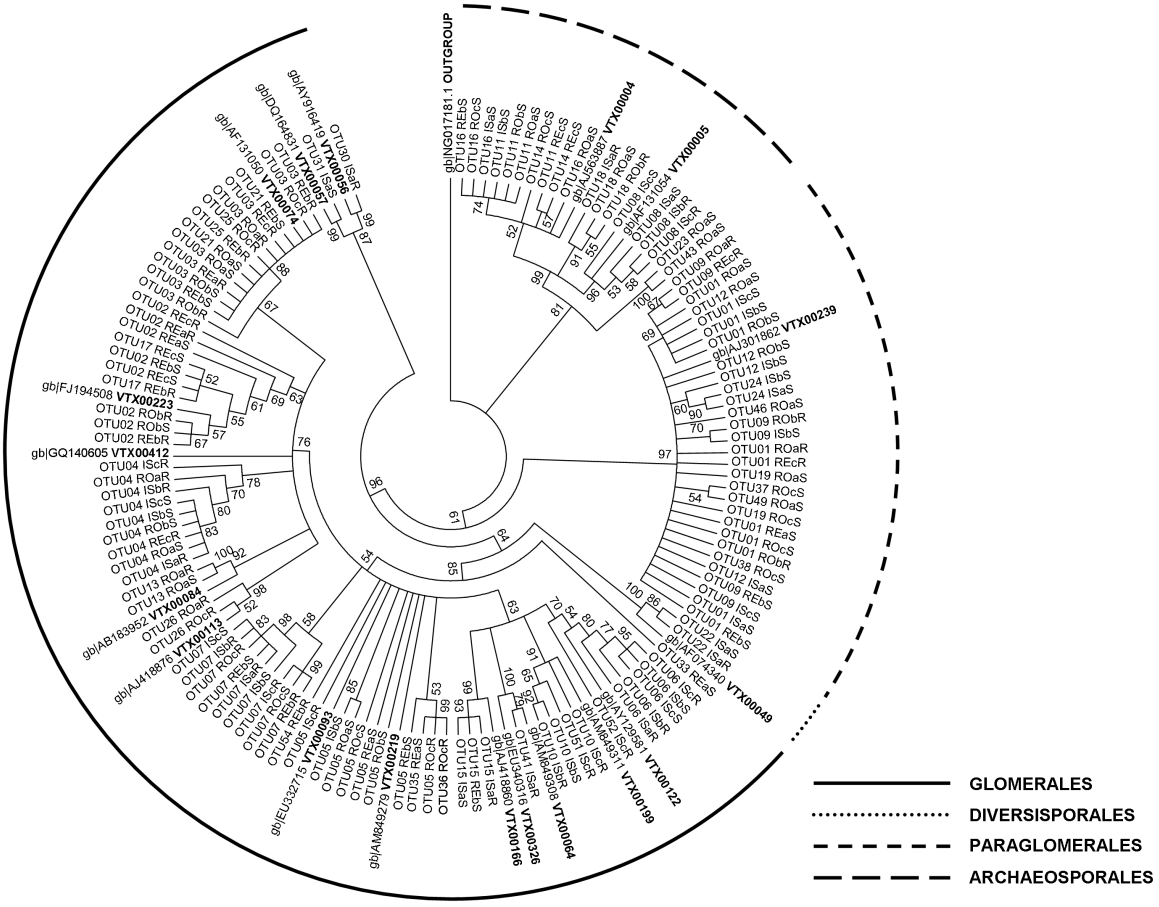
675 **Fig. S2** Typical AMF structures encountered in *C. japonica* roots – (a) an extra-radical spore, (b) an
676 intra-radical hypha, (c) intra-radical vesicles/spores, (d), (e) intra-cellular coils, (f) the main branch
677 of an arbuscule, (g) an arbuscule with very thin branching hyphae and (h) an entry-point and inter-
678 cellular running hyphae

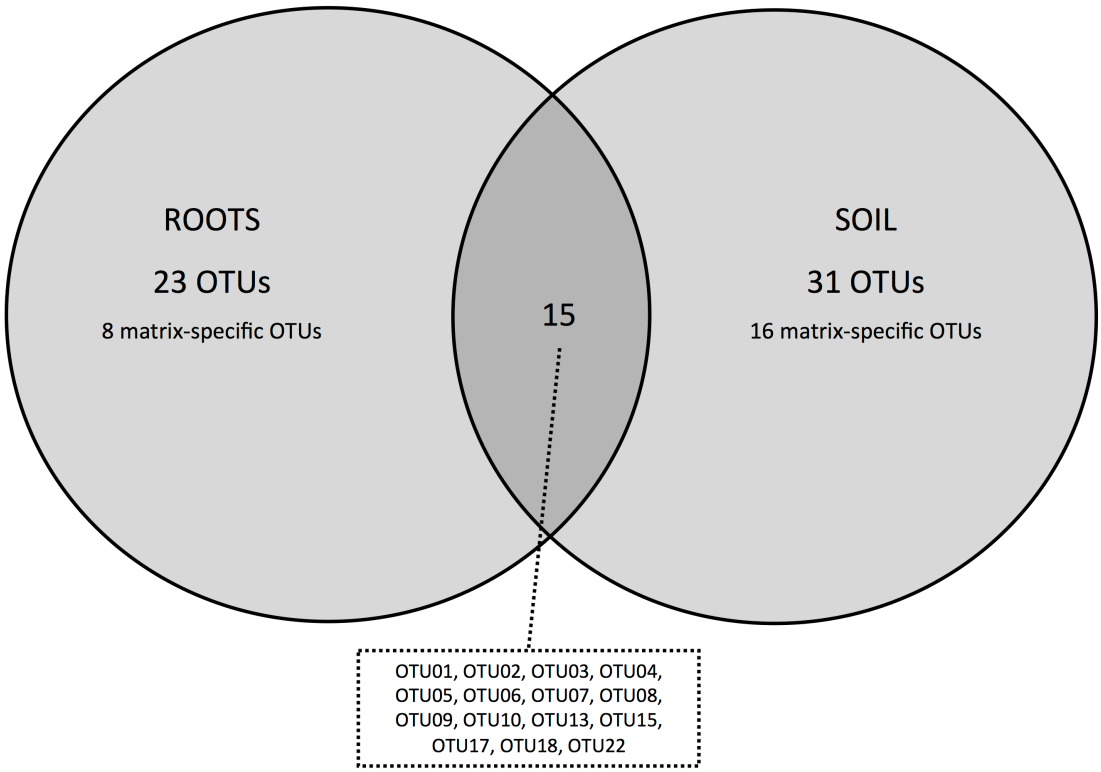
679

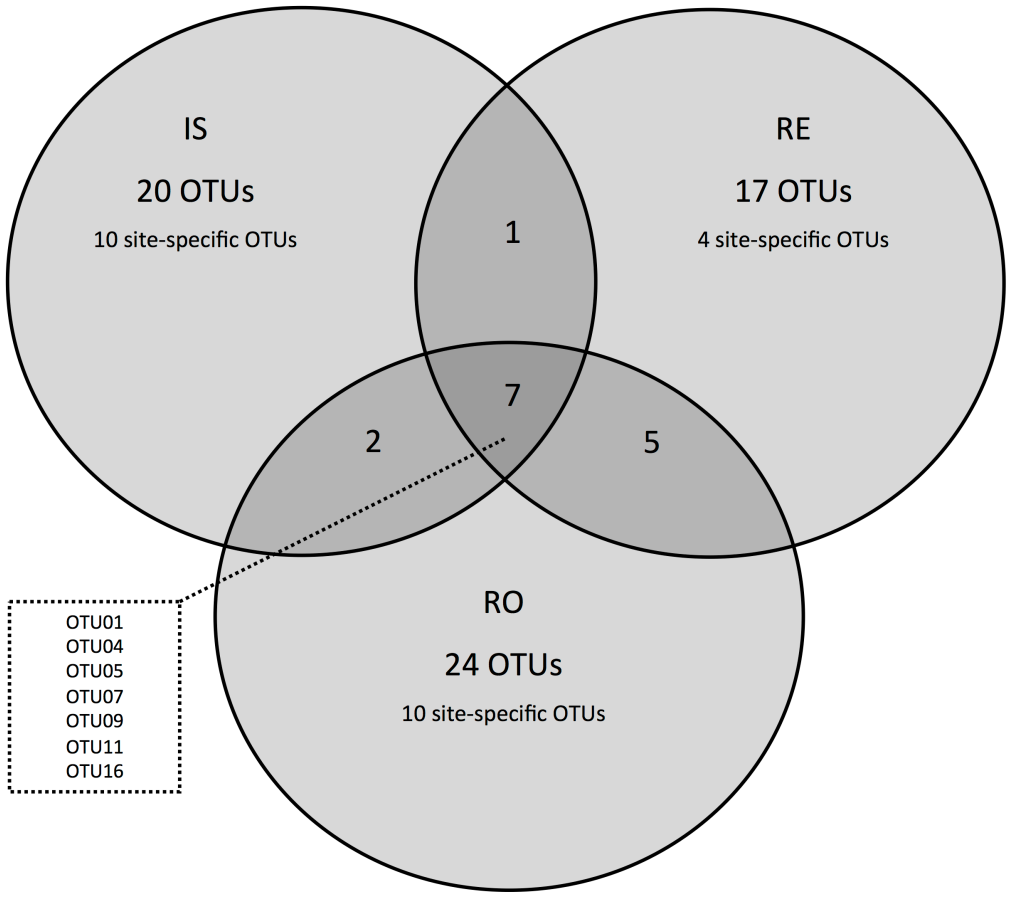
680 **Fig. S3** Rarefaction curves – The figure shows the OTU accumulation plotted as a function of the
681 number of sequences at the sample level.

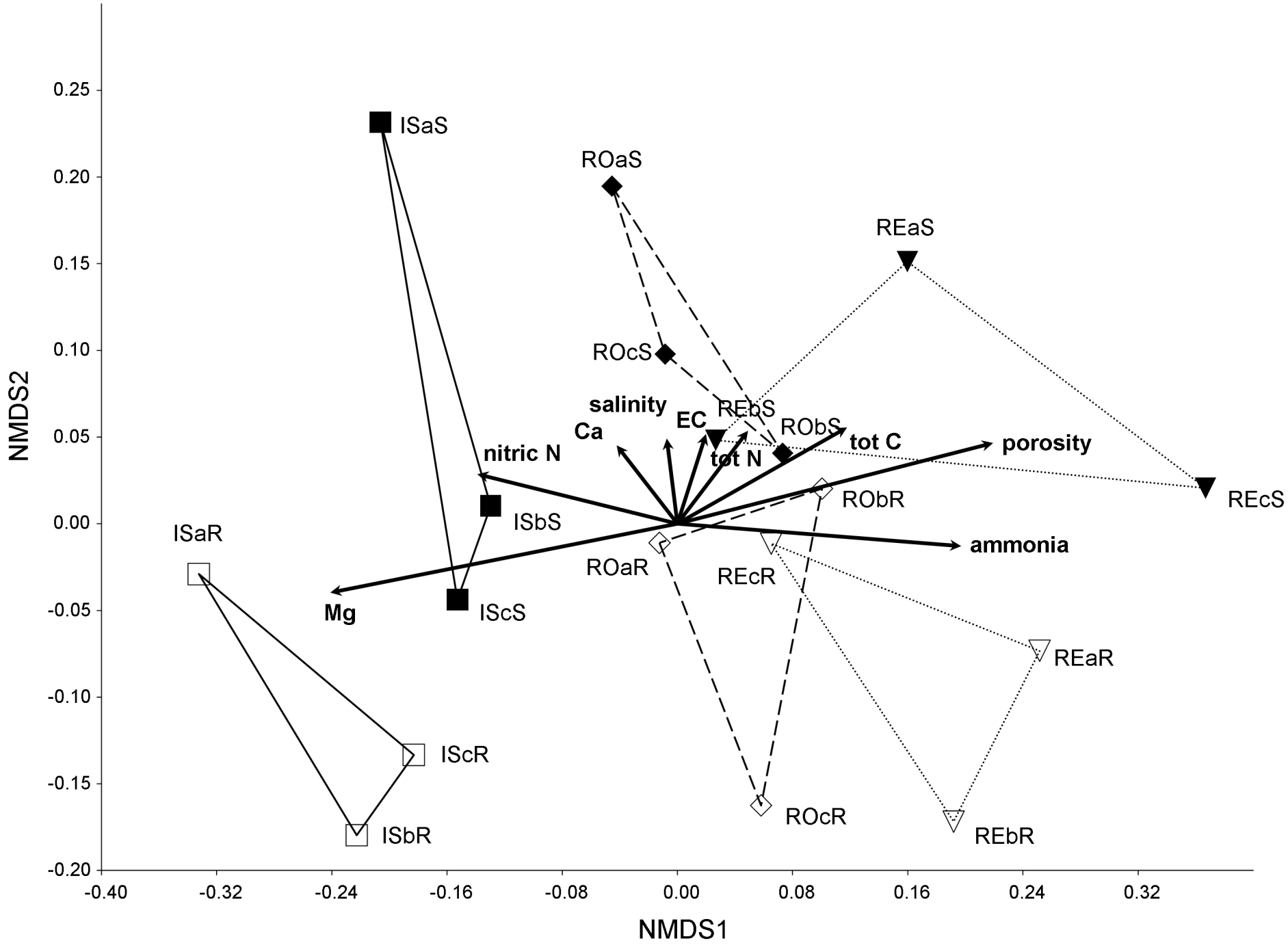
682

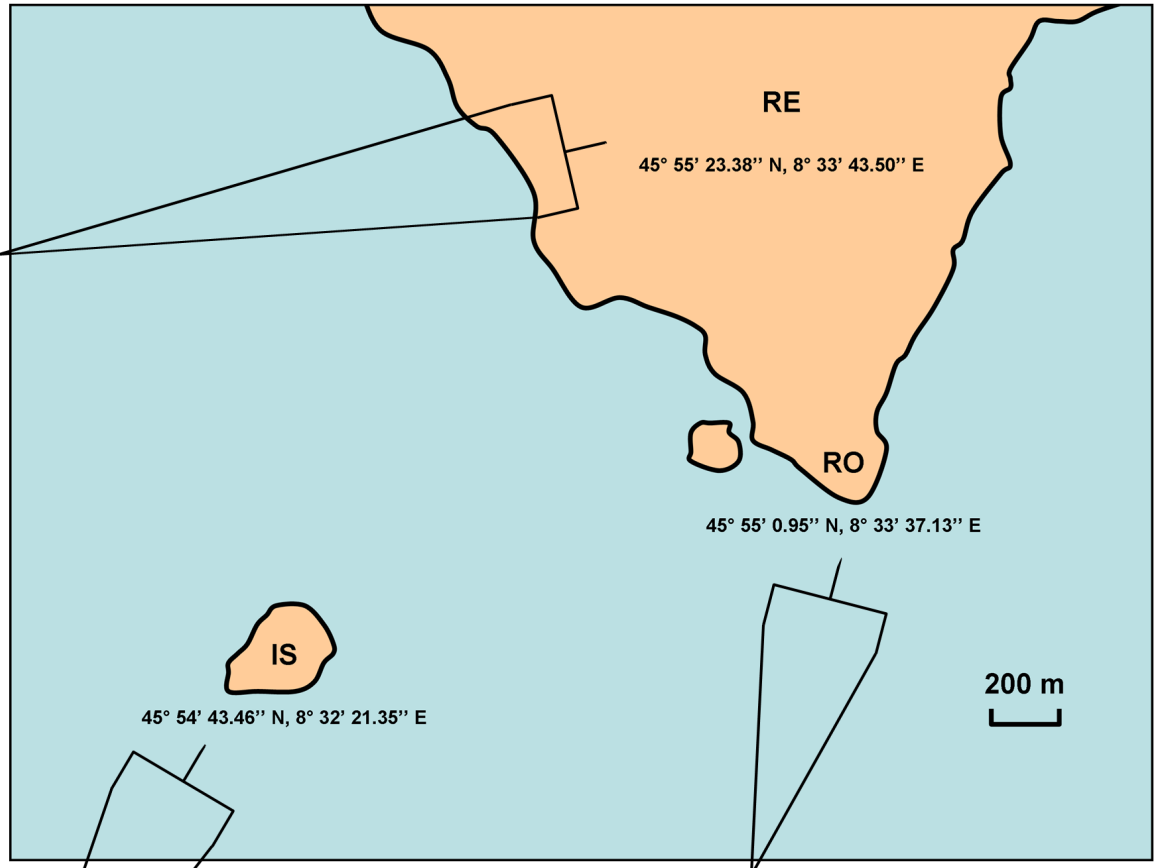

31

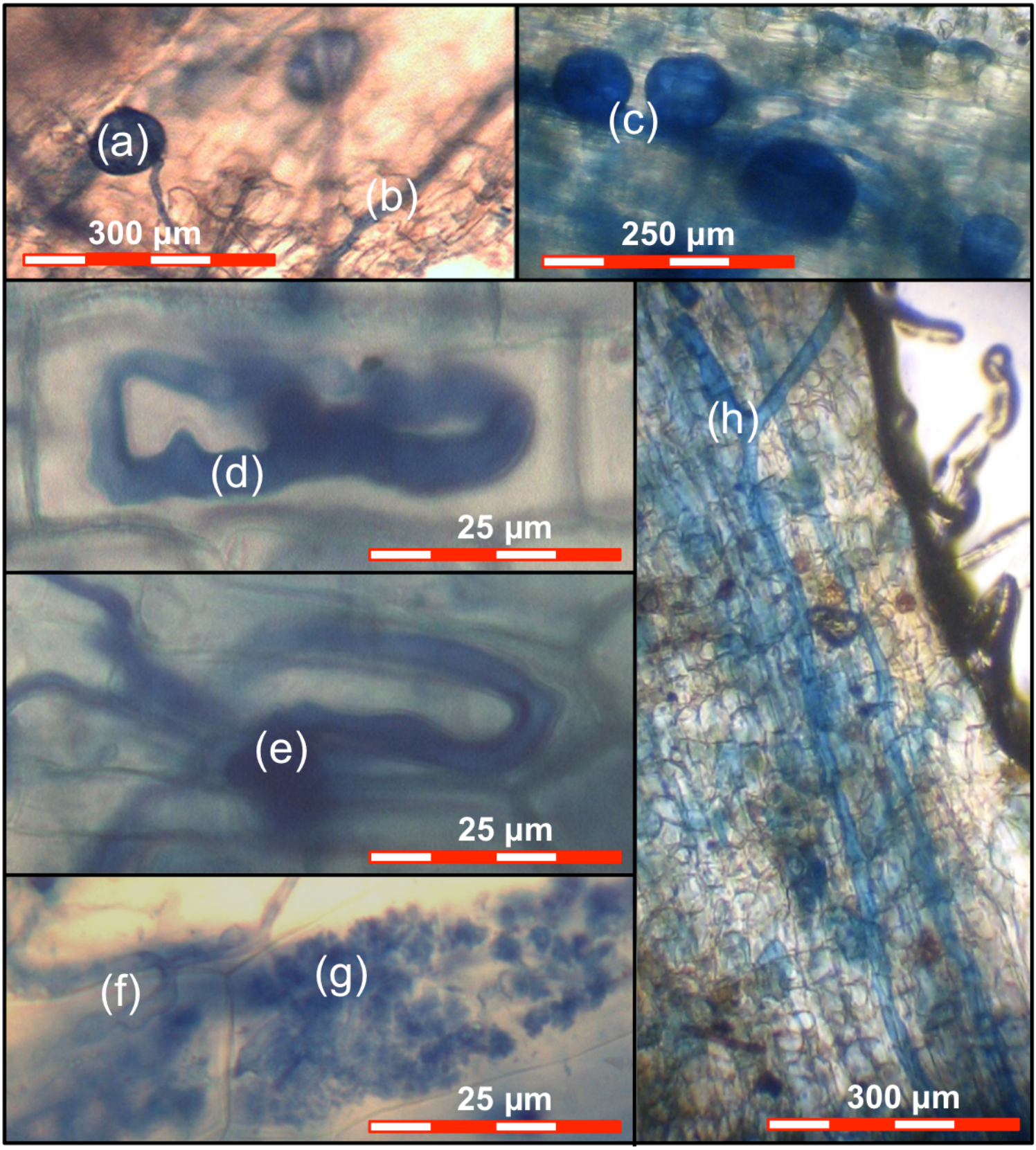

gb|NG017181.1 OUTGROUP
OTU16 REbS
OTU16 ROcS
OTU16 ISaS
OTU11 ISbS
OTU11 ROaS
OTU14 ROaS
OTU11 REcS
OTU14 REcS
OTU16 ROaS
gb|AJ563987 VTX00004
OTU18 ISaR
OTU18 ROaS
OTU08 ISaS
OTU08 ISbR
OTU08 ISaR
OTU08 ISbS
OTU08 ISaS
OTU08 ISbR
gb|AF131054 VTX00005
OTU23 ROaS
OTU43 ROaR
OTU09 REcR
OTU01 ROaS
OTU12 ROaS
OTU01 ISbS
OTU01 ISbS
OTU01 ROaS
gb|AJ301862 VTX00239
OTU12 ROaS
OTU12 ISbS
OTU24 ISbS
OTU24 ISaS
OTU46 ROaS
OTU09 ROaR
OTU09 ISbS
OTU01 ROaR
OTU01 REcR
OTU19 ROaS
OTU37 ROcS
OTU49 ROaS
OTU19 ROcS
OTU01 REaS
OTU01 ROcS
OTU01 ROaR
OTU38 ROcS
OTU12 ISaS
OTU09 REbS
OTU09 ISaS
OTU01 ISaS
OTU01 REbS
OTU22 ISaS
gb|AF074340 VTX00049
OTU22 ISaR
OTU06 REaS
OTU06 ISaR
OTU06 ISbR
OTU06 ISbS
OTU06 ISaS
gb|AY129088 VTX00122
gb|AM494931 VTX00199
OTU10 ISaR
OTU10 ISbS
OTU10 ISbR
OTU51 ISaR
OTU10 ISaS
OTU10 ISbR
gb|AM849308 VTX00064
gb|EU140316 VTX00326
OTU41 ISaR
gb|AJ418860 VTX00166
OTU15 REbS
OTU15 ISaR
OTU15 ISbS
OTU36 ROcR
OTU05 REbS
OTU35 REbS
gb|AM849279 VTX00219
OTU05 ROcS
OTU05 REaS
OTU05 ROcS
OTU05 ISbS
gb|EU332715 VTX00093
OTU05 ISaR
OTU07 REbR
OTU54 REbR
OTU07 ROcS
OTU07 ISbS
OTU07 ISaR
OTU07 ISbR
OTU07 ROcR
OTU07 ISaS
gb|AJ418876 VTX00113
OTU07 ISbR
OTU26 ROcR
gb|AB183952 VTX00084
OTU26 ROaR
OTU13 ROaR
OTU04 ISaR
OTU04 ROaS
OTU04 REcR
OTU04 ROcS
OTU04 ISbS
OTU04 ISaS
OTU04 ISbR
OTU04 ROaR
OTU04 IScR
gb|GQ140605 VTX00412
OTU02 REbR
OTU02 RObS
OTU02 RObR
gb|FJ194508 VTX00223
OTU17 REbR
OTU02 REcS
OTU02 REbS
OTU17 REaS
OTU02 REaR
OTU02 REcR
OTU03 ROcR
OTU03 REbS
OTU03 ROaR
OTU03 REaR
OTU03 ROaS
OTU25 REaR
OTU25 ROaR
OTU21 REaS
OTU03 REaS
gb|AF131050 VTX00074
gb|DQ164631 VTX00095
OTU03 ROcR
OTU37 ISaS
gb|AY916419 VTX00058
OTU30 ISaR
GLOMERALES
DIVERSISPORALES
PARAGLOMERALES
ARCHAEOSPORALES

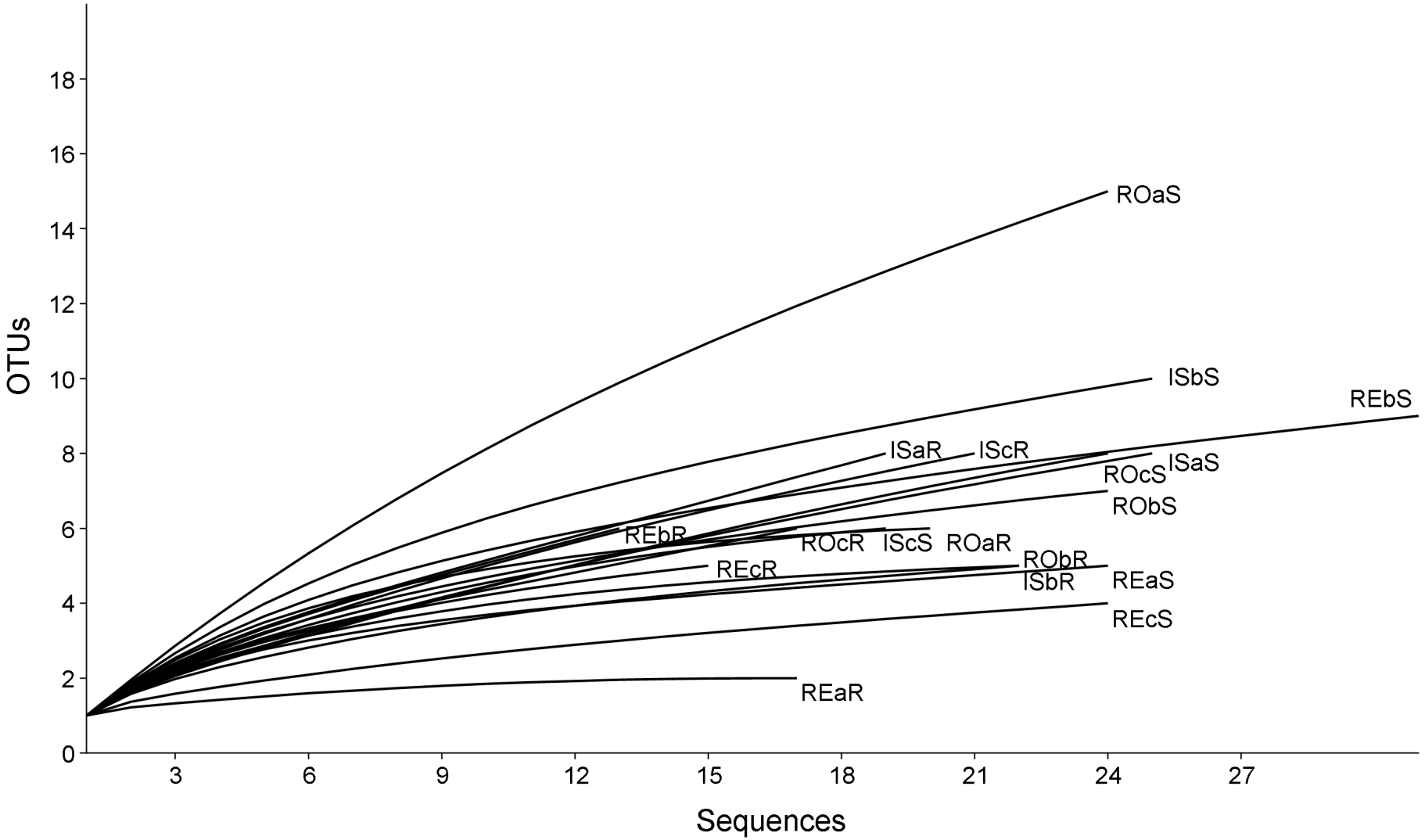

ROOTS
23 OTUs
8 matrix-specific OTUs
15
SOIL
31 OTUs
16 matrix-specific OTUs
OTU01, OTU02, OTU03, OTU04,
OTU05, OTU06, OTU07, OTU08,
OTU09, OTU10, OTU13, OTU15,
OTU17, OTU18, OTU22


IS
20 OTUs
10 site-specific OTUs
1
RE
17 OTUs
4 site-specific OTUs
7
2
5
RO
24 OTUs
10 site-specific OTUs
OTU01
OTU04
OTU05
OTU07
OTU09
OTU11
OTU16


NMDS2
NMDS1
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.40
-0.32
-0.24
-0.16
-0.08
0.00
0.08
0.16
0.24
0.32
ISaS
ROaS
REaS
ROcS
REbS
RObS
salinity
EC
Ca
tot C
porosity
nitric N
tot N
REcS
RObR
ISbS
ISaR
ammonia
ROaR
RECR
Mg
IScS
REaR
IScR
ISbR
ROcR
REbR


RE
45° 55’ 23.38’’ N, 8° 33’ 43.50’’ E
RO
45° 55’ 0.95’’ N, 8° 33’ 37.13’’ E
IS
45° 54’ 43.46’’ N, 8° 32’ 21.35’’ E
200 m


(a)
(b)
300 µm
(c)
250 µm
(d)
25 µm
(e)
25 µm
(f)
(g)
25 µm
(h)
300 µm


OTUs
Sequences
0
2
4
6
8
10
12
14
16
18
3
6
9
12
15
18
21
24
27
ROaS
ISbS
REbS
ISaR
IScR
ISaS
ROcS
ROcR
ISbR
REaS
REcS
REaR