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Species-rich but distinct arbuscular mycorrhizal communities in reforestation plots on degraded pastures and in neighboring pristine tropical mountain rain forest

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Abstract: For the first time in tropical mountain rain forest, arbuscular mycorrhizal fungal richness and community composition was investigated from planted seedlings of *Cedrela montana, Heliocarpus americanus, Juglans neotropica* and *Tabebuia chrysantha* in reforestation plots on degraded pastures. A segment of fungal 18S rDNA was sequenced from the mycorrhizas. Sequences were compared with those obtained from mycorrhizas of adult trees of 30 species in the neighboring, pristine tropical mountain rain forest. In total, 193 glomeromycotan sequences were analyzed, 130 of them previously unpublished. Members of Glomeraceae, Acaulosporaceae, Gigasporaceae and Archaeosporales were found in both habitats, with *Glomus* Group A sequences being by far the most diverse and abundant. *Glomus* Group A sequence type richness did not appear to differ between the habitats; a large number was observed in both. *Glomus* Group A sequence type composition, however, was found distinctly different. Seedlings were rarely colonized by fungi of the pristine forest but trapped a number of fungi known from other areas, which were rarely found in the pristine forest.

Resumen: Por vez primera en un bosque lluvioso tropical montano se investigó la riqueza de hongos micorrícicos arbusculares y la composición de la comunidad en plántulas de *Cedrela montana*, *Heliocarpus americanus, Juglans neotropica* y *Tabebuia chrysantha,* en parcelas de reforestación ubicadas en pastizales degradados. Se hizo la secuenciación de un segmento del ADNr fúngico de las micorrizas. Las secuencias fueron comparadas con las obtenidas de micorrizas de árboles adultos de 30 especies en el vecino y prístino bosque tropical lluvioso montano. En total se analizaron 193 secuencias de glomeromicotano, 130 de las cuales no habían sido publicadas previamente. Se encontraron miembros de Glomeraceae, Acaulosporaceae, Gigasporaceae y Archaeosporales en ambos hábitats, siendo las secuencias del Grupo A de Glomus por mucho las más diversas y abundantes. La riqueza de tipos de la secuencia del Grupo A de *Glomus* no pareció diferir entre hábitats; en ambos se observó un número mayor. Sin embargo, se encontró que la composición tipo de la secuencia del Grupo A *Glomus* era notablemente diferente. Las plántulas fueron colonizadas rara vez por hongos del bosque prístino, pero atraparon un número de hongos conocidos de otras áreas, los cuales sólo fueron hallados rara vez en el bosque prístino.

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Resumo: Pela primeira vez na floresta tropical sempreverde de montanha, a riqueza dos fungos micorrízicos arbusculares e a composição da comunidade foi investigada a partir de plântulas de *Cedrela montana, Heliocarpus americanus, Juglans neotropica e Tabebuia chrysantha* plantadas em parcelas reflorestadas em pastagens degradadas. Um segmento fúngico 18SrDNA foi sequenciado a partir das micorrízas. As sequências foram comparadas com as obtidas em micorrízas de árvores adultas de 30 espécies na floresta tropical primitiva sempreverde de montanha vizinha. No total foram analisadas 193 glomeromycotan sequências, 130 delas não publicadas anteriormente. Nos dois habitats foram encontrados membros das Glomeraceae, Acaulosporaceae, Gigasporaceae e Archaeosporales, sendo as sequências *Glomus* grupo A de longe a mais diversa e abundante. A riqueza do tipo de sequência *Glomus* grupo A não parece diferir entre os habitats; um grande número foi observado em ambas. Contudo, foi encontrado que a composição da sequência do tipo *Glomus* grupo A foi distintamente diferente. As plântulas eram raramente colonizadas pelos fungos da floresta primitiva mas fixaram fungos conhecidas de outras áreas, as quais eram raramente encontradas na floresta primitiva.

Key words: *Cedrela montana*, degraded pastures, Glomeromycota, *Heliocarpus americanus, Juglans neotropica*, neotropical mountain rain forest, reforestation, ribosomal 18S RNA gene, *Setaria sphacelata, Tabebuia chrysantha.*

Introduction

Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are the main mycobionts in grasslands and many tropical forests. While the historic use of spore-based morphospecies has suggested that there are relatively few species of AMF and that most species are ubiquitous generalists, DNA sequencing of AMF directly from mycorrhizas and the study of their molecular taxonomy has revealed a multitude of new and perhaps site-specific fungi (Aldrich-Wolfe 2007; Husband *et al*. 2002; Kottke *et al*. 2008; Wirsel 2004; Wubet *et al*. 2003, 2006a, 2006b). Sequence-based, comparative analysis of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe indicated lower numbers of fungal taxa in severely anthropogenically-altered habitats compared to tropical forests (Öpik *et al*. 2006). Loss of AMF species was observed in temperate forests converted to agriculture (Helgason *et al*. 1998) and was assumed to be even more serious in tropical lowland forests with a consequent negative impact on reforestation (Alexander & Lee 2005; Janos 1996). Conversion of forests into pastures in the tropics is typically accomplished by clear-felling followed by burning (slash-and-burn). Burn frequency and length of inter-fire intervals influence the degree of disturbance, and long-term repeated burning, according to the few published results, reduces arbuscular mycorrhizal abundance and diversity, especially in the upper few centimeters of soil (Bastias *et al*. 2006; Chen & Cairney 2002; Pattinson *et al*. 1999). However, the effect of fire on soil fungal communities has so far been unpredictable (see Cairney & Bastias 2007 for a recent review) and no information was available on such degraded, slowly recovering pastures in the neotropical mountain forest areas. Tree seedlings exposed to such degraded environments during reforestation may struggle to find appropriate mycobionts. Previous investigations revealed a distinct and highly diverse AMF community in the neotropical mountain rain forest (Kottke *et al*. 2008), but AMF potential of pastures neighboring the forest on the opposite river side, selected for reforestation with native tree species, was unknown. As part of the reforestation trial (Weber *et al*. 2008) we investigated, for the first time, AMF richness and composition of planted seedlings of four native tree species on three regeneration stages of the pastures. We considered the seedlings as "trap plants" *in situ* expecting that plants growing under natural conditions would reflect the mycorrhizal potential more accurately than plants used in pot cultures in the greenhouse. We compared AMF identity, richness and composition trapped by the seedlings in reforestation plots with our findings from adult trees in the neighboring, species-rich forest. We are well aware of the bias from comparing seedling associated fungi with those from adult trees, but we were unable to

sample mycorrhizal fungi of seedlings in the forest, since so few seedlings were observed on the dark forest floor.

We hypothesized (1) that the trap plants in the reforestation plots would be colonized by fewer AMF taxa than the trees in the pristine forest, (2) that the AMF community in the reforestation plots would be distinct from that in the forest, (3) that at least some of the forest AMF would be trapped by the planted seedlings and (4) that fungi in the pastures would be closely related to known AMF with widespread distributions.

Here we present the initial results of molecular phylogenetic analysis of AMF from the mycorrhizas in reforestation plots within fire-degraded pastures at a neotropical mountain site. We compare these results with our current knowledge about mycorrhizas in the neighboring pristine forest and published data on other sites.

Materials and methods

Study sites

The study sites are located between 1800 and 2200 m above sea level on the slopes above the San Francisco River, Cordillera Real, South Ecuador, (3° 58' S, 79° 4' W). The tropical mountain rain forest is preserved on the steep north-facing side of the river, but was cleared for cattle pasture at least 40 years ago using slash-and-burn techniques on the less steep slopes of the south-facing river bank. These pastures have been abandoned since *c.*1990 (Makeschin *et al.* 2008). Information on the plant composition of the forest, which is extra-ordinary rich in tree species, is presented in Homeier *et al.* (2008). Overviews on land-use gradients (Beck *et al.* 2008), reforestation experiments (Weber *et al.* 2008), climate (Bendix *et al.* 2008) and soils (Makeschin *et al.* 2008; Wilcke *et al.* 2008) are also available. Details on the sampling areas, recently abandoned pasture (R1), abandoned pasture covered by bracken (R2), and abandoned pastures covered by shrubs (R3) are given in Appendix Table 1 (data from Aguirre 2007). The different types of regenerating pasture were planted with six-month-old, nursery-raised seedlings of *Cedrela montana* Moritz ex Turcz., *Heliocarpus americanus* L., *Juglans neotropica* Diels and *Tabebuia chrysantha* (Jacq.) G. Nicholson, all local species of the tropical mountain rain forest.

Soils are similar on both sides of the San Francisco River, with low amounts of P and N in the mineral soil (Makeschin *et al.* 2008). Significant effects of land use on soils were documented by these authors. A strong initial loss of carbon after burning, presumably accompanied by losses of N and P, were found with the organic layer slowly regenerating during succession. Generally, soils are characterized by the accumulation of thick organic layers (8-35 cm) on top of the mineral soil in the pristine forest and large Ah horizons in the pastures. C/N ratios were similar in both habitats, but pH values were near four in the pristine forest and near five in the pastures. Accordingly, exchangeable K, Ca and Mg levels were approxi-mately four times higher, and Al, Fe, Mn levels were three times lower, in the pastures than in the forest site (Makeschin *et al.* 2008). Although plant-available nutrients are considered to be very low, the organic fraction of the soil is a large nutrient reservoir that can be mobilized by mycorrhizal fungi and other microbes (Wilcke *et al.* 2008).

Sampling

Sampling was carried out twice in the reforestation plots in the degraded pastures, one year and three years after planting. The survival rate of seedlings two years after planting was 94 % for *T. chrysantha*, 68 % for *C. montana*, 57 % for *H. americanus* and 44 % for *J. neotropica* (Aguirre 2007). Roots were sampled by tracing of single roots from the trunk from four to nine individuals of *C. montana, H. americanus, J. neotropica, T. chrysantha* and two individuals of the dominating grass, *Setaria sphacelata* (Schumacher) Moss (Table 1). Samples were also collected before planting from seven nursery-raised seedlings in total of *C. montana, Cinchona officinalis* L., *H. americanus,* and *Piptocoma discolor* (Kunth.) Pruski (Table 2) to obtain at least few data of the mycorrhizal community in the nursery. More seedlings were unfortunately not available for sampling. Seedlings were raised in a mixture of highland black soil, bed sand and forest humus (2:1:1) in 560 cm^3 polyethylene planting bags at a nursery at the Universidad Nacional de Loja, Ecuador. All substrates except forest humus were steam-fumigated before planting.

Roots were cleaned under tap water the same day they were harvested and degree of colonization was determined using standard staining methods (Haug *et al.* 2004). For each seedling from the abandoned pastures or nursery a mix from the excavated roots was used and five 1.5 ml tubes were each filled with three fine roots, each 1 cm in

Table 1. Sampled seedlings on the reforestation plots; numbers of successfully amplified tubes and glomeromycotan sequences obtained. DQ numbers indicate sequences published in Kottke *et al.* (2008); EU numbers represent new sequences; st = sequence type, numbers refer to Fig. 1.

Sampled seedlings	Tubes	*Glomus* Group A	*Glomus* Group B	Acaulosporaceae	Archaeosporales	Gigasporaceae	Paraglomeraceae
Tabebuia chrysantha 1	3	DQ336464 st22, EU152187 st24					
Tabebuia chrysantha 2	2	EU152197 st11			EU159176		
Tabebuia chrysantha 3	1	DQ336465 st21					
Tabebuia chrysantha 4	4	EU152165 st24, EU152190 st6		EU159133			
Tabebuia chrysantha 5	2	EU152170 st6, EU152171 st40					
Tabebuia chrysantha 6	3	EU152173 st42, EU152172 st11, EU152191 st11, EU152192 st25, EU152174 st37					
Tabebuia chrysantha 7	2	EU152177 st31, EU152193 st23					
Tabebuia chrysantha 8	5	EU152178 st21, EU152179 st17, EU152182 st24, EU152194 st23, EU152195 st31		EU159125	EU159164	EU159182	
Juglans neotropica 1	2	DQ336454 st11, DQ336456 st1, EU152185 st20, EU152136 st3	EU159162				
Juglans neotropica 2	1	DQ336457 st11 , EU152139 st40					
Juglans neotropica 3	2	DQ336458 st23, DQ336459 st16					
Juglans neotropica 4	1	EU152140 st3, EU152141 st1, EU152142 st43					
Juglans neotropica 5	1	EU152188 st20, EU152189 st39					
Juglans neotropica 6	1	EU152166 st32					
Juglans neotropica 7	1	EU152180 st20					
Juglans neotropica 8	1	EU152181 st39					
Juglans neotropica 9	3	EU152196 st24			EU159174		
Cedrela montana 1	1	DQ336444 st13, DQ336445 st22, DQ336446 st11	EU159161				

Contd....

Table 1. Continued.

Sampled seedlings	Tubes	*Glomus* Group A	*Glomus* Group B	Acaulosporaceae	Archaeosporales	Gigasporaceae	Paraglomeraceae
Cedrela montana 2	1	EU152184 st6					
Cedrela montana 3	1	DQ336448 st23, DQ336449 st24					
Cedrela montana 4	2	EU152183 st24		EU159124, EU159128			
Cedrela montana 5	2	EU152168 st21, EU152169 st6, EU152163 st5					
Cedrela montana 6	2	EU152164 st32		EU159129, EU159130			
Heliocarpus americanus 1	2	DQ336451 st24, EU152186 st4					
Heliocarpus americanus 2	1	DQ336452 st2, DQ336453 st32, EU152137 st38					
Heliocarpus americanus 3	3	EU152161 st39, EU152160 st1, EU152162 st20, EU152167 st39					
Heliocarpus americanus 4	1	EU152175 st39, EU152176 st3					EU159184
Setaria sphacelata 1	3	DQ336461 st2, EU152147 st20		EU159131, EU159132			
Setaria sphacelata 2	2	DQ336462 st24, EU152148 st11					EU159183
Number of sequences		63	2	8	3	1	2

Table 2. Sampled seedlings in the nursery; numbers of successfully amplified tubes and glomeromycotan sequences obtained. DQ numbers indicate sequences published in Kottke *et al.* (2008); EU numbers represent new sequences; st = sequence type, numbers refer to Fig. 1.

Sampled seedlings	Tubes	*Glomus* Group A	*Glomus* Group B	Acaulosporaceae	Archaeosporales	Gigasporaceae	Paraglomeraceae
Cedrela montana	3	DQ336437 st11	EU159156		EU159178		
Cinchona officinalis 1	1	DQ336438 st1					
Cinchona officinalis 2	1	EU152143 st8					
Heliocarpus americanus	1	DQ336440 st43			EU159177		
Piptocoma discolor 1	2	EU152149 st3	EU159158				
Piptocoma discolor 2	2		EU159159				
Piptocoma discolor 3	2		EU159160	EU159134			
Number of sequences		5	4	1	2	0	0

length (Tables 1 & 2). Tubes were installed on an electric dryer at about 50 °C for 12 hours and roots kept on silica gel for DNA isolation. This procedure was necessary because of the high air humidity in the tropics. Sampling in the forest was carried out in established plots along an altitudinal gradient comprising different forest types as described in Haug *et al.* (2004). The same amount of rootlets per individual tree was handled as described for the pasture samples. Samples were collected from 30 different tree species and up to four individuals per species (Table 3). Some of the sequences (33 %) were published in Kottke *et al.* (2008), but substantial numbers of samples were newly sequenced for this investigation (Table 3).

Processing of fungal DNA sequences

DNA was isolated from the dried root samples using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany). One to five tubes were processed per plant individual. The number of tubes yielding sequences is given in Tables 1-3. For phylogenetic analysis and molecular identification, we sequenced sections of the fungal nuclear gene coding for the small ribosomal subunit (18S; nucSSU). The following primer combinations were used in nested PCRs: *Glomus* Group A: first SSU128/SSU1536IH, second SSU300/GLOM1310 rc; *Glomus* Group B: first SSU 817/NS8, second SSU817/LETC1670rc; Acaulosporaceae: first SSU 817/NS8, second SSU817/ ACAU1660rc; Archaeosporales: first SSU817/SSU1536IH, second SSU 817/ARCH1375rc. Details on the primers and nested PCRs are given in Appendix. The sequences obtained were assigned to higher fungal groups with BLAST searches (Altschul *et al.* 1997) using the National Center for Biotechnology Information (NCBI) database (http: //www.ncbi.nlm.nih.gov). We checked for putative chimeric sequences (details in Appendix). The glomeromycotan sequences were deposited in GenBank; their accession numbers are given in Tables 1-3. Accession numbers starting with DQ (63 sequences) were published in Kottke *et al.* (2008). Accession numbers starting with EU (130 sequences) were deposited during this study.

Phylogenetic analysis for identification of fungi and sequence type definition of Glomus Group A

BLAST hits with sequence similarities ≥ 99 % were downloaded from GenBank and added to the dataset. We also included sequences from AM taxa identified from spores. Only one sequence was included in the final tree when several inserts of a cloned PCR product were very similar (1 to 5 bases different) and appeared together in a terminal cluster. Sequence alignments were done with MAFFT (Katoh *et al.* 2005). We used PAUP* version 4.0b10 (Swofford 2002) to estimate the phylogenetic relationships of the sequences obtained. Neighbour-Joining analyses (Saitou & Nei 1987) using the BIONJ modification (Gascuel 1997) with Kimura 2-distances were carried out and combined with bootstrap analyses (Felsenstein 1985) from 1000 replicates. Additionally, maximum-likelihood (ML) analysis using RAxML (Stamatakis 2006) was done with *Glomus* Group A sequences, with GTR+CAT as a DNA substitution model for heuristic search and GTR+G for final tree optimization, again combined with a bootstrap analysis from 1000 replicates. *Glomus* Group A sequences which showed sequence similarities ≥ 99 % were defined as a sequence type. In *Glomus* Group B, Archaeosporales and Gigasporaceae, sequence types were not defined because interspecific differences were not observed due to high sequence conservation within these groups. We also did not define sequence types in the Acaulosporaceae and Paraglomeraceae because only a few sequences of known species are currently available.

Evaluation of sequence-based diversity of Glomeromycota from the pastures, the nursery and the neighboring mountain rain forest

Presence or absence of individual Glomeromycota sequences were compiled in tables respecting habitat, plant species and plant individuals (Tables 1-3). The sequences and sequence types obtained from the two samplings in the reforestation plots were pooled because we did not observe interpretable differences among the two years.

Analysis of richness and community composition of Glomus Group A sequence types

We calculated a sample-based rarefaction accumulation curve with 95 % confidence intervals, and estimated the total sequence type richness of *Glomus* Group A with Chao 2 and Jackknife 2 using the software EstimateS, v.8.0.0 (Colwell 2006) set to "randomize samples without replacement".

Cluster analysis was performed on the presence/absence data matrix of the AM fungal

Table 3. Sampled tree species and numbers of individuals in the pristine forest; numbers of successfully amplified subsamples and glomeromycotan sequences obtained. DQ numbers indicate sequences published in Kottke *et al.* (2008); EU numbers represent new sequences; st = sequence type, numbers refer to Fig. 1.

Sampled species	Tubes	*Glomus* Group A	*Glomus* Group B	Acaulosporaceae	Archaeosporales	Gigasporaceae	Paraglomeraceae
Alzatea verticilliata	1	EU152150 st31					
Campnosperma panamensis	1	EU152151 st18, EU152152 st15					
Cedrela sp.	1	DQ336466 st2 , DQ336467 st4					
Clethra revoluta	2	DQ336469 st14, DQ336471 st11					
Clusia elliptica	1	DQ336474 st31					
Critoniopsis floribunda	1	EU152156 st41					
Faramea uniflora	1	EU152154 st18, EU152155 st41				EU159180	
Graffenrieda emarginata 1	1	DQ336477 st36, DQ336478 st33, DQ336479 st31		EU159141			
Graffenrieda emarginata 2	1	DQ336480 st31, DQ336481 st33		EU159127			
Graffenrieda emarginata 3	1	DQ336475 st14, DQ336476 st15, EU152135 st16		EU159123			
Graffenrieda emarginata 4	1			EU159137			
Guarea kunthiana 1	2	DQ336483 st12		EU159145			
Guarea kunthiana 2	2	DQ336482 st5			EU159163, EU159169, EU159171, EU159173		
Guarea cf. *kunthiana*	2	DQ336484 st41, DQ336485 st29, DQ336487 st41, EU152133 st28		EU159144	EU159166		
Guarea pterorhachis	1	DQ336488 st10, DQ336489 st9, EU152131 st35					
Guarea sp.	1	DQ336490 st41, DQ336491 st41, EU152132 st41					

Contd....

Table 3. Continued.

Sampled species	Tubes	*Glomus* Group A	*Glomus* Group B	Acaulosporaceae	Archaeosporales	Gigasporaceae	Paraglomeraceae
Heliocarpus americanus	1	DQ336493 st11, DQ336494 st14					
Hyeronima asperifolia 1	1	DQ336496 st19, DQ336497 st12					
Hyeronima asperifolia 2	2			EU159142	EU159165		
Hyeronima moritziana 1	2	DQ336499 st31, DQ336500 st33					
Hyeronima moritziana 2	1			EU159135			
Hyeronima oblonga	1	DQ336502 st5	EU159155			EU159179	
Hyeronima sp.	2	DQ336503 st34, DQ336504 st41, EU152134 st34		EU159136	EU159172		
Inga acreana 1	1	DQ336505 st24	EU159154				
Inga acreana 2	1	DQ336507 st29, EU152138 st28					
Inga acreana 3	1	DQ336508 st26					
Inga acreana 4	1	DQ336509 st14					
Meriana sp.	1	EU159149			EU159168		
Miconia punctata	1	EU152157 st41					
Myrsine latifolia	1	EU159151			EU159170		
Nectandra laevis 1	1	DQ336511 st30, DQ336513 st27, DQ336514 st26					
Nectandra laevis 2	2	DQ336516 st4, DQ336518 st2		EU159138			
Piper sp.	1	EU159152, EU159147		EU159143			
Podocarpus oleifolius 1	2	DQ336521 st15		EU159126, EU159139		EU159181	
Podocarpus oleifolius 2	1	EU152153 st16					
Podocarpus oleifolius 3	1	EU152146 st15					
Prumnopytis montana	1	EU152144 st9, EU152145 st7					
Prunus cf. opaca	2	EU152158 st34, EU152159 st15					
Purdiaea nutans	2	EU159148			EU159167		
Tabebuia chrysantha	2	EU159150	EU159157	EU159140	EU159175		
Vismia tomentosa	2	DQ336522 st15, DQ336523 st41, DQ336524 st27					
Vismia sp.	1	EU159153		EU159146, EU159122			
Number of sequences		69	3	16	11	3	0

sequence types of *Glomus* Group A comparing the AM fungal communities of the two habitats. The Ward clustering method with Squared Euclidean distances was implemented in SPSS v 14. We also carried out a chi-square test to determine whether the AM fungal communities differed between the two habitats. A Venn diagram was designed to display *Glomus* Group A sequence types in reforestation plots, nursery and pristine forest and to label the sequence types known from other investigations.

Results

Sequence-based composition of Glomeromycota in reforestation plots in degraded pastures and neighboring tropical mountain rain forest

In total, from both habitats and the nursery, 193 glomeromycotan sequences were obtained, 130 sequences published here for the first time (Tables 1-3). Fifty-six mycorrhizal samples of 29 individuals from the reforestation plots yielded sequences of *Glomus* Group A (63), *Glomus* Group B (2), Acaulosporaceae (8), Archaeosporales (3), Gigaspo-raceae (1) and Paraglomeracaeae (2) (Table 1). In the mycorrhizas of one individual seedling, up to eight glomeromycotan sequences were detected (Table 1). Fifty-five mycorrhizal samples of 42 tree individuals from the pristine forest belonging to 23 genera from 18 families yielded sequences of *Glomus* Group A (69), *Glomus* Group B (3), Acaulosporaceae (16), Archaeosporales (11) and Gigasporaceae (3) (Table 3). In the mycorrhizas of one individual tree, up to six glomeromycotan sequences were detected (Table 3). Twelve mycor-rhizal samples of seven individuals from the nursery seedlings belonging to four genera yielded sequences of *Glomus* Group A (5), *Glomus* Group B (4), Acaulosporaceae (1) and Archaeosporales (2) (Table 2).

Glomus Group A fungi were associated with nearly all the plant individuals under investigation, while *Glomus* Group B fungi were found only with seedlings of *J. neotropica* and *C. montana* from the reforestation plots, *C. montana* and *Piptocoma discolor* from the nursery, and three tree species in the pristine forest (Tables 1-3, Appendix Fig. 1). Members of Acaulosporaceae were detected in seedlings of *C. montana* and *T. chrysantha* from the reforestation plots and *P. discolor* from the nursery, but not in other seedlings (Tables 1 & 2; Appendix Fig. 2). Acaulosporaceae were also found associated with *S. sphacelata* and with eleven tree species in the pristine forest (Tables 1 & 3; Appendix Fig. 2). Members of Archaeosporales were found in seedlings of *T. chrysantha* and *J. neotropica* from the reforestation plots (Table 1; Appendix Fig. 3), and *C. montana* and *H. americanus* nursery plants (Table 2; Appendix Fig. 3). Archaeosporales seq-uences were also obtained from eight tree species in the pristine forest (Table 3; Appendix Fig. 3). Gigasporaceae were found only once on all the seedlings (*T. chrysantha*) and on three tree species in the pristine forest (Tables 1 & 3; Appendix Fig. 3). Paraglomeraceae were only found on *H. americanus* and *S. sphacelata* on the reforestation plots (Table 1; Appendix Fig. 3). An identical Gigasporaceae sequence (630 bp) was found with *Podocarpus oleifolius* from pristine forest and *T. chrysantha* from a reforestation plot (Appendix Fig. 3). Two further Gigasporaceae sequences, obtained from *Faramea uniflora* and *Hyeronima oblonga,* are also very similar (Appendix Fig. 3). Three of our sequences cluster with *Archaeospora trappei* (Archaeosporaceae), two sequences of the nursery form a sister clade to the Archaeosporaceae. The rest of our sequences are in clusters outside the known families of Archaeosporales. Considerably more Archaeosporales seq-uences were found in the forest than in the reforestation plots (Appendix Fig. 3). The two paraglomeracean sequences from *H. americanus* and *S. sphacelata* form a separate cluster adjacent to *Paraglomus brasilianum* and *P. occultum* (Appendix Fig. 3).

Glomus Group A sequence type-based richness and community composition

The *Glomus* Group A sequences (1035 bp) were analyzed with BIONJ and ML. Both phylogenetic trees showed very similar topologies and similar bootstrap values (Fig. 1). Sequence types were defined as sets of sequences with a sequence similarity of ≥ 99 %. In most cases, sequence types also formed monophyletic groups in our trees; sequence types 3, 15 and 29 are not monophyletic in the sequence trees. The 63 *Glomus* Group A sequences from the reforestation plots were grouped in 24 sequence types (Fig. 1; Table 4). Nineteen sequence types are composed of two to ten sequences; five sequence types consist of one sequence only. All seedlings from the reforestation plots shared fungal sequence types with other seedlings and these sequence type clusters consisted of sequences from different host species

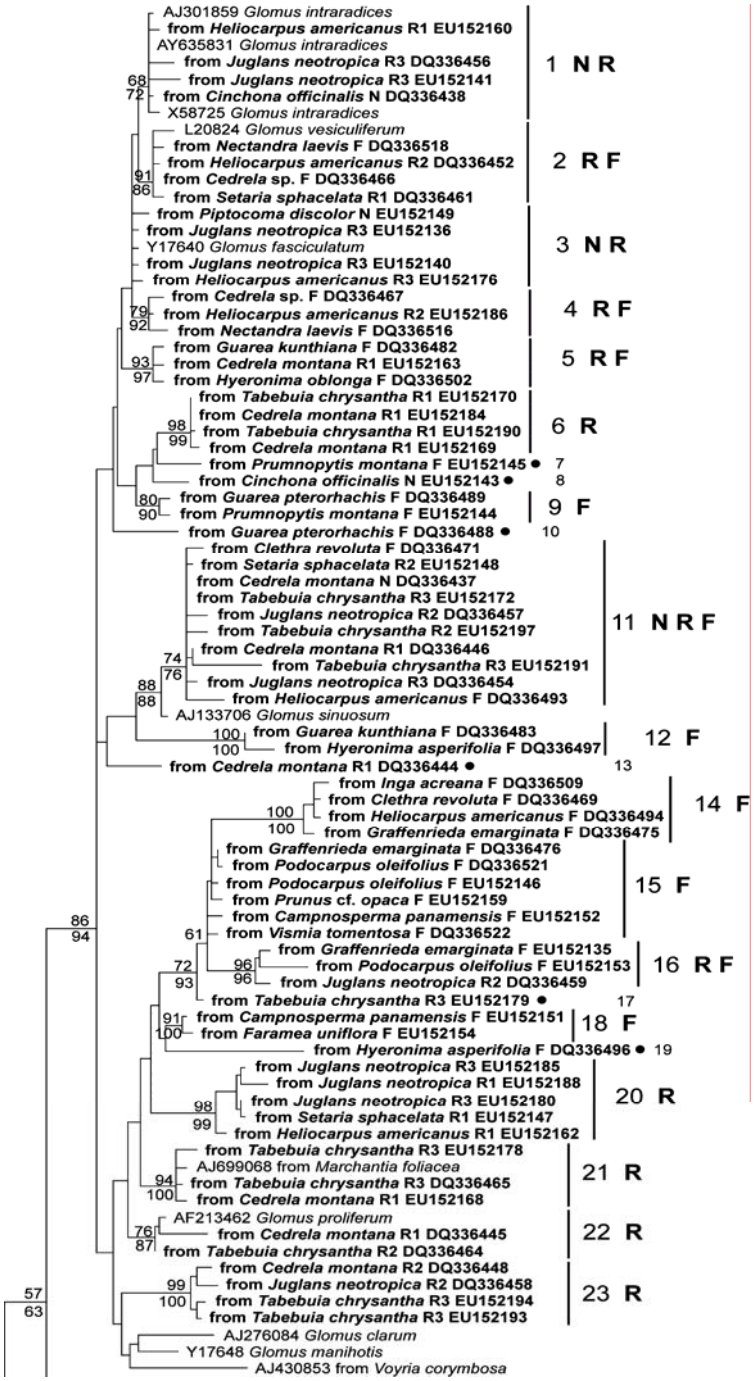
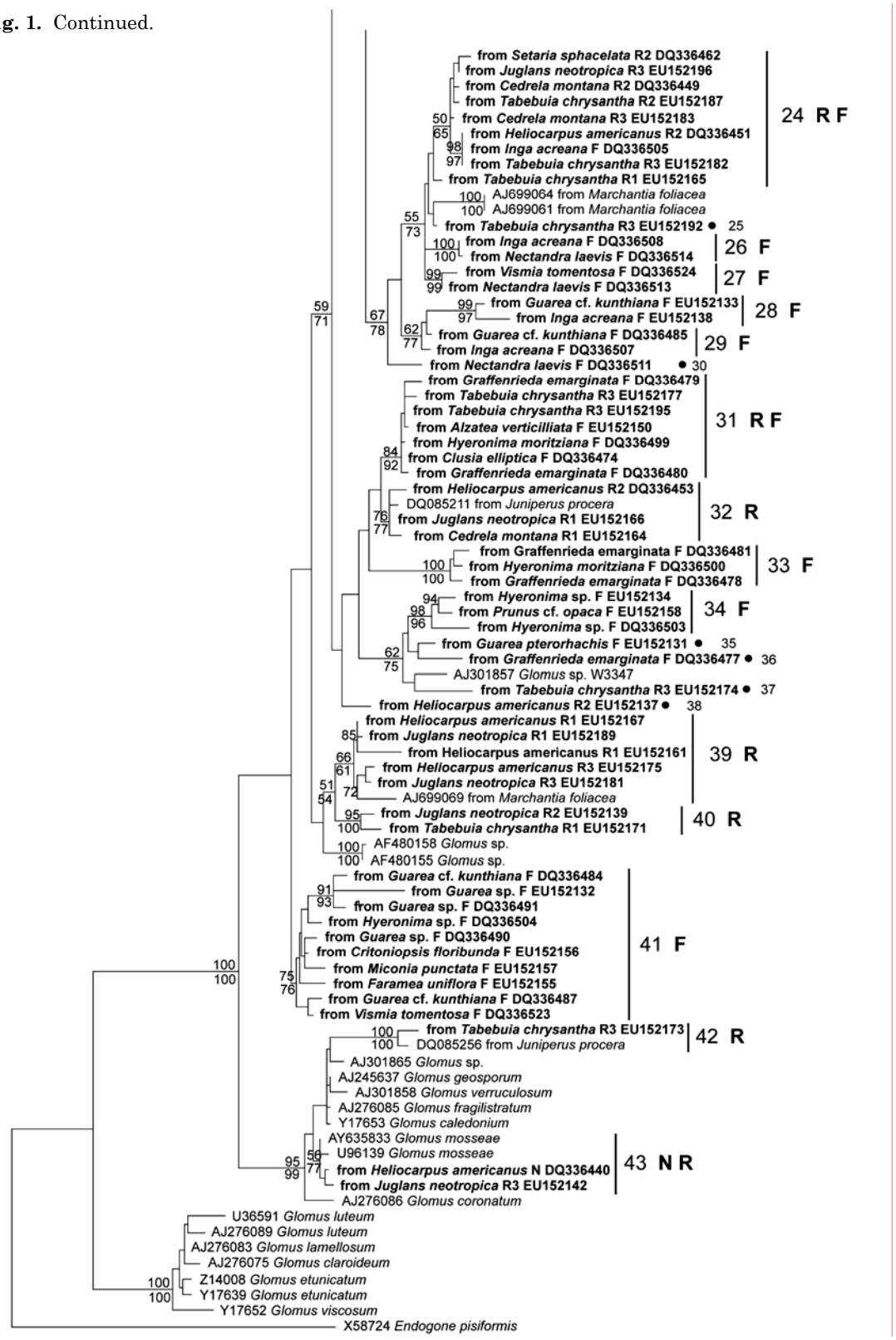


Fig. 1. Phylogenetic relationships of *Glomus* Group A sequences obtained by use of primers SSU300 and GLOM1310rc (in the second PCR) from mycorrhizas of tree seedlings in the reforestation plots (R1, R2, R3), in a nursery (N), and trees in the pristine forest (F) in South Ecuador. Sequences from the study sites are highlighted in bold. ML analysis was carried out on an alignment of nuclear DNA sequences coding for the small ribosomal subunit (nucSSU; 1088 characters). The tree was rooted with *Endogone pisiformis*. Numbers on branches designate bootstrap values (ML/BIONJ). Sequence types are based on sequence clusters with sequence similarities ≥ 99 % (see text) and are numbered serially. Letters indicate occurrence of sequence types: N-nursery, R-reforestation plots, F- pristine forest.

Fig. 1. Continued.



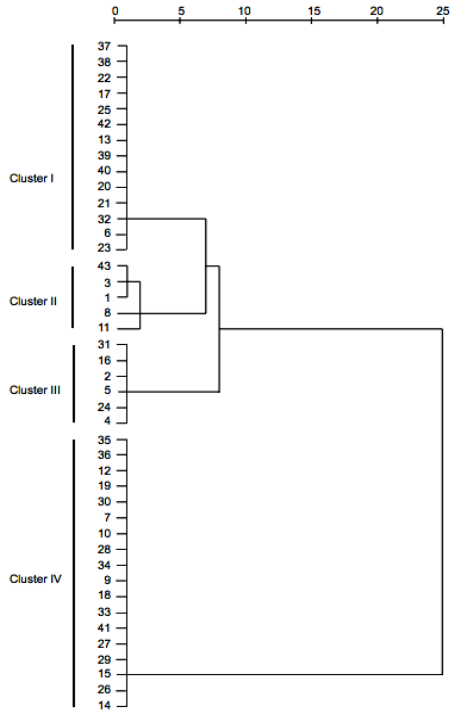


Fig. 2. Cluster analysis of *Glomus* Group A sequence types: Cluster I- Sequence types of the reforestation plots; Cluster II- Sequence types of the nursery, refore-station and forest sites; Cluster III- Sequence types of the reforestation and forest sites; Cluster IV- Sequence types of the pristine forest site.

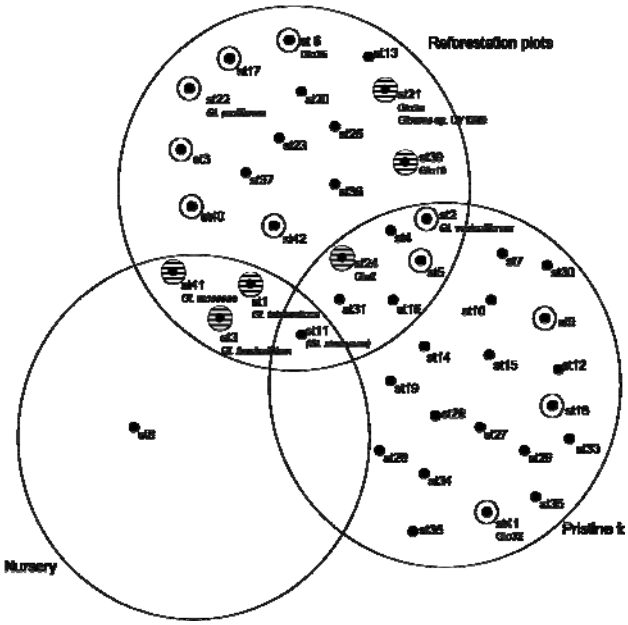


Fig. 3. Venn diagram of *Glomus* Group A sequence types in the nursery, in the reforestion plots and in the pristine forest. Black circles around black dots: sequence type known as identified morphospecies or showing ≥ 99 % similarity in the NS31-AM1 region with published sequences from other studies (for details see Table 4); circles shaded: sequence type belongs to the most frequently detected AM-taxa (Öpik *et al*. 2006).

(Fig. 1). Five sequence types from the reforestation plots correspond to known morphospecies (st 1 = *Glomus intraradices*, st 2 = *G. vesiculiferum*, st 3 = *G. fasciculatum*, st 22 = *G. proliferum*, st 43 = *G. mosseae*), nine are known as sequences from other environmental studies and ten do not match any currently published sequence (Table 4, Fig. 3). Sequence types 11 and 24 were found on all successional stages of the reforestation plots on degraded pastures (R1, R2 and R3). Sequence type 24 was associated with all four planted tree species and with *S. sphacelata* (Fig. 1). Sequence types 11 and 23 were verified for *C. montana, J. neotropica* and *T. chrysantha* (Fig. 1).

The 69 *Glomus* Group A sequences from the pristine forest (Table 3) were grouped in 25 sequence types (Fig. 1). Nineteen sequence types are composed of two to ten sequences; six sequence types consist of one sequence only. Nearly all individual trees share fungal sequence types with other trees, and sequence type clusters consist of sequences from different tree species (Fig. 1). One sequence type can be assigned to a morphospecies (st 2 = *Glomus vesiculiferum*), five are known as sequences from other studies and 19 do not match any currently published sequence (Table 4; Fig. 3).

Seven *Glomus* Group A sequence types were common in the reforestation plots and the forest; three of these are known from other studies (Table 4; Fig. 3). Only one of the nursery sequence types (st11) was found in the pristine forest. This sequence type occurred also in the reforestation plots and is thus the only sequence type found on all three sites (Fig. 3). Three further sequence types were common in the nursery and the reforestation plots (Fig. 3). In the reforestation plots, the known sequence types are high in

numbers (58 %), while in the pristine forest, the proportion of new sequence types is high (76 %) and that of known sequence types is low (24 %).

Richness estimation of *Glomus* Group A sequence types from the reforestation site and the pristine forest showed overlapping, thus not significantly different accumulation curves at 95 % confidence intervals. Accumulation curves did not reach their asymptotes in either habitat (Appendix Fig. 4). The expected richness (estimated with Chao 2 and Jacknife 2) was slightly higher for the reforestation site (Appendix Tables 4 & 5).

Ward cluster analysis was carried out based on sequence type occurrence and separated four distinct clades (Fig. 2). Cluster I contains 14 sequence types identified only from the reforestation plots. Cluster IV is characterized by 18 sequence types from the pristine forest only. Cluster II is composed of five sequence types occurring in the nursery, the reforestation plots and the forest site. Cluster III is composed of six sequence types shared between the reforestation plots and the forest site. Habitat was found to have a significant influence at $P < 0.0001$ ($X^2 = 200$; df. = 84) on the AM fungal composition.

Discussion

In this environmental study, we used molecular methods for identifying arbuscular mycorrhizal fungi directly from the mycorrhizas. We used SSU sequences because the amplification success was satisfactory and SSU is the only gene with a broad taxon sampling in Glomeromycota (Redecker & Raab 2006). We found a number of hitherto unknown AMF sequences in the reforestation plots and the pristine forest. As seen in other mycorrhizal community investigations (Husband *et al.* 2002; Öpik *et al.* 2003; Scheublin *et al.* 2004; Whitfield *et al.* 2004), *Glomus* Group A members dominated in both habitats. We cannot exclude that choice of primers caused a bias for this fungal group and future investigations may reveal importance of other AMF in the area. Using the primers currently available, we obtained insufficient numbers of sequences to define phylotyes in *Glomus* Group B, Acaulosporaceae, Gigasporaceae, Paraglomeraceae or Archaeosporales, and thus we could not carry out a comparative analysis on richness and community composition of these fungal groups but confined these analysis to *Glomus* Group A. We used sequence similarity of 99 % as a criterion to create sequence types in *Glomus* Group A. This high degree of similarity was nearly always found within well-supported monophyletic clades in our trees, corroborating our sequence type definition.

We expected much lower numbers of AMF in the reforestation plots according to previous findings at severely anthropogenically influenced sites (Alexander *et al.* 1992; Cairney & Bastias 2007; Janos 1996; Öpik *et al.* 2006). However, AMF from nearly all the taxonomic groups were present and fungal richness of *Glomus* Group A in the reforestation plots was equal to the richness in the neighboring forest and a tropical forest of Panama (Husband *et al.* 2002). Arbuscular mycorrhizal fungal potential in the reforestation plots on the degraded pastures in the tropical mountain forest area apparently differs from

Table 4. Occurrence of *Glomus* Group A sequence types in the habitats (N = nursery, R = reforestation plots on degraded pastures, F = pristine forest), number of sequences per habitat, and identification of sequence types by comparison with morpho-species or sequences in the SSU300-GLOM1310 or in the NS31-AM1 region from other investigations.

Sequence type	Number of sequences			Sequence type known as identified morpho-species or as sequence in the SSU300-Glom1310 region	Sequence type known as identified morpho-species or showing ≥ 99 % similarity with published sequences in the NS31-AM1 region (with host/ isolation source)
	N	R	F		
1	1	3	0	*Glomus intraradices*	*Glomus intraradices*
2	0	2	2	*Glomus vesiculiferum*	*Glomus vesiculiferum*
3	1	3	0	*Glomus fasciculatum*	*Glomus fasciculatum*

Contd...

Table 4. Continued.

Sequence type	Number of sequences			Sequence type known as identified morpho-species or as sequence in the SSU300-Glom1310 region	Sequence type known as identified morpho-species or showing ≥ 99 % similarity with published sequences in the NS31-AM1 region (with host/ isolation source)
	N	R	F		
4	0	1	2		EU350063 *Caragana korshinskii* EU332708 *Glycine max* EF041068 *Agrostis stolonifera* DQ357107 *Ammophila arenaria* AJ563882 *Phragmites australis* AY70 2066 grass roots AM746139 soil
5	0	1	2		DQ357081 *Ammophila arenaria* EU350066 *Caragana korshinskii*
6	0	4	0		AM412080 root tissue AY129603 Glo35 *Faramea occidentalis, Tetragastris panamensis*
7	0	0	1		
8	1	0	0		
9	0	0	2		EU417619 *Afrothismia winkleri*
10	0	0	1		
11	1	7	2	(*Glomus sinuosum* 98 %)	(*Glomus sinuosum* 98 %)
12	0	0	2		
13	0	1	0		
14	0	0	4		
15	0	0	6		
16	0	1	2		
17	0	1	0		AB183987 roots in forest
18	0	0	2		AB183953 roots in forest
19	0	0	1		
20	0	5	0		
21	0	3	0	AJ699068 *Marchantia foliacea*	AF485887 Glo3a *Glomus* sp. UY1225 AJ699068 *Marchantia foliacea*
22	0	2	0	*Glomus proliferum*	*Glomus proliferum*
23	0	4	0		
24	0	8	1		AJ854084 Glo2 *Ajuga reptans* AY969156 mixed hardwood soil
25	0	1	0		
26	0	0	2		
27	0	0	2		
28	0	0	2		
29	0	0	2		
30	0	0	1		
31	0	2	5		
32	0	3	0	DQ085211 *Juniperus procera*	DQ085211 *Juniperus procera*

Contd...

Table 4. Continued.

Sequence type	Number of sequences			Sequence type known as identified morpho-species or as sequence in the SSU300-Glom1310 region	Sequence type known as identified morpho-species or showing ≥ 99 % similarity with published sequences in the NS31-AM1 region (with host/ isolation source)
	N	R	F		
33	0	0	3		
34	0	0	3		
35	0	0	1		
36	0	0	1		
37	0	1	0		
38	0	1	0		
39	0	5	0	AJ699069 *Marchantia foliacea*	AJ563913 *Phragmites australis* AM746141 soil AJ854089 Glo18 *Ajuga reptans*
40	0	2	0		EF041100 Glo60 *Agrostis stolonifera* AM746145 soil
41	0	0	10		AY129609 Glo32 *Faramea occidentalis*
42	0	1	0	DQ085256 *Juniperus procera*	DQ085254 *Juniperus procera* AM849309 *Hepatica nobilis*
43	1	1	0	*Glomus mosseae*	*Glomus mosseae*

that found in other heavily anthropogenically-influenced sites. The man-made tropical mountain pastures may better be categorized as "grassland" according to the classification suggested by Öpik *et al.* (2006). These authors found no significant differences between tropical forests and grasslands in the average number of arbuscular mycorrhizal taxa per plant species. Highly diverse secondary vegetation is found in the surrounding areas of the abandoned pastures (Martinez *et al.* 2008) and may supply AMF propagules to the reforestation plots. Plant species richness was experimentally shown to support AMF diversity (van der Heijden *et al.* 1998). Investigation of the AMF community of the secondary woody vegetation in the surroundings is, however, needed to corroborate our assumption. *Glomus* A sequences from mycorrhizas of the dominating grass species *S. sphacelata* clustered with sequences from seedlings indicating that propagules are transferred from the pasture vegetation to the seedlings. In contrast, Aldrich-Wolfe (2007) found minimal sharing of mycobionts between tree seedlings and pasture grasses.

Our expectation of distinct fungal composition in the habitats was corroborated by the Ward cluster analysis on presence/absence data of *Glomus* Group A sequence types. Distinct differences in fungal composition resulted also from a global overview on molecular defined AMF by Öpik *et al.* (2006). Most of the *Glomus* Group A sequence types were found either in the reforestation plots or in the pristine forest. Only a small number of fungal sequence types occurred in both habitats. Thus, the seedlings, during the first three years, trapped only a very limited number of fungi so far known from the pristine neighboring forest. Husband *et al.* (2002) found a change of AMF associations during successive field studies on tropical tree seedlings, indicating that plant age could influence the fungal community. Only further observations of the seedlings and comparative investigation of AMF of remnant trees and other woody plants in the surroundings of the reforestation plots can show if forest adapted fungi are still present or were definitely lost during degradation of the habitat. Such future investigations would also contribute to lowering the potential bias of only investigating tree seedlings for AMF potential of a site. Seedlings may be preferentially colonized by fast growing AMF and thus, we may have missed some of forest adapted AMF on the

degraded pastures.

We did not observe differences in sequence type presence among the three succession stages of the reforestation plots on the degraded pastures or along the altitudinal gradient in the pristine forest. The database may still be too limited to detect such differences. The occurrence of only one sequence type common between pristine forest and nursery may indicate difficulties in cultivating forest AMF in the nursery. However, this result needs to be corroborated by further sampling. The occurrence of widespread fungi like *Glomus intraradices, G. fasciculatum* and *G. mosseae* in the nursery seedlings was most likely due to inefficient soil sterilization.

Our results indicate strong habitat influence on AMF community, corresponding to conclusions by Öpik *et al.* (2006) in their global analysis. Although the two habitats generally share the same climate - nearly permanent high precipitation and moderate temperature - roots in the upper few centimeters of the pasture soils in the reforestation plots are more frequently stressed by water deficiency and high temperature from intense solar radiation. Even more importantly, the differences in soil nutrients and the occurrence of a large Ah horizon on the reforestation plots versus a thick humus layer in the pristine forest may influence the AMF communities. Some of the fungi may have been imported by human activities from other continents and are now spread by *S. sphacelata*, as this grass species is predominantly planted by local people to establish the pastures after burning.

Of the 13 most frequently detected AM fungal taxa (Öpik *et al.* 2006, Table 2) the following are found on the reforestation plots: *Glomus intraradices/fasciculatum* (st 1, st 3), *G. mosseae* (=st 43), *Glomus* sp. UY1225 (=st 21), Glo18 (=st 39) and Glo2 (=st 24). In the pristine forest only one (Glo2 = st 24) of these globally occurring AM fungal taxa was detected (Fig. 3). All these taxa are generalists colonizing a wide host range (Helgason *et al.* 2007), which is enlarged with every new evaluation (this study; Liu *et al.* 2009; Öpik *et al.* 2008). The occurrence on the degraded pastures confirms the better resilience of these taxa (Helgason *et al.* 2007). Concerning these comparisons one has to keep in mind, however, that several genotypes/strains/subspecies may be hidden behind (Croll *et al.* 2008; Mathimaran *et al.* 2008). No host-specific fungal sequence types are obvious from the data of the reforestation plots supporting the above mentioned observations and Öpik *et al.* (2006). However, host-fungus preferences, as found in studies of semi-dry tropical forest (Wubet *et al.* 2006a, 2006b) or in legumes and non-legumes (Scheublin *et al.* 2004) may be obscured in our investigation because of still insufficient sampling.

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Appendix

Detailed methods for molecular identification of arbuscular mycorrhizal fungi

We used SSU817 as a forward primer with GLOM1310rc primer in a first attempt, by which we obtained 127 sequences of *Glomus* Group A (data not shown). However, because of the high similarity of about 550 nucleotides, the phylogenetic trees were poorly resolved. To improve the phylogenetic resolution by using longer sequences, we used SSU128 or SSU300 as forward primers in the first PCR in subsequent attempts (Tables 2 & 3). We also tried to use the SSU300 forward primer with the reverse primers LETC1670rc, ACAU1660rc and ARCH1375rc, but no PCR products were obtained. Amplification with the primers ACAU 1660rc, LETC1670rc, ARCH1375rc, GIGA5.8R was conspicuously less successful than with GLOM 1310rc. Even an amplification success did not guarantee that the sequence belonged to the Acaulosporaceae, *Glomus* Group B, Archaespor-ales or Gigasporaceae, respectively.

The PCR reaction volume was 50 µl, with concentrations of 3 mM $MgCl_2$, 200 µM of each dNTP (Life Technologies, Eggenstein, Germany), 0.5 µM of each of the primers (Biomers, Ulm, Germany), 1 U Taq Polymerase (Life Technologies), amplification buffer (Life Technologies), 0.2 µl 1 % BSA (bovine serum albumin; Sigma) and 1 µl DNA extract in the first PCR, and 0.5 µl of the first PCR products for the second PCR. Three microliters of each reaction were separated on a 1.5 % agarose gel and stained with ethidium bromide prior to direct sequencing or cloning. Amplified PCR products were cloned with the Invitrogen TA Cloning

Kit (Life Technologies) following the manufacturer's instructions. Inserts were reamplified from clones using the M13 primers by picking twelve positive bacterial clones with a toothpick and placing them directly into the PCR reaction mixture. After gel electrophoresis up to 12 positive cloned amplification products were cleaned with QIAquick (Qiagen, Hilden, Germany). Direct sequencing of PCR products was performed using the forward PCR primer as the sequencing primer; for cloned products the M13F primer was used. After preliminary analysis, the second strand was sequenced with the reverse PCR primer M13R. Cycle sequencing was conducted using the ABI PRISM Dye-Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol, but with the reaction volume halved and the kit diluted 1:6. Electrophoresis and data sampling were performed on an automated sequencer (ABI 3100; Applied Biosystems). Sequences were edited and contigs constructed using Sequencher software (version 4.1, Gene Codes, Ann Arbor, Michigan). The sequences obtained were assigned to higher fungal groups with BLAST searches (Altschul *et al.* 1997) against the National Center for Biotechnology Information (NCBI) database (http://www.ncbi. nlm.nih.gov). We checked for putative chimeric sequences using the program Pintail (Ashelford *et al.* 2005) and we compared sequence segments with GenBank accessions using a BLAST search. About 16 % of sequenced clones had portions of sequences that matched other taxa (plants, ascomycetes, basidiomycetes). These sequences were excluded.

Appendix Table 1. The characteristics of the three reforestation plots (source: Aguirre 2007).

Characteristics	R1: Pasture	R2: Fern	R3: Shrub
Altitude (m a.s.l.)	1800 - 2100	1850 - 2100	2000 - 2200
Inclination (%)	53 (6-90)	69 (10-100)	44 (5-55)
Vegetation cover (%)	100	100	80 - 100
Dominant life-forms	grasses	fern and few shrubs	shrubs, fern and herbs
Actual use before planting	livestock farming (cattle pasture)	early successional state dominated by fern	advanced successional state dominated by shrubs
Shannon-Index	0.87 (0.20 - 1.380)	0.84 (0.58- 1.26)	1.89 (0.87 - 3.00)
Topography	irregular and steep	irregular and steep	irregular and steep
Remnant trees	*Piptocoma discolor, Isertia laevis, Tabebuia chrysantha*	*Nectandra membranacea, Inga* sp.	*Vismia ferrruginea, Tabebuia chrysantha, Clethra* sp.

Appendix Table 2. Primer names, sequences and references.

Primer Name	Sequence (5´-3´)	Reference
SSU128	GGA TAA CCG TGG TAA TTC TAG	designed for this study
SSU1536IH	RTT GYA ATG CYC TAT CCC CA	Borneman & Hartin 2000, modified
SSU300	CAT TCA AAT TTC TGC CCT ATC A	designed for this study
GLOM1310rc	TAA CAA TGT TAG RCC TAG CT	Redecker 2000
ACAU1660rc	CCG ATC CGA GAG TCT CA	Redecker 2000
LETC1670rc	ACT CAC CGA TCG CCG ATC	Redecker 2000
ARCH1375rc	TCA AAC TTC CGT TGG CTA RTC GCR C	Russell et al. 2002
NS8	TCC GCA GGT TCA CCT ACG GA	White et al. 1990
NS5	AAC TTA AAG GAA TTG ACG GAA G	White et al. 1990
ITS4	TCC TCC GCT TAT TGA TAT GC	White et al. 1990
GIGA5.8R	ACT GAC CCT CAA GCA KGT G	Redecker 2000
GIGAIH	CCC ATC ACG ATG AAR TTT CA	designed for this study

Appendix Table 3. Primer combinations and annealing temperatures used for the PCRs.

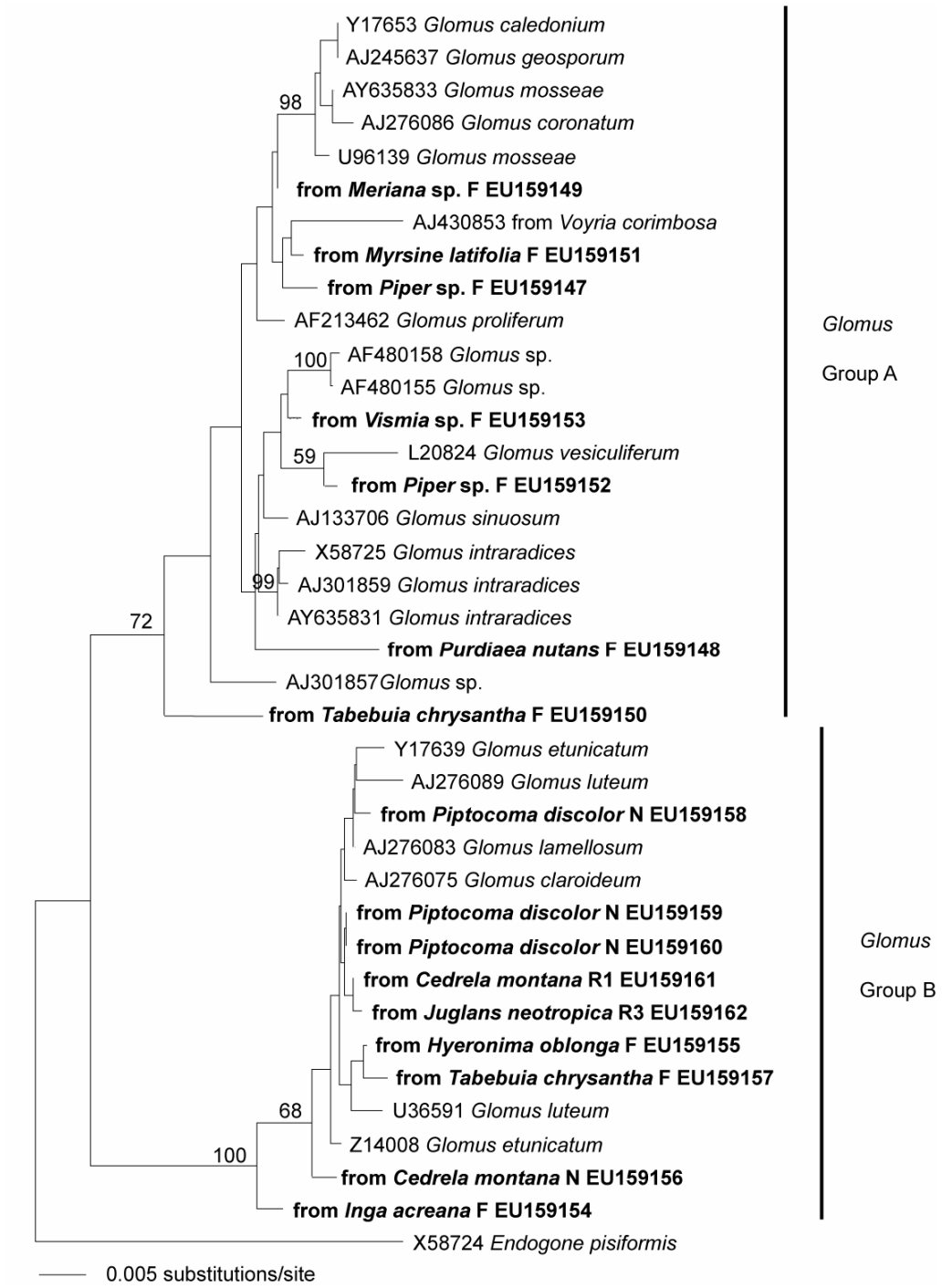
Fungal group	1st PCR	2nd PCR	Annealing temp. (°C)
Glomus Group A	SSU817-SSU1536IH	SSU817-GLOM1310rc	50
	SSU128-SSU1536IH	SSU128-GLOM1310rc	50
	SSU128-SSU1536IH	SSU300-GLOM1310rc	50
Glomus Group B	SSU817-NS8	SSU817-LETC1670rc	50
Acaulosporaceae	SSU817-NS8	SSU817-ACAU1660rc	50
Archaeosporales	SSU817-SSU1536IH	SSU817-ARCH1375rc	50
Gigasporaceae	NS5-ITS4	NS5-GIGA5.8R	50
	SSU817-NS8	SSU817-GIGAIH	50

Appendix Table 4. Sequence type richness estimation in the pristine forest (*n* number of analyzed PCR products, *Sobs* observed richness obtained by resampling without replacement).

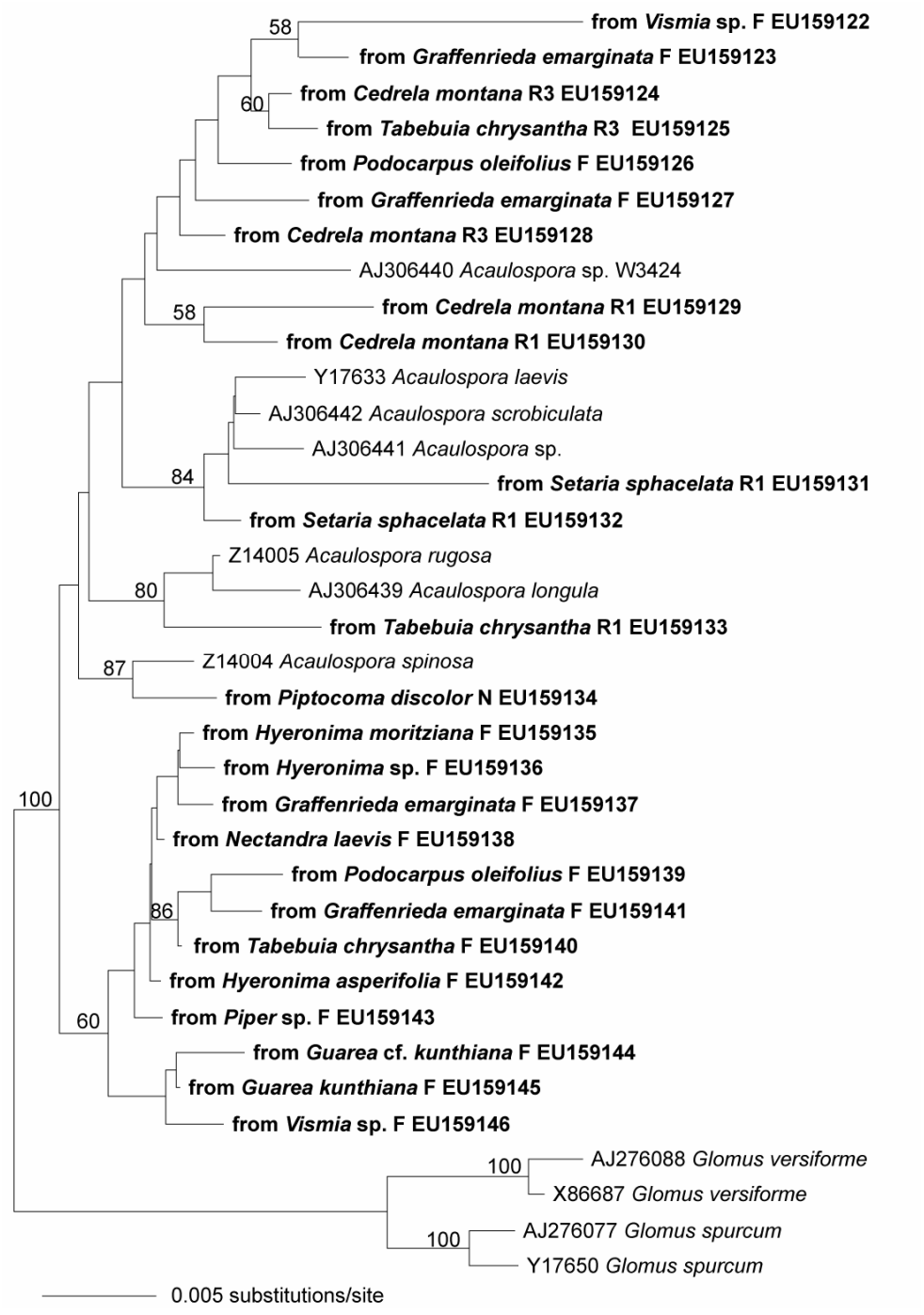
n	*Sobs*	*Chao 2*	*Jackknife 2*
10	6.00	37.06	15.44
25	20.32	30.85	35.29
42	25.00	27.42	31.91

Appendix Table 5. Sequence type richness estimation in the reforestation plots (*n* number of analyzed PCR products, *Sobs* observed richness obtained by resampling without replacement).

n	*Sobs*	*Chao 2*	*Jackknife 2*
10	9.24	24.89	13.79
25	16.55	27.71	29.01
42	20.97	29.89	34.08
57	24.00	30.82	37.59

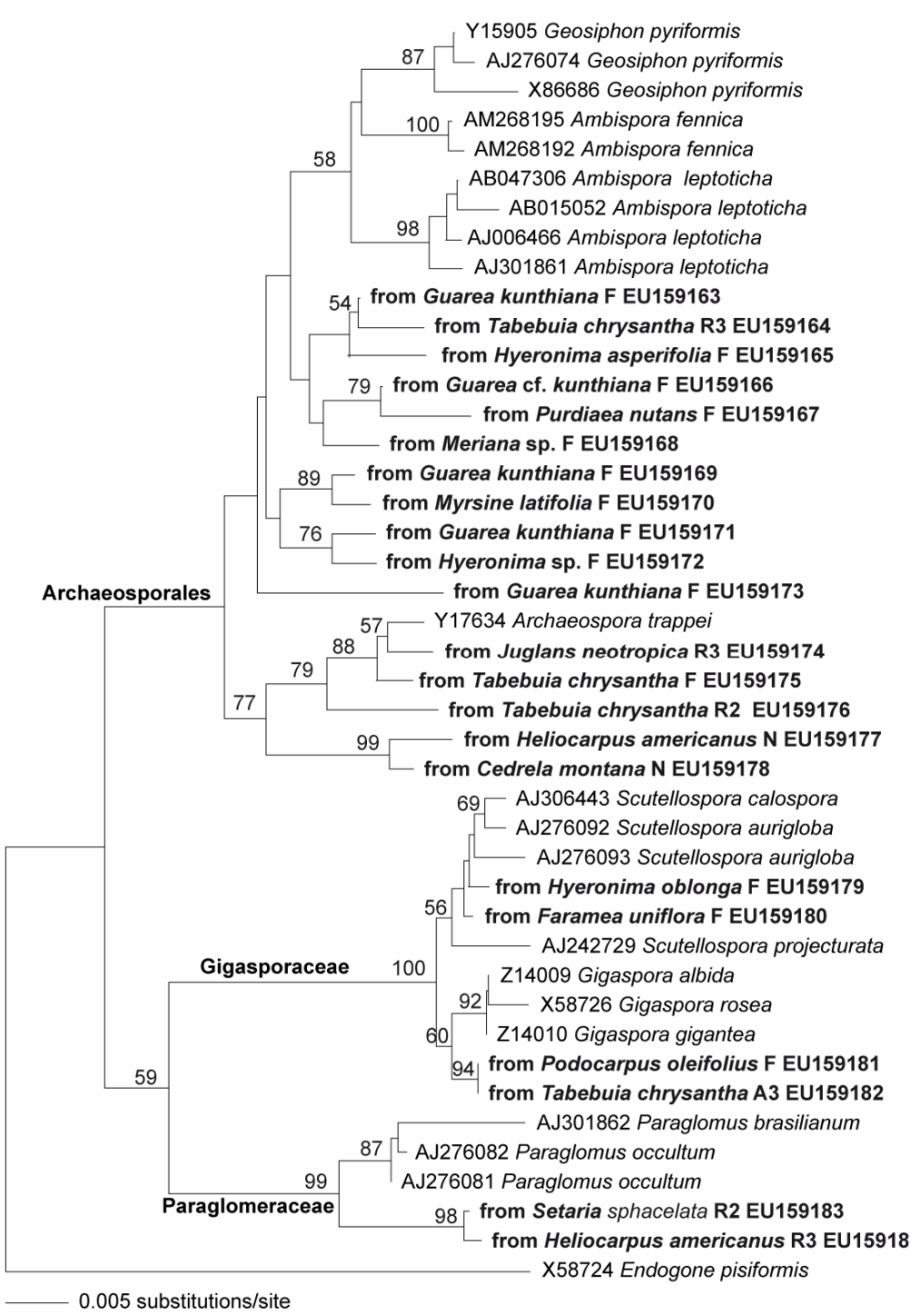


Appendix Figure 1. *Glomus* Group A and *Glomus* Group B sequences obtained from mycorrhizae of tree species on reforestation plots (R1, R2, R3), pristine forest (F) and a nursery (N) in South Ecuador by use of primers SSU817 - GLOM1310rc or SSU817 - LETC1670rc respectively (in the second PCR). BIONJ analysis was carried out from an alignment of nuclear DNA sequences coding for the small ribosomal subunit (nucSSU; 912 characters). The tree was rooted with *Endogone pisiformis*. Numbers on branches designate bootstrap values.

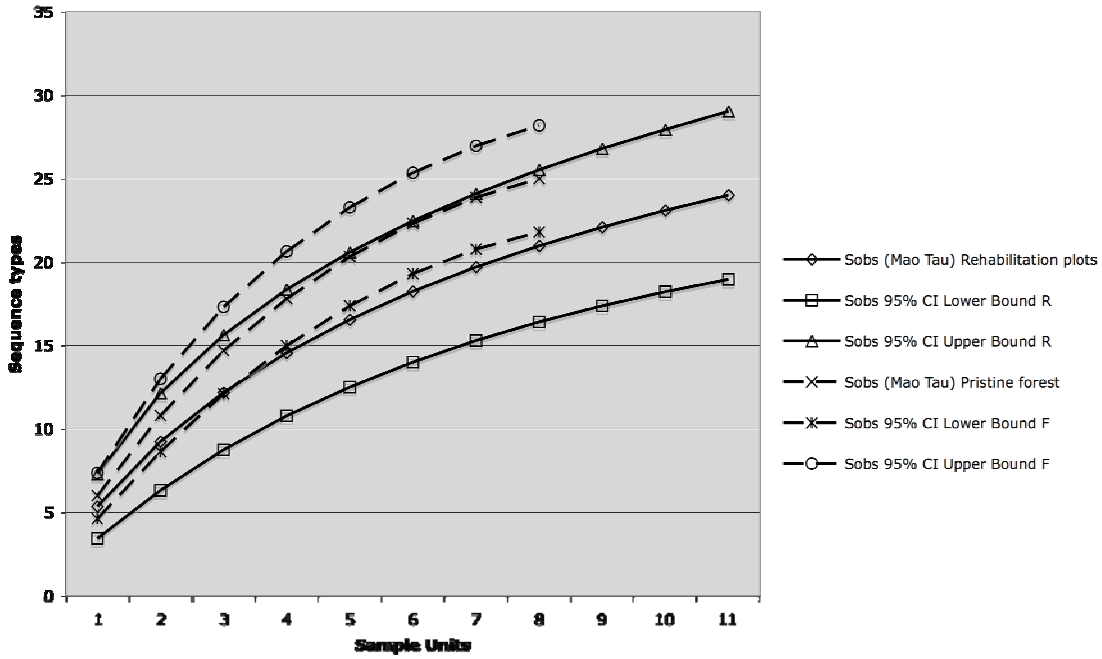


Appendix Figure 2. Acaulosporaceae sequences obtained by use of primers SSU817 and ACAU1660rc (in the second PCR) from tree species mycorrhizas sampled in rehabilitation plots (R1, R2, R3), pristine forest (F) and a nursery (N) in South Ecuador. BIONJ analysis was carried out from an alignment of nuclear DNA sequences coding for the small ribosomal subunit (nucSSU; 900 characters). The tree was rooted with four sequences of the Diversisporaceae. Numbers on branches designate bootstrap values.

Twelve sequences of Acaulosporaceae from the pristine forest cluster together. Each investigated *Graffenrieda emarginata* individual showed an Acaulosporaceae sequence (Table 3).



Appendix Figure 3. Archaeosporales, Gigasporaceae and Paraglomeraceae sequences obtained by use of primers SSU817 and ARCH1375rc, ACAU1660rc, LETC1670rc (in the second PCR) from mycorrhizas of tree species on reforestation plots (R1, R2, R3), pristine forest (F) and a nursery (N) in South Ecuador. BIONJ analysis was carried out from an alignment of nuclear DNA sequences coding for the small ribosomal subunit (nucSSU; 631 characters). The tree was rooted with *Endogone pisiformis*. Numbers on branches designate bootstrap values.



Appendix Figure 4. Sequence type accumulation curves with 95 % confidence intervals for the reforestation plots and pristine forest. Sobs (number of sequence types observed by resampling without replacement).

Appendix References

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