Arbuscular mycorrhizal fungi for reforestation of native tropical trees in the Andes of South Ecuador

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Contents

Contents **3**

Abbreviations **5**

Zusammenfassung **7**

Abstract **9**

1 Introduction **11**

1.1 Arbuscular mycorrhiza and reforestation in the tropics 11

1.2 The *Glomeromycota* 12

1.3 Investigation site 14

1.4 Aim of the study 15

2 Materials and Methods **16**

2.1 Sampling sites 16

2.1.1 Tropical mountain rain forest, abandoned pasture and afforestation site 16

2.1.2 Tree nursery experiments at the Universidad National de Loja, South Ecuador 16

2.1.3 Nursery experiments performed with AMF inoculum produced in the framework of this study 18

2.1.4 Nursery experiment No. 3 19

2.1.5 Nursery experiment No. 4 and No. 4A 22

2.1.6 Statistical analysis 25

2.2 Setting up fungal cultures 25

2.2.1 Inoculum production 28

2.3 Inoculum efficiency test 29

2.4 Identification of AMF 29

2.4.1 Morphological examination of spores 30

2.4.2 Molecular characterization of the fungi from cultures, nursery and reforestation sites 30

2.4.3 454 GS FLX Titanium amplicon sequencing 33

3 Results **38**

3.1 Characterization of AMF cultures from Ecuador 38

3.1.1 Morphological characterization of fungal cultures (isolates) 38

3.1.2 Molecular characterization of AMF from Ecuador 50

3.2 Nursery experiment No. 3 63

3.2.1 Efficiency of inoculum tested on *Plantago lanceolata* as host plant 63

3.2.2 Inoculum effect in the nursery 63

3.2.3 Reforestation success by AMF inoculation 77

3.2.4 Tracing of AMF by 454 GS FLX sequencing 95

3.3 Nursery experiment No. 4 and No. 4A 113
3.3.1 Inoculum efficiency tested on *Plantago lanceolata* as host plant 113
3.3.2 Inoculum efficiency in Nursery experiment No. 4 114
3.3.3 Effect of inoculation on *Cedrela montana* in Nursery experiment No. 4A 124

4 Discussion 129

5 Conclusion 141

5.1 Outlook 142

6 References 143

7 Acknowledgement 154

8 Appendix 155

8.1 Figures and tables 155

8.2 Manuals 213

8.2.1 Amplicon Library Preparation Method Manual - GS FLX Titanium Series 213

8.2.2 TCB No. 005-2009 – Using Multiplex Identifer (MID) Adaptors for the GS FLX Titanium Chemistry – Extended MID Set 220

Curriculum vitae 228

Publication list 229

Symposia and Conferences 230

Eidesstattliche Erklärung 231

Abbreviations

AFTOL	Assembling the Fungal Tree of Life
AM	arbuscular mycorrhiza
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
approx.	approximate(ly)
Att	attempt
a.s.l.	above sea level
BEG	International Bank for the *Glomeromycota*
bp	base pair(s)
BS	bootstrap support
BSA	bovine serum albumin
ca.	circa
cf.	Latin: confer (English: compare)
comb. nov.	Latin: combinatio nova (English: new combination)
CTAB	cetyltrimethylammonium bromide
DAOM	Agriculture and Agri-Food Canada National Mycological Herbarium
d.e.	data equal
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxyribonucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
e.g.	Latin: exempli gratia (English: for example)
Fisher-LSD	Fisher's Least Significant Difference
i.a.	Latin: inter alia (English: among other things)
INVAM	International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi
ITS	internal transcribed spacer
kb	kilo base pair(s)
LB	lysogeny broth (see Bertani, 1951)
LSU	large subunit
mt	mitochondrial
MAFFT	Multiple Alignment using Fast Fourier Transform
MID	Multiplex Identifier
MUCL	Mycothèque de l'Universite Catholique de Louvain

Mya	million years ago
NEB	New England Biolabs
No.	number(s)
PCR	polymerase chain reaction
rDNA	ribosomal DNA
RFLP	restriction fragment length polymorphism
rcf	relative centrifugal force
RNA	ribonucleic acid
ROC	root organ culture
rRNA	ribosomal RNA
SB	sodium borate
sensu	English: in the sense of
SSU	small subunit
sp.	species (singular; plural: spp.)
Taq	*Thermus aquaticus*
TE	Tris-EDTA
Tm	melting temperature
Tris	Tris(hydroxymethyl)aminomethane
Tukey's HSD	Tukey's Honestly Significant Difference
U	unit (of enzyme activity)

Zusammenfassung

Die arbuskuläre Mykorrhiza (AM), eine Symbiose zwischen Pilzen und Pflanzenwurzeln, wird von mehr als 80% aller Landpflanzen ausgebildet. In dieser Pilz-Wurzel-Symbiose versorgt der Pilzpartner die Pflanze mit Nährstoffen und Wasser und erhält im Gegenzug Kohlenhydrate. Insbesondere die Phosphatversorgung der Pflanzen kann dadurch verbessert werden. Besonders in Gebieten mit nährstoffarmen Böden, wie den Tropen, ist dies von entscheidender Bedeutung. Ein Großteil aller Baumarten der Tropen ist mit AM-Pilzen (AMP) assoziiert. Im südecuadorianischen, tropischen Bergregenwald, im Gebiet der Forschungsstation Estacíon Científica San Francisco (ECSF) zeigte sich, dass 98% der untersuchten Bäume mit diesen Pilzen mykorrhiziert sind. Diese hohe Mykorrhizaabhängigkeit macht Wiederaufforstungsversuche einheimischer Baumarten schwierig. In einem Vorversuch im Rahmen eines vorangegangen Projektes der Deutschen Forschungsgemeinschaft (DFG FOR402 Projekt A6 - Kottke, Oberwinkler) konnte das Wachstum von Jungpflanzen in der Baumschule der Universidad Nacionál de Loja, Südecuador, durch Inokulation mit AMP aus im Wald gesammelten Boden und Mykorrhizen positiv beeinflusst werden. Auf diesem Vorversuch aufbauend, wurde ein Konzept zur Versorgung von Jungpflanzen einheimischer Baumarten mit AMP entwickelt. Vor allem die Sterblichkeitsraten der Jungpflanzen während der Anzucht und nach dem Auspflanzen sollten reduziert werden. Es sollte aber mit definierten, einheimischen AMP gearbeitet werden. Die eingebrachten AMP sollten während der Baumschulphase und nach der Auspflanzung in den Wurzeln der Jungpflanzen nachverfolgt werden. Ziel war es die bestgeeigneten Pilze für die jeweilige Baumart zu bestimmen.

In der vorliegenden Arbeit wurden zunächst die AMP, die mit Sämlingen von *Cedrela montana* und *Heliocarpus americanus* in der Baumschule assoziiert waren in Topfkulturen isoliert und auf Artebene identifiziert. Die erhaltenen AMP wurden morphologisch mittels ihrer Sporen und Myzelstrukturen, sowie molekularbiologisch anhand eines ca. 3 kb großen Fragmentes der nukleären ribosomalen DNA charakterisiert. AMP der Gattungen *Rhizophagus*, *Claroideoglomus*, *Acaulospora*, *Archaeospora*, *Scutellospora* und *Ambispora* wurden identifiziert. Die Isolate wurden auf *Plantago lanceolata* als Wirtspflanze zur Inokulumproduktion vermehrt. Ein Gemisch all dieser Pilzarten wurde in der oben genannten Baumschule für die Mykorrhizierung der einheimischen Baumarten *C. montana*, *H. americanus* und *Tabebuia chrysantha* verwendet. Nach einer sechsmonatigen Baumschulphase wurde ein Teil der Pflanzen auf einer brachliegenden Weidefläche ausgepflanzt und weiter beobachtet. Um die Ergebnisse der Inokulierung während der Baumschul- und Aufforstungsphase nachzuverfolgen, wurden zu je zwei Zeitpunkten

Wachstumsdaten erhoben und Wurzelproben genommen.

Der Großteil der isolierten und in der Baumschulphase eingesetzten AMP konnten mittels 454 GS FLX Titanium Sequenzierung auf Artebene identifiziert und in den Sämlingen und Jungpflanzen auf den Aufforstungsflächen nachgewiesen werden. Zusätzlich wurden bis zu 11 weitere nicht durch den Inokulum-Mix eingebrachte AMP gefunden (u.a. *Glomus macrocarpum*, *Rhizophagus irregularis*, *Acaulospora brasiliensis*-like, *Rhizophagus* sp., *Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., *Archaeospora* sp. und *Scutellospora* sp.). Die Inokulierung mit AMP zeigte eine signifikant reduzierte Sterblichkeitsrate von *C. montana* und *T. chrysantha* in der Baumschulphase und der ausgepflanzten *T. chrysantha* Jungpflanzen auf den Aufforstungsflächen. Die mit AMP inokulierten Sämlinge zeigten in der Baumschulphase teilweise erhöhtes Wachstum. Auf den Aufforstungsflächen zeigte sich eine reduzierte Sterblichkeitsrate, aber keine weitere positive Beeinflussung des Wachstums im Unterschied zu den nicht inokulierten, jedoch teils mykorrhizierten Kontrollpflanzen.

In einem weiteren Experiment, im kleinen Maßstab, wurden Präferenzen zwischen den Pilzarten und den Baumarten näher untersucht. Dazu wurde eine Auswahl der oben erwähnten, identifizierten AMP als individuelle Inokula in der Baumschule angewendet. Die Ergebnisse zeigten eine positive Beeinflussung der gemessenen Wachstumsparameter der Sämlinge im Vergleich zu den Kontrollpflanzen und deutliche Wachstumsunterschiede zwischen den einzeln eingebrachten AMP und den jeweiligen Baumarten.

Eine zukünftige Optimierung des verwendeten AMP-Inokulums für die jeweiligen Baumarten ist aufgrund dieser Ergebnisse möglich. Laufende Wiederaufforstungen in Ecuador mit den einheimischen, tropischen Baumarten *C. montana*, *H. americanus* und *T. chrysantha* können damit unterstützt werden.

Abstract

Arbuscular mycorrhiza (AM), a symbiosis between fungi and plant roots, is formed by more than 80% of land plants. In this fungus-root-association the fungal partner provides nutrients and water to the plant and gains carbohydrates in exchange. Especially phosphor supply to the plant is improved. In areas of nutrient-poor soils like the tropics this fact is of great importance. The vast majority of tropical tree species are associated with AM fungi (AMF). In the south Ecuadorian tropical montane rainforest, in the area of the research station Estacíon Científica San Francisco (ECSF), 98% of the examined tree roots were found colonized by AMF. The high mycorrhiza dependency impedes reforestation attempts by native tree species. In a preliminary experiment in the framework of a former project of the German Research Foundation (DFG RU402 project A6 - Kottke, Oberwinkler) an improved growth performance was shown for nursery tree seedlings at the Universidad Nacionál de Loja, South Ecuador, when these seedlings were raised with addition of forest soil and mycorrhizal roots. Based on this nursery experiment (herein named No. 1), an elaborated concept of growing indigenous tree seedlings for experimental reforestation was developed. The specific aim was to reduce the mortality of the seedlings by introduction of defined, Ecuadorian AMF in the nursery phase. The introduced AMF should be monitored in the seedling roots during the nursery phase and on the reforestation plots and related to seedling performance of different tree species.

In this study AMF associated with nursery seedlings of *Cedrela montana* and *Heliocarpus americanus* were isolated in pot cultures and subsequently identified. The obtained AMF were characterized morphologically by spore and mycorrhizal structures and molecular biologically identified on the basis of an approximately 3 kb long fragment of the nuclear ribosomal DNA. AMF belonging to the genera *Rhizophagus*, *Claroideoglomus*, *Acaulospora*, *Scutellospora*, *Archaeospora* and *Ambispora* were identified. The fungi were multiplied using *Plantago lanceolata* as host to produce inoculum for nursery applications. A mixture of all the AMF was applied at the Ecuadorian nursery to inoculate seedlings of *C. montana*, *H. americanus* and *Tabebuia chrysantha*. After a nursery phase of 6 months, part of the tree seedlings were planted on abandoned pastures on experimental reforestation plots. Growth data and root samples of all tree species were collected two times, respectively. All the isolated and applied AMF were traced on species level during the nursery and the reforestation phase by using 454 GS FLX Titanium sequencing. Up to 11 further AMF species were detected, which had not been introduced by the inoculum mixture (e.g. *Glomus macrocarpum*, *Rhizophagus irregularis*, *Acaulospora brasiliensis*-like, *Rhizophagus* sp.,

Claroideoglomus sp., *Funneliformis* sp., *Diversispora* sp., *Archaeospora* sp. and *Scutellospora* sp.). Inoculated plants of *C. montana* and *T. chrysantha* showed a significant reduced mortality rate during the nursery phase. Inoculated *T. chrysantha* seedlings showed a significantly reduced mortality rate on the reforestation plots. The AMF inoculated seedlings showed partial increased growth parameters in the nursery phase. A reduced mortality rate was observed on the reforestation plots, but no further positive effects when compared to the non-inoculated, but also mycorrhizal control plants.

An additional small-scale nursery experiment was carried out to investigate potential AMF-plant preferences. Identified AMF were selected and individually applied to tree seedlings raised in the Ecuadorian nursery. Results showed improved plant performance when compared to the controls. Distinct growth differences were observed between different AMF inocula and respective tree species.

On the basis of these results, future optimization of AMF-inoculum for different tree species will be possible. Current reforestation attempts by indigenous tropical tree species like *C. montana*, *H. americanus* und *T. chrysantha* in Ecuador will profit from AMF inoculation during the nursery phase.

1 Introduction

1.1 Arbuscular mycorrhiza and reforestation in the tropics

Ecuador is one of the hottest hotspots in biodiversity (Brummit & Lughadha 2003). Jorgensen & León-Yánez (1999) described more than 2,700 tree species native to Ecuador. However, more and more tropical forest is destroyed by slash and burn and replaced by pastures for cattle-breeding (Mosandl *et al.* 2008). The deforestation rate in Ecuador is one of the highest in South America (FAO 2006) and deforestation is becoming a serious problem. Land-use concepts that would make natural forests more valuable for farmers are lacking (Knoke *et al.* 2008, 2009).

Reforestation in Ecuador is currently focused on fast growing foreign species such as *Eucalyptus* spp. and *Pinus* spp. for commercial interest. Still little is known about afforestation of native tree species (Aguirres Mendoza 2007). Due to a lack of knowledge, rising of native tree seedlings is difficult (Stimm *et al.* 2008). Reforestation of native tree seedlings is further hampered as the vast majority of tropical trees need association with fungi placed into the phylum *Glomeromycota* (Schüßler *et al.* 2001) which form so-called arbuscular mycorrhiza (AM) with the tree roots (Wang & Qui 2006, Zangaro *et al.* 2000). The symbiosis was documented for a vast number of tropical trees (Janos 1987, 1996) and for the Ecuadorian tropical mountain rainforest (Kottke & Haug 2004, Kottke *et al.* 2004, Kottke *et al.* 2008). Arbuscular mycorrhiza forming fungi (AMF) were found to associate unspecific with diverse plants, which can be colonized by diverse AMF species (Aldrich-Wolfe 2007, Börstler *et al.* 2010, Haug *et al.* 2010, Öpik *et al.* 2006, Wubet *et al.* 2003). Arbuscular mycorrhiza can improve plant growth performance, resistance to drought stress and pathogens through improved plant uptake of P, N (Smith & Read 2008) and other nutrients (Cavagnaro 2008). The symbiosis is therefore crucial in tropical mountain forests where the nutrient availability of acidic soils is low (e.g. P and N). In the forest of the Reserva Biológica San Francisco (RBSF) phosphorus is bound in organic layers (Makeschin *et al.* 2008) and thus not directly available to plants (Wilcke *et al.* 2001, 2002, 2008). The uptake and storage of different toxic soil chemicals such as arsenic make these fungi interesting for restoration of polluted or degraded sites, also in Ecuador (Elahi *et al.* 2010, Jankong & Visoottiviseth 2008, Wubet *et al.* 2003, 2009).

Several studies revealed plant or habitat preferences of AMF (Croll *et al.* 2008, Geml *et al.* 2008, Haug *et al.* 2010, Martínez-García & Pugnaire 2011, Öpik *et al.* 2006, 2009). Different AMF may show distinct beneficial effects depending on plant species and experimental conditions (Klironomos 2003, van der Heijden *et al.* 1998a). Loss of certain AMF species

through human or natural disturbances may therefore be of disadvantage for forest regeneration on such sites. Application of AMF inocula on restoration sites was found to have positive influence on vegetation coverage (Noyd *et al.* 1995, 1996, Smith *et al.* 1998). Another possibility is the reforestation of AMF-inoculated tropical tree seedlings on such degraded areas. Several studies showed that growth performance increased and mortality decreased when nursery raised tropical tree seedlings were inoculated with AMF (Allen *et al.* 2003, Guadarrama *et al.* 2004, Turjaman *et al.* 2006, Urgiles *et al.* 2009). Inoculation of tropical tree seedlings by AMF would further significantly reduce the planting shock.

The question is however, if native AMF are necessary or if worldwide easy to grow generalists should be preferred in tropical nurseries. Only a small proportion of the AMF indicated from molecular findings are available and are easy to culture. Commercial inocula vary enormously in their effectivity depending on host plant, growth conditions and AMF species composition (Corkidi *et al.* 2004, Tarbell & Koske 2007). Field soil inoculum may even perform better than commercial inocula (Rowe *et al.* 2007). Invasive fungal species may have negative impact on local communities and rare or endangered species (Wilcove & Master 2005, Gurevitch & Padilla 2004). Indigenous AMF may prevent spreading of foreign species into ecosystems (Pringle *et al.* 2009) and are edaphically adapted to local conditions and tree species. No AMF from the area of Southern Ecuador were available at the beginning of this study. It was therefore necessary to isolate, characterize and identify native AMF from the area and to test their inoculation potential at the given conditions.

1.2 The *Glomeromycota*

The AMF were placed in an own monophyletic phylum, *Glomeromycota,* by Schüßler *et al.* (2001). A recent update of genera and species by Schüßler & Walker (2010) rearranged the *Glomerales* into five genera called *Glomus*, *Funneliformis* (former *Glomus* group Aa), *Rhizophagus* (former *Glomus* group Ab), *Sclerocystis* and *Claroideoglomus* (former *Glomus* group B) as previously indicated by Schwarzott *et al.* (2001). The latest update was done by Redecker *et al.* (2013) to clarify the recent glomeromycotan taxonomy. The “consensus” classification of Redecker *et al.* (2013) included the new valid genera *Dentiscutata* and *Cetraspora* (formerly *Scutellospora*) within the *Gigasporaceae* and *Septoglomus* (formerly a part of *Funneliformis*) in the *Glomeraceae* and rejections of questionable genera and families (see Fig. 1). Only a part of the described AMF is available as cultures (Krüger *et al.* 2012). In the beginning AMF were mostly characterized morphologically by the appearance of their spores and mycorrhizal structures. Since molecular techniques became available characterization by different molecular markers such as β-tubulin (Msiska & Morton 2009), two RNA polymerase II subunits (RPB1 and 2; James *et al.* 2006, Redecker & Raab 2006),

elongation factor 1 (EF1), mitochondrial LSU rDNA (Börstler *et al.* 2010, Sýkorová *et al.* 2012) and different regions of the rRNA gene (Krüger *et al.* 2012, Stockinger *et al.* 2010) were suggested to identify AMF. Classification of glomeromycotan fungi resulted in several taxonomical revisions in the last years (e.g., Oehl *et al.* 2008, Morton & Msiska 2010, Schüßler *et al.* 2011, Redecker *et al.* 2013). In this study the recent taxonomy according to Schüßler & Walker (2010) and Redecker *et al.* (2013) is used.

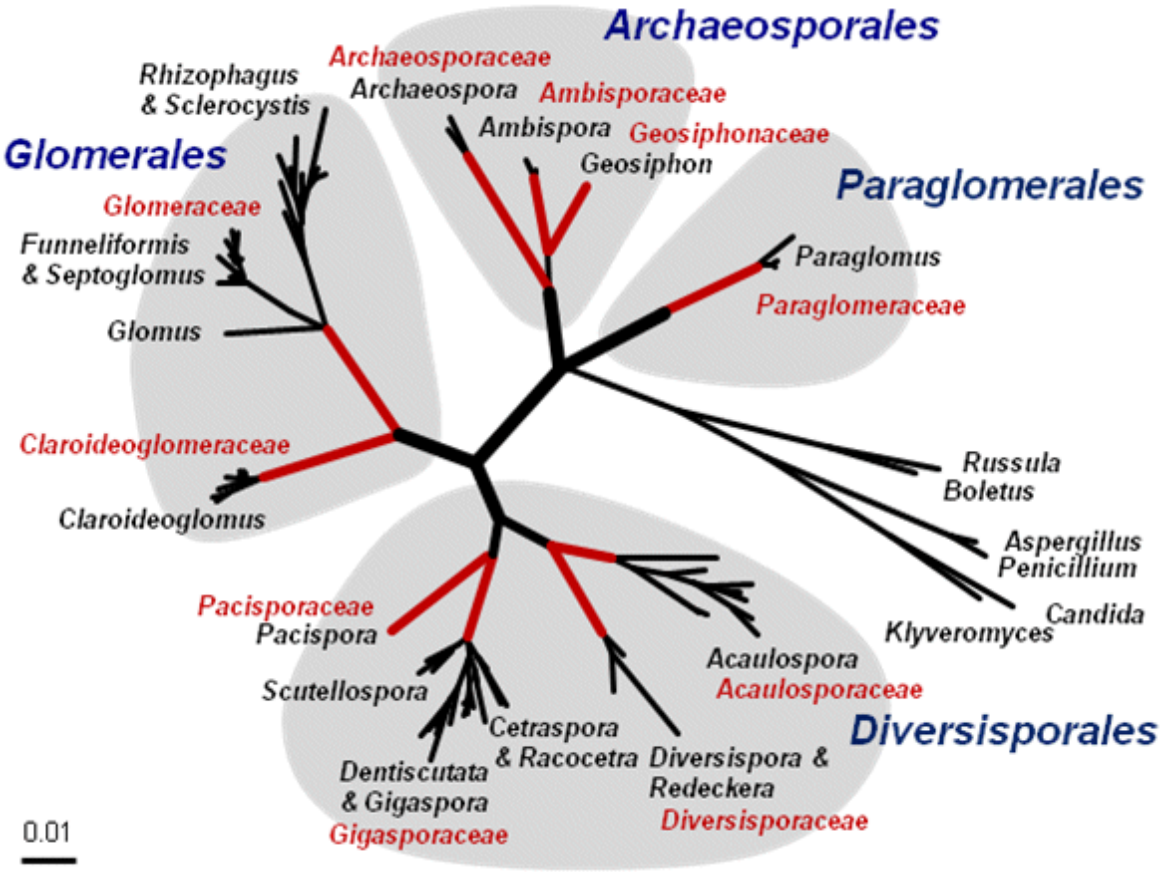


Fig. 1: Phylogenetic tree of the *Glomeromycota*. Phylogenetic tree showing the recent classification after Schüßler & Walker 2010 and Redecker *et al.* 2013. Orders are labeled in blue, families in red and genera in black. Different members of the Dikarya were used as outgroup.

Morphological identification of AMF by spore characteristics solely often leads to misidentification. In some cases species were even placed in the wrong order (e.g. *Acaulospora brasiliensis*, Krüger *et al.* 2011). Characterization of AMF in this study was therefore done by combining morphological and molecular methods and the nuclear ribosomal RNA gene region was used for molecular analysis. The full small subunit (SSU), the internal transcribed spacers (ITS) 1 and 2 with the interjacent 5.8S and a part of the large subunit (LSU) rDNA (SSU_{full}-ITS-LSU_{part}) were sequenced. The fragment is approximately 3 kb long and provides a robust phylogeny (Stockinger *et al.* 2010, Krüger *et al.* 2012). PCR primers described in Schwarzott & Schüßler were used to amplify the near full SSU rDNA

(2001). The SSU_{part}-ITS-LSU_{part} rDNA fragment was amplified with an AMF-specific primer set covering all main AMF lineages while discriminating against contaminations (e.g. *Asco-* and *Basidiomycetes*) published in Krüger *et al.* (2009).

New molecular methods of massive parallel sequencing are now widely used for community analyses of AMF (e.g. Tedersoo *et al.* 2010, Moora *et al.* 2011, Lekberg *et al.* 2012). The 454 GS-FLX Titanium sequencing (Roche) of amplicons was chosen to monitor the nursery applied AMF and their persistence over time. AMF can be identified by implementation of amplicon reads (ca. 400 bp long) into a large 'backbone' alignment based on 3 kb SSU_{full}-ITS-LSU_{part} rDNA sequences of the Ecuadorian AMF (Stockinger *et al.* 2010, Krüger *et al.* 2012).

1.3 Investigation site

The research area in the South of Ecuador is part of the Podocarpus National Park located between Loja and Zamora, Zamora-Chinchipe Province. In this region the research station Estación Científica San Francisco (ECSF) is surrounded on one side of the valley by natural tropical montane rainforest and on the other side by pastures. The area is under investigation now for 15 years (DFG research unit 402 and 816), different groups investigated for example the pastures and their soil (Makeschin *et al.* 2008, Wilcke *et al.* 2008), the plant species richness in the tropical forest (Homeier *et al.* 2008), climate (Bendix *et al.* 2008) and the effectivity of reforestation of the pastures (Aguirres Mendoza 2007, Weber *et al.* 2008). The pastures are poor of usage and have to be maintained constantly, otherwise bracken fern is overgrowing and makes them useless (Hartig & Beck 2003, Roos *et al.* 2010). Experimental reforestation plots are located on the pastures beside the ECSF (RU816, project C2.1 Günter, Mosandl, Stimm, Weber). Several native tree species (e.g., *Alnus acuminata*, *Cedrela montana*, *Heliocarpus americanus*, *Tabebuia chrysantha*, *Juglans neotropica*) and foreign *Eucalyptus* spp. and *Pinus patula* were investigated. Tree species were selected according to their potential and value for farmers (potential crop trees). Survival, growth and impact on the pastures were compared in Aguirres Mendoza (2007).

Former research in the area of the RBSF revealed high AMF abundance. Nearly all investigated native tree species (112 from 115) formed AM (Kottke *et al.* 2008). Haug *et al.* (2010) investigated also AMF diversity on the reforestation plots at the pastures of the RBSF. The authors found mainly AMF sequences (SSU rDNA) belonging to *Glomus* group A (including *Glomus*, *Funneliformis*, *Rhizophagus* and *Sclerocystis*), also few sequences related to *Claroideoglomeraceae* (*Glomus* group B), *Acaulosporaceae*, *Gigasporaceae*, *Paraglomeraceae* and *Archaeosporales*. Interestingly, AMF richness was similar on the reforestation plots and the neighboring pristine forest, but only few fungal sequences were

found in both areas. On contrast, other studies of anthropogenic influenced, disturbed areas found decline of fungal richness (Alexander *et al.* 1992, Janos 1996, Öpik *et al.* 2006, Cairney & Bastias 2007).

Former reforestation attempts by use of native tree seedlings on the pastures of the RBSF area resulted in high mortality rates (ca. 50%). *Tabebuia chrysantha* seedlings showed the highest survival rate. These seedlings had been raised in the nursery on substrate mixed with natural forest soil and therefore were colonized by mycorrhizal fungi (Aguirre Mendoza 2007). Effects of mycorrhizal roots and fertilizer to native tree seedlings were investigated in an Ecuadorian nursery by Urgiles *et al.* (2009). Inoculation by mycorrhizal roots showed improved growth performance of the tree seedlings compared to the control. To identify suited AMF for reforestation, several nursery experiments were conducted to investigate effects of native potential crop tree seedlings by inoculation of local AMF and/or fertilization and potential plant-AMF preferences.

1.4 Aim of the study

I. We wanted to clarify which AMF would be efficient for reforestation purpose of native, tropical trees in the tropical montane rainforest area of Southern Ecuador.

II. We wanted to learn if specific AMF-plant associations performed better than others under the given nursery conditions and if this would also hold true for field conditions.

III. By using molecular techniques we aimed to identify and characterize native AMF and to trace their persistence in the nursery and under field conditions.

To reach these aims three potential crop tree species native to Ecuador were selected for nursery and planting experiments and inoculated in the early seedling phase in the nursery. The applied inoculum was produced in pot cultures, in a semi-closed system. Through tracing the introduced Ecuadorian AMF over the nursery and the reforestation phase we wanted to identify which AMF are best suited for which of the three native tree species by 454 GS-FLX Titanium sequencing.

2 Materials and Methods

2.1 Sampling sites

The AMF identified and tested for efficiency in this investigation stem from different field plots, from nursery experiments and from trap cultures as described in the following.

2.1.1 Tropical mountain rain forest, abandoned pasture and afforestation site

Field samples were collected in the area of Reserva Biológica San Francisco (RBSF) located between 1800 – 2200 m a.s.l. on the slopes of San Francisco River, Cordillera Real, halfway between Loja and Zamora, South Ecuador (3° 58' S, 79° 4' W). In March, April and September 2006, 29 soil samples containing AMF spores and roots were collected by Arthur Schüßler from beneath individual trees in the montane rainforest, from grass and bracken fern on an abandoned pasture as well as on afforestation sites planted by a 2-year-old *Cedrela montana.* A part of these samples were searched for AMF spores directly after sampling in Ecuador. Soil was suspended in water, decanted, wet sieved through a 250 µm sieve and spores collected by use of a stereomicroscope were stored at 4°C in water. Samples were transferred in cooled packs (approx. 4-8°C) to Germany and used for set-up of AMF cultures.

Additional *Podocarpus oleifolius* (personal communication J. Homeier) root samples were collected in September 2007 sampled beside T2 (pathway 2 in the RBSF) at mark T2-1325 and T2-620 corresponding to altitudes of approximately 2116 and 2233 m. Roots were stored in 70% EtOH, transported to Germany and used for molecular analysis of the AMF contained in the roots and/or nodules (modified lateral roots).

2.1.2 Tree nursery experiments at the Universidad National de Loja, South Ecuador

Different experiments were performed in the Ecuadorian nursery utilizing the fungal inoculum produced by the AMF described herein. A first, preliminary experiment was carried out using tree seedlings of *Inga acreana*, *Tabebuia chrysantha*, *Cedrela montana* and *Heliocarpus americanus* to trap mycorrhizal fungi from forest humus in the nursery. Mycorrhizal roots of these plants were used in the first nursery experiment (No. 1) to inoculate again the *C. montana* and *H. americanus* (Urgiles *et al.* 2009). Table 1 gives information on the tree

species, inoculation and fertilizer applied in the Nursery experiment No. 1 and the code of the different samples as used in this study, modified after Urgiles *et al.* (2009). Results showed significant benefit for seedling growth, but AMF community remained unstudied.

In a second step, mycorrhizal roots were, therefore, sampled from the six months old seedlings of *Cedrela montana* and *Heliocarpus americanus* of Nursery experiment No. 1 by Arthur Schüßler and used to set up individual fungal cultures. These cultures were later used for inoculum production in Germany and in the subsequent nursery experiments No. 2, 3, 4, 4A and 5. Nursery experiment No. 3, No. 4 and No. 4A will be described in the following chapter, whereas No. 2 and 5 will be described by Urgiles *et al.* (2013a, 2013b, both in preparation).

Code	Tree species	Treatment
N1	*Cedrela montana*	Inoculated with mycorrhizal roots from *Cedrela montana*
N2	*Heliocarpus americanus*	Inoculated with mycorrhizal roots from *Heliocarpus americanus*
N3	*Cedrela montana*	Inoculated with a mix of mycorrhizal roots from *Cedrela montana, Heliocarpus americanus, Tabebuia chrysantha* and *Inga acreana*
N4	*Heliocarpus americanus*	Inoculated with a mix of mycorrhizal roots from *Cedrela montana, Heliocarpus americanus, Tabebuia chrysantha* and *Inga acreana*
N5	*Cedrela montana*	Inoculated with a mix of mycorrhizal roots from *Cedrela montana, Heliocarpus americanus, Tabebuia chrysantha* and *Inga acreana* + low fertilization
N6	*Heliocarpus americanus*	Inoculated with a mix of mycorrhizal roots from *Cedrela montana, Heliocarpus americanus, Tabebuia chrysantha* and *Inga acreana* + low fertilization
N7	*Cedrela montana*	Inoculated with mycorrhizal roots of *Cedrela montana* + high fertilization
N8	*Heliocarpus americanus*	Inoculated with mycorrhizal roots of *Heliocarpus americanus* + high fertilization

Table 1: Setting of the Nursery experiment No. 1. The used mycorrhizal root inocula resulted from seedlings of four native tree species grown on a substrate containing humus soil from the tropical mountain rain forest. A slow release Osmocote fertilizer was used in two different fertilization strength, low (0.25 g) and high (0.5 g). Table modified after Urgiles *et al.* (2009).

2.1.3 Nursery experiments performed with AMF inoculum produced in the framework of this study

The subsequent nursery experiments (No. 3, 4, 4A) were performed under nursery standard conditions, if not stated otherwise. The standard nursery substrate consisted of 75% mine sand and 25% black soil mixed together and disinfected by steam, filled into 500 g plastic bags. The nursery substrate was acidic with high amounts of nitrogen, medium P_2O_5, low K_2O, high amount of organic matter and exchangeable bases (Ca^{2+}, Mg^{2+}), K^+ was medium to low (Table 2, for further details see also Appendix Fig. A2). Analysis of the used standard nursery substrate (sand-soil mixture) was performed at UNL, Loja.

			Available elements [µg/ml]			Exchangeable bases [meq/100ml]		
Substrate type	**pH (in water)**	**Organic matter [%]**	**N**	**P_2O_5**	**K_2O**	**Ca^{2+}**	**Mg^{2+}**	**K^+**
steam sterilized	4.38	6.27	78.38	13.15	41.00	4.93	0.90	0.25

Table 2: Physico-chemical analysis of the standard nursery substrate after steam sterilization. pH, organic matter, available elements and exchangeable bases after the steam sterilization are shown.

”Slow release Osmocote” fertilizer (Substral Osmocote Rosen-Dünger, Scotts Celaflor GmbH, Mainz, Germany) was used containing: 15% nitrogen, 8% phosphorus (P_2O_5), 11% potassium (K_2O), 0.9% magnesium, 1.9% sulfur, 0.002% boron, 0.4% iron, 0.005% manganese, 0.018% molybdenum, 0.017% zinc. The fertilizer was mixed with the nursery substrate before seedlings were transplanted. The low strength fertilization comprised 0.25 g fertilizer mixed with 500 g nursery standard substrate per plastic bag. High strength fertilization included 0.5 g fertilizer per plastic bag.

Seeds were collected in Ecuador and provided by the forestry group Project C2.1 of the DFG RU812. Seedlings were raised, inoculated and transplanted in the nursery by Narcisa Urgiles and co-workers.

Measuring tree seedling growth and mycorrhization rate

Growth data of all surviving tree seedlings including height, root collar diameter (RCD), number of leaves and mortality rate was detected 3 and 6 months after inoculation in the nursery at UNL. Out-planted tree seedlings on the reforestation plots were measured and sampled two times (one plant per plot in June and November 2009), representing different seedling ages dependent on tree species due to transplanting time in the nursery (Table 4).

Number of leaves was counted and height and root collar diameter (RCD) were measured, the latter 1 cm above the substrate surface.

Additionally, a destructive sampling of up to 21 tree seedlings per treatment took place in the nursery and on the reforestation plots. Destructively sampled seedlings were analyzed for fresh weight and biomass of root, shoot and leaves, leaf area, mycorrhization rate and nutrient content of leaves and roots. The sampled seedlings were removed from the experiment, washed and scanned to obtain pictures of the leaves, shoots and the root system. The fresh weight of roots, leaves and shoots was measured. A part of the root system was cut into 10 pieces of 0.5 cm (each in 3 replicates), stored in vials with 80% EtOH and transported to Germany for later DNA extraction. Another part of the roots was used for staining (15 root segments of 2 cm length). To get the dry weight (biomass), root, shoot and leaves were dried in an oven at 60ºC over 24 hours (nursery samples) or 120°C over 10 days (reforestation samples) and weighed afterwards. Leaf area was analyzed via Scion Image software or the Fiji image processing package (including ImageJ).

Nutrient analysis of roots and leaves was performed after drying 24 hours at 103°C in an oven. Up to six seedling roots or leaves per treatment were mixed, pestled to fine powder, sampled into glass vessels and processed at the Technical University of Munich, Institute of Silviculture. The seedling roots and leaves were analyzed for the following nutrients: K, Ca, Mg, Al, Cu, Fe, Mn, Na, P, Zn, B, S, N%, S%, H% and C%. Due to a changed analysis method at the TU Munich, the analysis of S% and H% was excluded in the last processed samples.

Mycorrhization rates in percentage (Trouvelot *et al.* 1986) were estimated after hot (Kormanik & McGraw 1982) or cold staining (Grace & Stribley 1991) with methyl blue. In some cases, especially for the seedlings from the reforestation plots, an additional clearing of the roots by 1% hydrogen peroxide solution for 5 min at 60°C was necessary.

The recording of the raw data in the nursery was carried out by Narcisa Urgiles with help of Paul Lojan, the author and co-workers. Additionally, in September 2009, Arthur Schüßler and Manuela Krüger helped with scanning of the plants and cutting of roots. All data collected on the reforestation plots and complete analysis of the nursery experiment data, described herein was performed by the author.

2.1.4 Nursery experiment No. 3

Experimental design

The experimental design of Nursery experiment No. 3, as shown in Table 3, consisted of five treatments applied to *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha*.

Inoculum was harvested from the cultures Att1449-5, -10, -12, Att1450-1, Att1451-6, -8, Att1452-6 and Att1456-1, -7, -11 and applied as a mixture (Table 7). Inactivation of the AMF inoculum was done by steam sterilization (heat-killed). Inoculation of the tree seedlings was done in different strength, due to different growth periods of the AMF cultures. Seedlings of *C. montana* received 15 g of a weaker AMF inoculum, due to a limitated growth period and harvest of the cultures after 22 days. Seedlings of *H. americanus* and *T. chrysantha* received 8.5 g AMF inoculum from cultures grown for 70 and 92 days. The inoculum was mixed with the nursery substrate per bag. Conditions for inoculum production are given in chapter 2.2.1.

Treatment number	Code	Description
T0	Control	Control treatment, no AMF inoculum added
T1	HF	High fertilization, no AMF inoculum added
T2	-AMF + LF	Low fertilization, addition of heat-killed AMF inoculum
T3	+AMF + LF	Low fertilization, addition of AMF inoculum
T4	+AMF	No fertilization, addition of AMF inoculum mixture

Table 3: Treatments of Nursery experiment No. 3. The used inoculum resulted from the fungal cultures characterized in this study. Three native tree species namely *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* were treated with a long term fertilizer in two different fertilization strength, low (0.25 g) and high (0.5 g), and/or inoculation. AMF inoculum was a mixture of the cultures Att1449-5, -10, -12, Att1450-1, Att1451-6, -8, Att1452-6 and Att1456-1, -7, -11, in equal amounts. Sterilized standard nursery substrate (sand-soil mixture) was used in all treatments.

The experimental design in the nursery experiment was conducted as randomized complete blocks. Three blocks served as three irrigation lines in the greenhouse. Each block was assigned as one complete replicate, comprising the same amount of tree seedlings but ordered randomly (White 1984). Each block consisted of 7 replicates including 10 plants per treatment in every replicate per tree species (Fig. 2, Table 3). In total 3150 seedlings of *C. montana*, *H. americanus* and *T. chrysantha* were used for this experiment (3 blocks × 7 replicates × 5 treatments × 10 plants = 1050 seedlings per tree species). This allowed sampling and monitoring of sufficient tree seedlings and mycorrhizas in the nursery. A part of the *H. americanus* seedlings (126 plants per treatment) was already transferred for hardening to the research station before reaching the age of 6 months in the nursery, due to the fixed time schedule of the forestry group. Thus, only a reduced set of *H. americanus*

seedlings (63 plants per treatment) remained in the nursery for the 6 months-sampling and measurement.

Because of a modification in the water regime (change from automatic to manual watering, due to uneven watering through plugged sprinklers) after the first nursery sampling, the humidity in the nursery decreased. The low humidity caused a mite attack of the tree seedlings, therefore it cannot be excluded that the seedlings somehow reacted to this, affecting the statistical analyses.

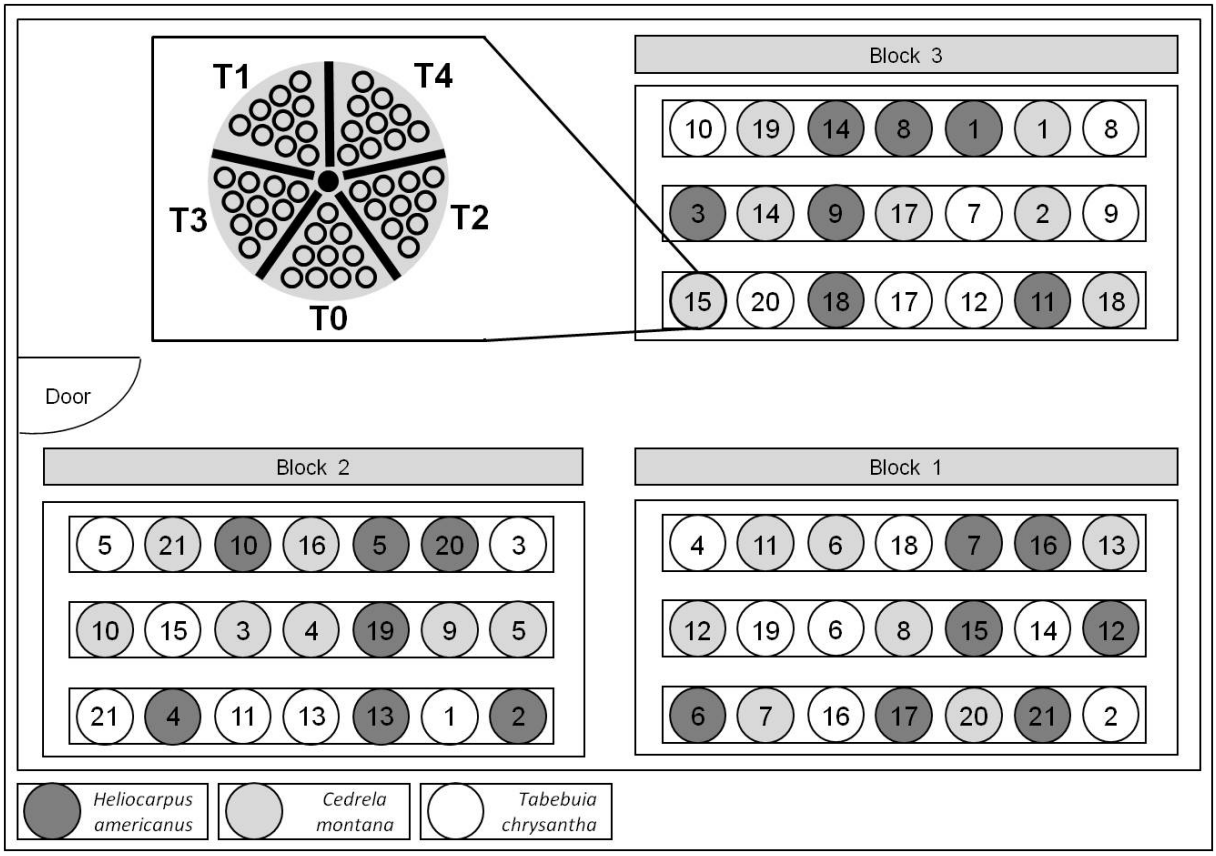


Fig. 2: Set-up of the Nursery experiment No.3 in the greenhouse of the UNL nursery, Loja, Ecuador. Each replicate consists of five treatments with 10 plants, randomly distributed and divided by plastic borders. The circle in the upper left corner exemplifies one replicate. Treatments are **T0:** control, **T1:** high fertilization, **T2:** low fertilization + heat-killed AMF inoculum, **T3:** low fertilization + AMF inoculum, **T4:** AMF inoculum-only; placed under a sprinkler head and divided by plastically borders. The fungal cultures Att1449-5, -10, -12, Att1450-1, Att1451-6, -8, Att1452-6, Att1456-1, -7, -11 were used in equal amounts as inoculum mix (AMF cocktail).

Afforestation at RBSF

After a nursery growth phase of up to 12 months the plantlets were transported by the forestry group to the ECSF for hardening in a small greenhouse. Two to three months later the tree seedlings were planted on 120 reforestation plots (map shown in Appendix Fig. A1)

established on abandoned pastures close to the ECSF under the directive of Ximena Palomeque (Palomeque 2012). In June 2009 and November 2009 measurements of plant growth were carried out on the reforestation plots. In total 6 plants per treatment were sampled at two sampling points. In June one plantlet was removed for analysis and in November a neighboring plant was sampled. Biomass was only measured for leaves and shoot. Seedling roots were stored in EtOH and transported to Germany, therefore solely nutrient analysis of leaves was done.

Two samplings during the nursery phase and two on the reforestation plots were carried out. After 3 and 6 months sampling of the tree seedlings in the nursery took place. On the reforestation site the samplings of one plant per plot was done in June and November 2009. Details are given in Table 4.

		Tree species					
		Cedrela montana		***Heliocarpus americanus***		***Tabebuia chrysantha***	
Sampling number	**Place**	**Sampling date**	**Age of seedlings**	**Sampling date**	**Age of seedlings**	**Sampling date**	**Age of seedlings**
Trans-planting	Nursery, UNL	Mar. 2008	0 mo.	May 2008	0 mo.	Jun. 2008	0 mo.
1		Jun. 2008	3 mo.	Aug. 2008	3 mo.	Sept. 2008	3 mo.
2		Sept. 2008	6 mo.	Nov. 2008	6 mo.	Nov.- Dec. 2008	6 mo.
Out-planting	Reforestation plots, RBSF	Mar. 2009	12 mo.	Dec. 2008	7 mo.	Jan. 2009	7 mo.
3		Jun. 2009	15 mo.	Jun. 2009	13 mo.	Jun. 2009	12 mo.
4		Nov. 2009	18 mo.	Nov. 2009	16 mo.	Nov. 2009	15 mo.

Table 4: Sampling dates and age of seedlings (in months) during Nursery experiment No. 3.

2.1.5 Nursery experiment No. 4 and No. 4A

This experiment was conducted to investigate possible plant-AMF preferences and was carried out by use of seven individual AMF inocula. The standard substrate used in this experiment was the same as in the Nursery experiment No. 3, except that the black soil was sterilized twice and only low fertilization was used. AMF cultures used are given in Table 7. No statistical analysis was carried out due to low numbers of tree seedlings.

Nursery experiment No. 4

In a first part, all the three native tree species *Cedrela montan*a, *Heliocarpus americanus* and *Tabebuia chrysantha* were used. Individual inocula were harvested from the cultures Att1449-5, -10, -12, Att1450-1, Att1451-8, -18 (sister culture to Att1451-6), Att1455-2 and

Att1456-7 (Table 6). Nursery experiment No. 4 consisted of two treatments with low fertilization strength (0.25 g) as shown in Table 5. Each treatment consisted of 5 plants for *C. montana*, 7 plants for *H. americanus* and 8 plants for *T. chrysantha*, placed in two blocks (Fig. 3). Tree seedlings were inoculated with 3.4 g of the individual AMF per plant. The different inocula were applied by point inoculation, into a planting hole with the seedling transplanted directly on the inocula. After 3 and 6 months, the height, RCD and the number of leaves from each seedling were taken. Destructive sampling was done after 6 months of 5 plants per inoculum and treatment for scanning and measurement of fresh weight, biomass and mycorrhization rates.

Treatment number	Code	Description
T1	+AMF	Low fertilization, addition of AMF inoculum
T2	-AMF	Low fertilization, addition of heat-killed AMF inoculum

Table 5: Treatments of Nursery experiment No. 4. The used inoculum resulted from the fungal cultures Att1449-5, -10, -12, Att1450-1, Att1451-8, -18 (sister culture to Att1451-6), Att1455-2 and Att1456-7 applied in equal amounts. Three native tree species namely *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* were treated with low fertilization strength (0.25 g) and inoculation. Double sterilized standard nursery substrate (sand-soil mixture) was used in all treatments.

Nursery experiment No. 4A

This experiment was only done with *Cedrela montana*. Individual inocula resulted from the fungal cultures characterized in this study and were harvested from the cultures Att1449-5, -10, -12, Att1450-1, Att1451-8, Att1455-2 and Att1456-7 (Table 7). Nursery experiment No. 4A consisted of four treatments, shown in Table 5. The experimental design consisted of 63 plants in total. Each of the four treatments included 9 plants arranged in 2 replicates (Fig. 3). Tree seedlings were inoculated with 6 g of the individual AMF inocula per treatment. Measurements were carried out as above.

Treatment number	Code	Description
T1	-AMF	Heat-killed AMF inoculum, no fertilization
T2	+AMF	AMF inoculum, no fertilization
T3	-AMF + LF	Low fertilization, addition of heat-killed AMF inoculum
T4	+AMF + LF	Low fertilization, addition of AMF inoculum

Table 6: Treatments of Nursery experiment No. 4A. The used inoculum resulted from the fungal cultures Att1449-5, -10, -12, Att1450-1, Att1451-8, Att1455-2 and Att1456-7 applied in equal amounts, but individually. One native tree species *Cedrela montana* was treated with no or low fertilization strength (0.25 g) and inoculation. Double sterilized standard nursery substrate (sand-soil mixture) was used in all treatments.

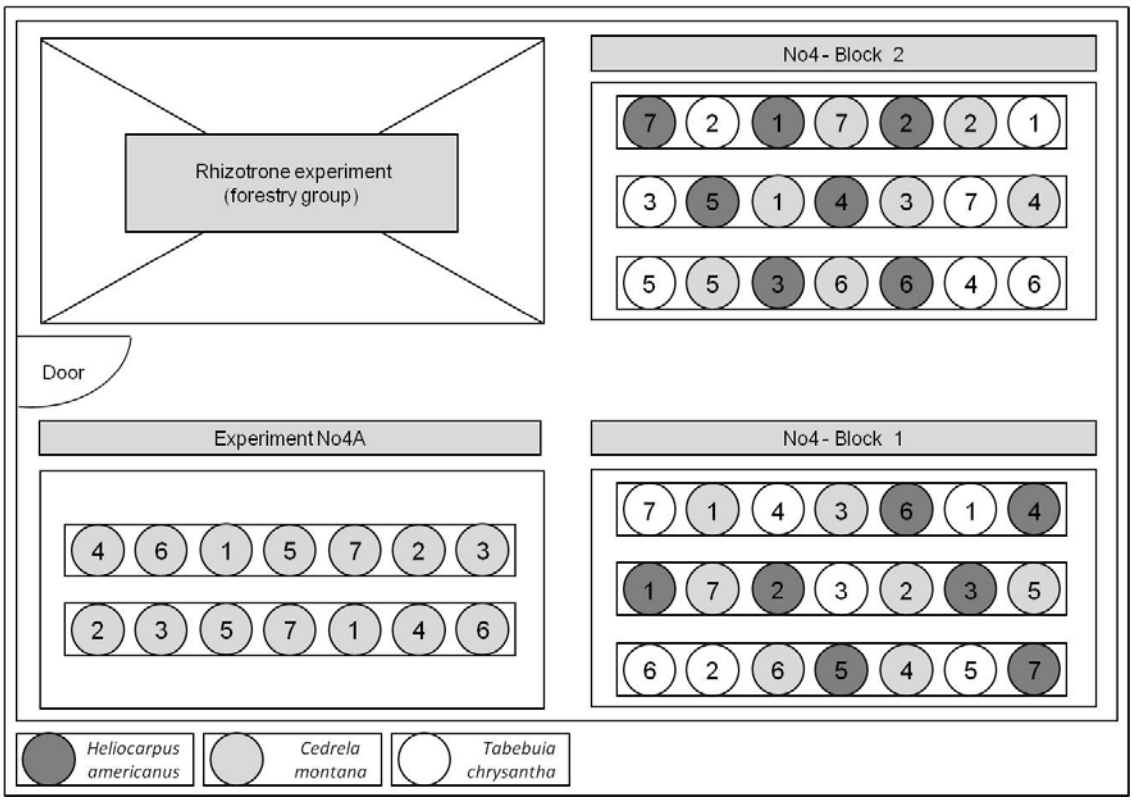


Fig. 3: Set-up of Nursery experiment No. 4 and No. 4A in the Ecuadorian nursery. Nursery experiment No. 4 on the right side is arranged in 2 blocks, No. 4A on the left side on one bench only.

Recording of the raw data was done by Narcisa Urgiles and co-workers. Analysis of the data was carried out by the author.

2.1.6 Statistical analysis

A one-way ANOVA was carried out with all data sets except for Nursery experiment No. 4 and No. 4A, due to low seedling numbers. The different treatments were tested for significance in growth via the Tukey's honestly significant difference (HSD) test ($P<0.05$). Additional test of significance via the Fisher's least significant difference (LSD) test ($P<0.05$) were done to reveal potential tendencies in the growth data. An additional two-way ANOVA was calculated testing the dependencies of the growth parameters on AMF and fertilizer on different significance levels ($P<0.1$, 0.05, 0.01 and 0.001). Statistical analysis was carried out by use of SPSS 18 and StatGraphics Plus v3.1 software.

2.2 Setting up fungal cultures

Trap culturing of the sampled roots and spores was established in a growth chamber of the Institute of Botany, Technical University Darmstadt. Trap cultures were established either in pots (Ø 8 cm) filled with sterile sand placed in sunbags (Sigma-Aldrich, USA) or small plastic greenhouses. Inoculum was applied as root fragments or spores into a hole in the middle of the pot when inserting a host plant. *Plantago lanceolata* raised from seeds, pre-treated with 0.7 M NaOCl solution under sterile conditions, were used as host plants. 35 trap cultures were prepared from the different nursery root samples of Nursery experiment 1, N1-N8 (Table 1). Three to five pots per sample were prepared. Additional four trap cultures originating from an afforestation site of *Cedrela montana* were established (E35-1, -2, -3, and E34/E36/E47). Three subculture of sample E35 were made stemming from the >250 µm sieving fraction, whereas three spore samples originating from the <250 µm sieving fraction (E34, E36 & E37; see Table 7) were mixed and cultured as one single trap culture in an individual sunbag. These AMF trap cultures were set up by Arthur Schüßler in April 2006.

The pots were watered with deionized water (pH 6) and checked for AMF colonization and spore formation in June 2007.

Single spore, multi spore or root fragment cultures

Culturing of the individual *Glomeromycota* isolates was carried out in Germany at the greenhouse of the Genetics, Department Biology I, Ludwig-Maximilians-University Munich, from 2007 to 2010. A first survey of AMF spores was obtained from trap cultures together with Christopher Walker. Detailed information is given in Table 7. The numbering and identifiers of the cultures are named according to the database of Christopher Walker. Every culture obtained an Att-number (Att = attempt) linked with the according vouchers, spore characters and other information. Successful trap cultures were renamed as Att-numbers

(1449-0 to 1456-0, see Table 7). Descendants are labeled with the according Att- and sub-number.

After setting up all sub-cultures, host plants in parent cultures in poor conditions were repotted. Therefore *Plantago lanceolata* seedlings of the cultures Att1455-0 and Att1456-0 were carefully dug out from the old pot, the roots of the trap plant were washed to prevent spreading of contaminations and the plant was placed in a new pot with sterilized substrate closed within a sunbag (see also Table 7). In total 67 cultures were set up either as single spore, as multispore or root fragment culture. Multispore cultures were established with a maximum of 80 spores per culture. Root fragment cultures were established when spores could only be observed inside the roots (intraradical spores). The inoculum (spore(s) or root fragment) was placed directly on the roots of *P. lanceolata* seedlings. The seedlings were raised in Ø 10 cm pots filled with autoclaved mixture of 4:1(v/v) sand (gravel sand, 0-4 mm washed, Kieswerk Klardorf GmbH & Co Produktions KG, Schwandorf, Germany) to Oil Dri (US-Special, TypIII R, EugenTrost GmbH & Co KG Puchheim, Germany). Cultures were placed in sunbags, in a plant growth chamber with approximately 23°C day and 18°C night temperature under a 14 and 10 hours light regime. After 14 months plants were checked for AMF colonization and spore production. Cultures with abundant spores were chosen as starter for inoculum production (see Table 7).

Table 7: Culture attempts, origin and usage in the nursery experiments. Rejuvenated parent cultures are crossed out and the subsequent culture is shown in brackets. Numbers of spores used for set-up of the multispore cultures are written in brackets in the according column. AMF species names are temporary, respecting morphological and molecular characterization of the fungal cultures.

	Culture number		Culture numbers			Application in Nursery experiment				
Origin	**Pri-mary**	**Parent culture**	**Single spore**	**Multispore**	**Root fragment**	**No. 3**	**No. 4**	**No. 4A**	**AMF species**	**Genus**
Spores from rhizosphere sample of *C. montana* reforestation plot, >250µm	E35-3	~~Att1455-0~~ (=1455-1)	Att1455-2 to 1455-6				Att1455-2	Att1455-2	*De. savannicola*	*Dentiscutata*
Spores from rhizosphere sample of *C. montana* reforestation plot, <250µm	E34/ E36/ E37	Att1450-0	Att1450-1 to 1450-5	Att1450-6 (50 spores)		Att1450-1	Att1450-1	Att1450-1	*Acaulospora* sp. nov.	*Acaulospora*
Roots of *C. montana* from Nursery experiment No. 1 (sample N5)	E43-4	Att1451-0	Att1451-1 to 1451-5	Att1451-6 (12 spores)	Att1451-7 to 1451-11	Att1451-6 Att1451-8	Att1451-18 Att1451-8	Att1451-8	*Cl. etunicatum*-like *Rhizophagus* sp.	*Claroideoglomus* *Rhizophagus*
Roots of *H. americanus* from Nursery experiment No. 1 (sample N2)	E45-2	~~Att1456-0~~ (=1456-12)	Att1456-6 to 1456-16		Att1456-1 to 1456-5	Att1456-1 Att1456-7 Att1456-11	Att1456-7	Att1456-7	*Rhizophagus* sp. *Ar. trappei*-like *Cl. etunicatum*-like	*Rhizophagus* *Archaeospora* *Claroideoglomus*
Roots of *H. americanus* from Nursery experiment No. 1 (sample N4)	E46-3	Att1449-0	Att1449-1 to 1449-10, Att1449-12 to 1449-16	Att1449-11 (50 spores)	Att1449-17 to 1449-21	Att1449-5 Att1449-10 Att1449-12	Att1449-5 Att1449-10 Att1449-12	Att1449-5 Att1449-10 Att1449-12	*Diversispora* sp. *Cl. etunicatum*-like *Ambispora* sp.	*Diversispora* *Claroideoglomus* *Ambispora*
Roots of *H. americanus* from Nursery experiment No. 1 (sample N6)	E47-3	Att1452-0	Att1452-1 to 1452-5	Att1452-6 (80 spores)		Att1452-6			*Ar. trappei*-like	*Archaeospora*

To produce sufficient inoculum, cultures were transferred into larger pots with a diameter of 18 cm filled with autoclaved substrate consisting of 4 parts sand (DORSOLIT 0.60-1.20 mm No.7 silica sand fire dried, BayWa AG Dachau) and 1 part Oil Dri. The following cultures were selected for inoculum production: Att1449-5, Att1449-10, Att1449-12, Att1450-1, Att1451-6, Att1451-8, Att1452-6, Att1456-1, Att1456-7, Att1456-11 and Att1455-2. Table 6 shows the successful established culture and their usage in the nursery experiments. Duplicates of these cultures were established in February 2008 and April 2010 by dividing the cultures, transferring half of the substrate and half of the host plants into new pots (Ø 18 cm), and placing them into sunbags.

2.2.1 Inoculum production

The AMF cultures used for inoculum production in the nursery experiments were grown in the greenhouse of the LMU in Germany. Pot cultures of *Plantago lanceolata* plants colonized with individual AMF species of the cultures Att1449-5 (*Diversispora* sp.), Att1449-10 (*Cl. etunicatum*-like), Att1449-12 (*Ambispora* sp.), Att1450-1 (*Acaulospora* sp. nov.), Att1451-6 (*Cl. etunicatum*-like), Att1451-8 (*Rhizophagus* sp.), Att1452-6 (*Ar. trappei*-like), Att1455-2 (*De. savannicola*), Att1456-1 (*Rhizophagus* sp.), Att1456-7 (*Ar. trappei*-like) and Att1456-11 (*Cl. etunicatum*-like) were harvested for inoculum production. AMF species names are temporary, respecting the current morphological and molecular characterization of the fungal cultures shown in this study. The *Plantago lanceolata* plants were carefully removed from the pots. Half of the substrate was transferred to a sterile container. About two thirds of the total root systems of the plants were sampled and transferred to the container. The pots, which still contained half of the substrate, were then filled up with autoclaved substrate. The *P. lanceolata* plants which still had one third of rootlets left were replanted into prepared pots and placed in a sunbag for maintenance of the inoculum source. The sampled roots were cut in small pieces and mixed with the substrate. The mixture was covered by a mesh and dried for 1-2 days at room temperature positioned on the clean bench or on a clean working bench. This inoculum, consisting of growth substrate, spores, hyphae and roots of the host plants was used for the inoculation in the nursery at UNL, Loja. More than 40 kg of inoculum were harvested at 27.02.2008, 15.04.2008, 04.05.2009, 03.06.2009 and transported to Ecuador. Additional 19 kg of inoculum was also harvested for nursery experiment No. 2 and 5 performed in the Ecuadorian nursery (UNL, Loja) which will be described in Urgiles *et al.* (2013a, 2013b, both in preparation).

2.3 Inoculum efficiency test

UNL nursery, Loja, Ecuador

Test-cultures of *Plantago lanceolata* and mixed inoculum were established in Oct. 2007 to check the effectivity of the applied AMF inoculum mixture in the Ecuadorian nursery, UNL; Loja. The mixed inoculum consisted of substrate (sand-Oil Dri) containing AMF spores, hyphae and mycorrhizal roots of *Plantago lanceolata* of the following cultures: Att1449-5 (*Diversispora* sp.), Att1449-10 (*Cl. etunicatum*-like), Att1449-12 (*Ambispora* sp.), Att1450-1 (*Acaulospora* sp. nov.), Att1451-6 (*Cl. etunicatum*-like), Att1451-8 (*Rhizophagus* sp.), Att1452-6 (*Ar. trappei*-like), Att1456-1 (*Rhizophagus* sp.), Att1456-7 (*Ar. trappei*-like) and Att1456-11 (*Cl. etunicatum*-like). 12 cm pots were filled with standard nursery substrate and three *P. lanceolata* plants inserted. A triangular hole was dug and the inoculum was placed in the hole. Four different amounts of the AMF inoculum mixture were applied (0.5, 1, 2 and 4 g per pot). Each variant was replicated three times. Three replicates of the same inoculum concentration were placed together in a sunbag. The test-cultures were harvested after 8, 10 and 12 weeks and analyzed in the framework of Nursery experiment No. 3. Mycorrhization rates of 5 root pieces, 2 cm each, were estimated in classes after Trouvelot *et al.* (1986).

Set up of the cultures was carried out by the author with help of Narcisa Urgiles and Paul Lojan. Mycorrhization rates were estimated by Narcisa Urgiles and Paul Lojan.

LMU greenhouse, Munich, Germany

Additionally test-cultures of the Ecuadorian AMF were established at LMU using *Plantago lanceolata* as host and the inocula Att1449-5 (*Diversispora* sp.), Att1449-10 (*Cl. etunicatum*-like), Att1449-12 (*Ambispora* sp.), Att1450-1 (*Acaulospora* sp. nov.), Att1451-8 (*Rhizophagus* sp.), Att1455-2 (*De. savannicola*) and Att1456-7 (*Ar. trappei*-like). In an 8 cm pot, filled with substrate consisting of 3 parts sand (DORSOLIT) and 1 part Oil dry (US special, Typ III R), three *P. lanceolata* plants were placed on the sides of a triangular hole. 6 g inoculum per culture was given into the hole. All three replicates of one inoculum were place together into one sunbag. The three replicates were named A, B and C. The ‘test-cultures’ were harvested after 3 and 6 months and analyzed in the framework of Nursery experiment No. 4. Mycorrhization rates of 15 root pieces each 0.5 cm was taken after Trouvelot *et al.* (1986).

2.4 Identification of AMF

The successfully established AMF cultures were characterized by their fungal structures and molecular analysis of a part of the rRNA gene.

2.4.1 Morphological examination of spores

Substrate and roots of the prepared single spore, multispore and root fragment cultures were sampled and checked for spore occurrence and mycorrhization. Root samples were stained with methyl blue either by hot (Kormanik & McGraw 1982, Brundrett *et al.* 1984) or cold staining (Grace & Stribley 1991, Koske & Gemma 1989, Walker & Vestberg 1994) to observe mycorrhization. Substrate was suspended in water, swirled, decanted and wet-sieved (32 µm mesh; Retsch) and spores decanted into small petri dishes. An initial examination was carried out by use of a dissecting microscope. Spores were collected individually and embedded in PVLG (Koske & Tessier 1983) with and without Melzer's reagent (Brundrett *et al.* 1994) and examined by use of a compound microscope at magnifications up to 1250 fold. Remaining spores were collected in 200 µl PCR tubes and stored at -20°C until DNA extraction.

The morphological characterization was done in collaboration with Christopher Walker. Spore color of fresh material was examined in water under a dissecting microscope (Olympus D2 SZH) by application of reflected light at a color temperature of 3100 K (cold light source Schott KL1500, Schott AG, Germany) and magnifications up to 50 fold. Spore color was determined using color charts from the Royal Botanic Garden Edinburgh (RBG, Anon 1969) or Munsell (Anon 1990) by comparing color simultaneously at the same light conditions. Spore size, length and breadth were measured for up to 142 spores (per AMF culture) either with a calibrated eyepiece or with the LAS AF software (Leica) and an inverted Leica DMI6000B microscope. Spore sizes are given in mean values including minima and maxima values in brackets. To optimize visibility of the wall components of crushed spores the cover slip was slightly turned clockwise, to reveal the inner walls by moving them outside the spore. In some cases the wall structure was documented as muronyms giving the types of the wall components by the method of Schenck & Perez (1990). Slides containing stained roots and/or spores were partly stored as vouchers in the collection of Christopher Walker and registered in the according database with a voucher number (W-number, see Appendix Table A2). The remaining slides were stored in the laboratory of the Ludwig-Maximilians-University Munich, Department Biology I, Genetics. Spore measurements and photographs were taken by the author, Christopher Walker and Arthur Schüßler.

2.4.2 Molecular characterization of the fungi from cultures, nursery and reforestation sites

DNA from the AMF established as culture was extracted from single spores or mycorrhizal roots when no extraradical spores were observed. DNA was also extracted from root samples of the tree seedlings stemming from the Nursery experiment No. 1 and of *Podocarpus oleifolius* roots sampled in the forest of the RBSF (see Appendix Table A3).

DNA was extracted from up to 5 cleaned single spores crushed by a filter tip in a 200 µl vial using a minimum of water. DNA from spores was obtained either via magnetic beads treatment (Dynabead DNA DIRECT Universal Kit, Invitrogen, Karlsruhe, Germany; as described in Schwarzott & Schüßler 2001) or by adding of 10 µl 5x PCR buffer (GoTaq buffer, Promega, Germany) and incubation at 95°C for 15 minutes (Naumann *et al.* 2010). 2 µl DNA extract was used in the subsequent PCR reaction, the remaining DNA extracts was stored at -20°C for later usage.

Roots were cut in 10 root pieces of 0.5 cm length and stored in 80% EtOH at -20°C. Before DNA extraction, roots were washed in absolute EtOH and dried 1h at 60°C, in a sunbag, to prevent contamination. After the drying step roots were soaked in 100 µl molecular biological grade water (Applichem, Germany), transferred to a 2 ml vial. A tungsten carbide bead (Ø 3 mm, Qiagen) was placed in each tube and tubes were frozen in liquid nitrogen immediately. The roots were disrupted using the Tissue Lyser (Retsch bead mill MM300, Qiagen) with precooled Teflon adaptors (in liquid nitrogen), two times for 3 minutes at 30 Hz until the roots were completely ground to fine powder. DNA from roots was extracted using either the DNeasy Plant Mini Kit (Qiagen) following manufacturer's instructions or a CTAB (cetyltrimethyl-ammoniumbromide) based protocol modified after Allen *et al.* (2006), as described in Krüger *et al.* 2009. DNA was stored at 4°C for direct use, or at -20°C for long term storage.

The molecular characterization used the nuclear rDNA region as molecular marker including the whole SSU (≤1800 bp), the internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) and a part (500-800 bp) of the LSU rDNA. Generally, PCR amplification of the SSU rRNA gene (ca. 1800 bp) was carried out according to Schwarzott & Schüßler (2001), using the primers GeoA1/ART4 in the first PCR and GeoA2/Geo11b (5' ACT TTT ACT TCC TCT AAA YGA CC 3') in the second PCR. The SSU_{part}-ITS-LSU_{part} rDNA fragment was amplified using the AMF specific primers SSUmAf/LSUmAr in the first PCR (ca. 1.800 bp) and SSUmCf/LSUmBr in the nested PCR (ca. 1.500 bp; Krüger *et al.* 2009). The primers SSUGlom1 (Renker *et al.* 2003), NDL22 (van Tuinen *et al.* 1998), LR4+2bp (Stockinger et al. 2009), AML1 and AML2 (Lee *et. al* 2008), SSU128 (Haug *et al.* 2010) or ITS1Frc (reverse complementary of ITS1F: Gardes & Bruns 1993) were additionally used for some of the samples.

Cloning was carried out either with the TOPO-TA or the TOPO blunt cloning kit (Invitrogen, Germany) after the manufacturer's instructions, but reduced amount of chemicals. Components for the ligation reaction where reduced to ⅓ and the competent cells were divided and 25 µl instead of 50 µl were used for transformation. Up to 48 clones per PCR product were checked via colony PCR with the GoTaq DNA Polymerase and the 5× Green GoTaq reaction buffer (Promega, USA) according to the manufacturer's instructions using

primers M13R-24mod (5' CCA GGG TTT TCC CAG TCA CGA CG 3') and M13F-24mod (5' TGA GCG GAT AAC AAT TTC ACA CAG G 3'). Cycling conditions were as follows: 5 min initial denaturation at 95°C, 40 cycles of 30 s denaturation at 95°C, 30 s annealing at 65°C and 1 min elongation at 72°C, followed by a final 10 min elongation at 72°C.

After the cloning and the colony PCR, the products which showed according insert length on a 1% agarose gel (in 1xSB or 1xTE), were checked by RFLP with the restriction enzymes MboI, RsaI and HinfI (all New England BioLabs Inc.). Different patterns of the inserts were analyzed and up to 3 clones per pattern were sequenced to cover a large part of the intraspecific sequence variability (Krüger *et al.* 2009).

The selected clones were cultured in 5 ml LB medium over night at 37°C and 300 rpm. The following plasmid preparation was either done with the NucleoSpin Plasmid kit or the NucleoSpin Multi-8 Plasmid kit (both Macherey & Nagel, Düren, Germany), using a vacuum manifold for higher throughput. DNA quantification of the plasmids was done with the Eppendorf Photometer (Eppendorf AG, Hamburg, Germany) or the NanoDrop 1000 (ThermoScientific Inc.) Sequencing was done in 7 µl total volume containing the sequencing primer and the correctly concentrated DNA (ca. 1000 ng plasmid) in the Sequencing Service Unit of the LMU Munich by Cycle, Clean and Run with BigDye v3.1 on an ABI 3730 capillary sequencer.

Molecular methods are the same as published in Krüger *et al.* (2009, 2012) and Stockinger *et al.* (2009, 2010).

Phylogenetic analysis

Sequences were quality checked by use of SeqAssem and aligned by hand to a Master alignment by Align (www.sequentix.de). The sequences of the Ecuadorian samples were aligned with a selection of representative species and the according reference sequences. The subset of >4300 sequences in the database was assembled during the last 15 years in the research group of Arthur Schüßler and is in large parts published in Krüger *et al.* (2012). This set of sequences served also as backbone dataset (as reference alignment) for the analysis of the 454 sequence reads (see chapter 2.4.3)

SSU consensus sequences were made using the strict consensus rule. Variable sites were coded by the according IUPAC nucleotide code. The consensus SSU sequences were concatenated with the individual SSU-ITS-LSU fragments obtained from one culture excluding identical sequences. This resulted in an approximately 3 kb long rDNA fragment spanning the whole SSU, ITS and a part of the LSU rDNA region, which provides robust phylogeny and species-level resolution for glomeromycotan fungi (Krüger *et al.* 2012). In total 292 individual sequences of the Ecuadorian AMF cultures were concatenated to 84 approx.

3 kb long fragments. These concatenated sequences were annotated with the accession number of the respective SSU-ITS-LSU fragment and a "_R" standing for "reference" ("R-sequences"). Further 155 sequences originating either from environmental samples (*Podocarpus oleifolius*) or roots from nursery plants (N1-N8) were reduced to 19 individual sequences, excluding identical sequences. Up to now the following sequences were deposited in the EMBL database under the accession numbers HE962432-77 (*De. savannicola* Att1455-2, confidential until 06. Dec. 2014).

Maximum likelihood trees were calculated with RAxML ver. 7.2.8 (Stamatakis *et al.* 2008) through the CIPRES Science Gateway (Miller *et al.* 2010, www.phylo.org/portal2) using 1000 bootstraps and the GTRGAMMA model for bootstrapping and tree inference. The annotationsa in phylogenetic trees were batch replaced using Align, visualized and exported from FigTree v1.2.1 into Microsoft Office PowerPoint 2007, where trees were edited manually and branches with BS below 60% were reduced to polytomies.

2.4.3 454 GS FLX Titanium amplicon sequencing

Sample preparation

The DNA-root-extracts from the Ecuadorian tree seedlings (see CTAB-DNA extraction) were used for analysis via 454 sequencing. A new forward primer LSUD2mod was designed for this method. The primer binds at the beginning of the D2 domain of the LSU rRNA gene and was designed to discriminate against plants and other non-AMF organisms. Due to the restricted length of approximately 400-450 bp useable primer binding sites were limited, therefore a degenerated primer was designed appropriate to use in nested PCR with prior amplification of the AMF specific primers SSUmAf/LSUmAr. The sequence of LSUD2mod is as follows 5' GTG AAA TTG TTR AWA R 3'.

Polymerase chain reactions

Three independent PCRs per sample were performed to decrease potential PCR bias.

1. PCR (total volume 15 µl)	7.5 µl	2x Phusion HF Master Mix (Finnzyme)
	0.75 µl	SSUmA (10 pmol)
	0.75 µl	LSUmA (10 pmol)
	0.075 µl	BSA (10 mg/ml; NEB)
	4.425 µl	Mol. bio. water (Roth)
	+ 1.5 µl	DNA template (1:10 diluted)

The PCR program was performed on a Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with a 5 min initial denaturation at 99°C; 20 cycles of: 10 s denaturation

at 99°C, 30 s annealing at 60°C and 1 min elongation at 72°C; followed by a final 10 min elongation at 72°C.

After the first PCR was finished, the nested PCR was done right after so that the tubes with the 1.PCR product are only opened under a separate nested PCR bench. In the second PCR 25 Multiplex Identifiers (MID) were used to run up to 25 samples in one 1/8 run of the GS FLX sequencer. MID sequences were provided in the Roche manual "TCB No. 005-2009 – Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry – Extended MID Set" (see chapter 8.2.2). MIDs which showed an identical base at the 3'-end of the used AMF primers were excluded (see Appendix Table A2). Also the LSUmB (Krüger *et al.* 2009) and the LSUD2mod primer had to be adapted to the 454 sequencing each with a calibration part (four bases - TCAG) and the so-called primer A or B from Roche, which bind to the beads (for further information see Appendix Table A1 and chapter 8.2.2).

Second PCR (total volume 25 µl)	12.5 µl	2x Phusion HF Master Mix (Finnzyme)
	1.25 µl	454-LSUmB (10 pmol)
	0.125 µl	BSA (10 mg/ml; NEB)
	9.625 µl	Mol. bio. water (Roth)
	+ 1.25 µl	454-LSUD2mod-MID1-36 (10 pmol)
	+ 0.25 µl	DNA template (from 1. PCR)

The PCR program was performed on a Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with a 5 min initial denaturation at 99°C; 20 cycles of: 15 s denaturation at 99°C, 30 s annealing at 65°C and 20 s elongation at 72°C; followed by a final 10 min elongation at 72°C.

PCR product analysis, clean-up and measuring

To check if the amplification of each sample was successful 5 µl of the nested PCR product were loaded onto a 1% agarose gel (either in 1xSB or 1xTE). If the band of the amplicon was weak or not visible on the gel, additional 10 nested PCR cycles for these products were done. When all products were successfully amplified the 3 replicates of the nested PCR were pooled together into a new PCR plate (or tubes).

A subsequent PCR clean-up was performed according to manufacturer's instructions with the Nucleo Spin Exctract II Kit (Machery-Nagel). The measuring of the DNA concentration was done with the Quant-iT PicoGreen kit (Invitrogen) at the Helmholtz Zentrum München, Environmental Genomics, following the instructions of the Roche manual for Library Quantitation (see chapter 8.2.1). For photometrical analysis a Spectra MAX Gemini EM-Photometer was used, where samples in 96 well plates can be measured at once. The software used was SoftMaxPro 4.6 and fluorescence top reads were set as follows,

extinction: 480 nm and emission: 520 nm with 6 reads per well. The output file was a standard text file which was further processed with Excel.

Dilution and pooling of DNA

The measured photometrical data were arranged in Excel sheets and according to the measured DNA standards a linear regression line was calculated to get the DNA amounts corresponding to the measured fluorescence. Measured samples with a DNA concentration lower than 2 ng/ µl were re-done, starting with a new nested PCR. To calculate the molecules per µl the formula shown below, was used (for more details see Amplicon Library Preparation Method Manual (Roche), section 3.3.3 Amplicon Dilution and Pooling; Appendix chapter 8.2.1).

$$\text{Molecules}/_{\mu l} = \frac{\text{sample concentration}\left[{}^{ng}/_{\mu l}\right] \times 6.022 \times 10^{23}}{656.6 \times 10^{9} \times \text{amplicon length [bp]}}$$

Dilution and pooling of the samples was done following the instructions of the Amplicon Library Preparation Method Manual from Roche, with the slight change that all samples with DNA concentrations below 5 ng/µl were concentrated to 5 ng/µl and then further processed. The samples were given to Dr. Marion Engel in the Helmholtz institute, she made the emPCR and the 454 sequencing run, the author assisted and helped during the whole process once. Afterwards the raw and quality files were processed via the shotgun pipeline of the GS FLX sequencer (Roche).

Data analysis

In total 100 samples per tree species (5 root samples per time point and treatment, 25 samples per sample point per tree species) were analyzed in ½ (4/8) run of the GS FLX sequencer, if not stated otherwise. In total 497,374 sequence reads were analyzed and 27,963 clusters were manual checked for the corresponding replicate or plot number (see Table A9, A10 and A110). Sequence reads which occurred only once (singletons) or twice were excluded from the analysis. Analysis of the enormous bioinformatic data of 454 sequences was done by web-based and command-line based programs as follows.

RDP's pyrosequencing pipeline (http://pyro.cme.msu.edu/) - Pipeline initial process

This web-based interface, especially the pipeline initial process "(…) includes sorting the raw reads into those from each of the original samples, trimming off the key tag and primers, and removing sequences of low quality."[1] Several output folders are exported by the program

1 The Ribosomal Database Project's Pyrosequencing Pipeline, Center for Microbial Ecology @ Michigan State university, http://pyro.cme.msu.edu/init/form.spr, last accessed 23.01.2012

according to the taqs used (here: MID1-36) and several statistic files. First the raw file and the quality file of the 454 sequencing was uploaded followed by an additional taq file, containing all sample names and their correlation to the used taqs and the gene preset (16S rRNA) was changed to other. The interface provides also a possible insertation of the forward and the reverse primer, since only uni-directional read sequencing was used only the forward primer was added before processing the data.

The Minimum sequence length was set to 300, minimum average exponential quality-score was set to 15 and the forward primer sequences was not cut to improve automatical aligning via mafft afterwards.

Adding of identifiers

All names of the 454 sequences were shortened to five unique characters of the original, an identifier including the replicate or plot-number and the treatment number was added via search and replace in MS Word. For example, the 454 sequence named GEHGW5N06HHY7J, stemming from replicate 5 and treatment 1, was renamed to HHY7J_5-T1. Sequences of all five samples belonging to one treatment at one sample point were put together into a file and analyzed together.

454 Replicate filter (http://ribo0.mmg.msu.edu/replicates/)

"This tool clusters and filters out artificially replicated sequences in 454 data. It returns a fasta file of unique sequences and a list of the sequences in each cluster. This tool is described in Gomez-Alvarez *et al*. 2009."[2] The uploaded fasta file was clustered by the following settings, sequence identity cutoff was set to 0.97 and length difference requirement was used as given (0). All three output files, including the file with the unique clustered sequences, the summary of the sequences and all the fasta clusters were saved.

Aligning of sequences by MAFFT via Cygwin

The aligning of the short 454 sequences to the backbone alignment, consisting of approximately 3 kb long rDNA sequences (SSU-ITS-LSU) including the Ecuadorian sequences from the isolates, Nursery experiment No. 1 and *Podocarpus oleifolius* root samples, MAFFT v6.861b was used via the Cygwin command-line based interface. After testing different settings, the best results could be achieved with a gap opening penalty (op) of 3 and offset (gap extension penalty, ep) of 0.123. MAFFT was used to align the short 454 sequences (extracted_clusters_unique.fas-file) according to tree species, sample time and treatment to the backbone alignment of glomeromycotan sequences (GLOMEROMYCOTA_backbone.fas -file). The following command line was used; mafft --op 3 --ep 0.123 --add extracted_clusters_unique.fas

2 Schmidt-Lab, 454 Replicate Filter, Gomez-Alvarez V, Teal TK, Schmidt TM (2009) Systematic artifacts in metagenomes from complex microbial communities. The ISME Journal 3: 1314-17, http://ribo0.mmg.msu.edu/replicates/, last accessed 23.01.2012

GLOMEROMYCOTA_backbone.fas > output.fas. The output fasta file is then prepared for phylogenetic analysis by applying a mask into Align (Sequentix, Germany – www.sequentix.de) and converting the file to phylip format for further analysis.

Due to the enormous amount of short sequences, which had to be aligned to the backbone dataset the local installed MAFFT program (commando line based) revealed a limited calculation power of aligning more than ca. 1,500 sequences. The limitation seemed to be dependent on the amount and the length of the sequences (personal communication with member of the MAFFT help desk). To overcome this problem in some cases a limited number of sequences per species of the backbone alignment (published in Krüger *et al.* 2012 and provided on the webpage www.amf-phylogeny.com) were used for calculating the phylogenetic tree, covering the intraspecific variability of each AMF species. In some cases these reduced sequence datasets still exceeded the limitation of MAFFT, thus the short 454 sequences were divided in two parts and calculated separately. This maybe will become obsolete as a new "--addfragments"-command (herein not tested) is provided now on the MAFFT homepage (http://mafft.cbrc.jp/alignment/software), which adds short sequence fragments to a master alignment.

Calculating of phylogenetic trees with RAxML, visualization by FigTree

The RAxML program ver. 7.2.8 (Stamatakis *et al.* 2008) was used on the CIPRES Science Gateway (Miller *et al.* 2010, www.phylo.org/portal2) to calculate all maximum likelihood phylogenetic trees with the combined alignment, using 1,000 bootstraps and the GTRGAMMA model for bootstrapping and tree inference. Annotations in the phylogenetic trees were batch replaced via Align and afterwards trees were visualized in FigTree v1.2.1. At last the tree data was copied into an Excel sheet and analyzed manually for the different AMF species, checking all cluster files for the corresponding replicates or plots.

3 Results

3.1 Characterization of AMF cultures from Ecuador

3.1.1 Morphological characterization of fungal cultures (isolates)

Eleven Ecuadorian AMF were identified from cultures with *Plantago lanceolata* as plant host, when not stated otherwise. Nine AMF cultures stem from the trap cultures of mycorrhizal roots of *Cedrela montana* and *Heliocarpus americanus* originating from the Nursery experiment No. 1 (described in Urgiles *et al.* 2009). Two cultures (Att1450-1, Att1455-2), originated from an afforestation site with experimental plots of *C. montana* in the area of the RBSF.

A summary of the morphological characteristics is shown in Table 8, for further details see Appendix Table A2. AMF species names are temporary, respecting morphological and molecular characterization of the fungal cultures and may be changed in future publications.

3.1.1.1 *Diversispora* sp. (Att1449-5)

The *Diversispora* sp. culture (Att1449-5) was successfully established from one single glomeromycotan spore (single spore isolate). The culture produced few hyphae with tiny glomoid spores, which were hyaline, whitish or yellow-brownish. The spores stick to the mycelium and appear single or in loose clusters. No spores were formed in roots. Wound healing of the hyphae was observed, a feature known in the genus *Diversispora*. Arbuscules and hyphae were observed in stained roots of the host plant *Plantago lanceolata* (Fig. 4, E, F) showing the successful colonization by the *Diversispora* sp. The spores of the isolate Att1449-5 are globose to subglobose to broad ellipsoid to ellipsoidal, 102 µm in length (25 to 180 µm) – 102 µm in breadth (27 to 166 µm), hyaline to white to very pale brown in color (see Table 8). The spores showed no reaction of the wall components in PVLG-Melzer's.

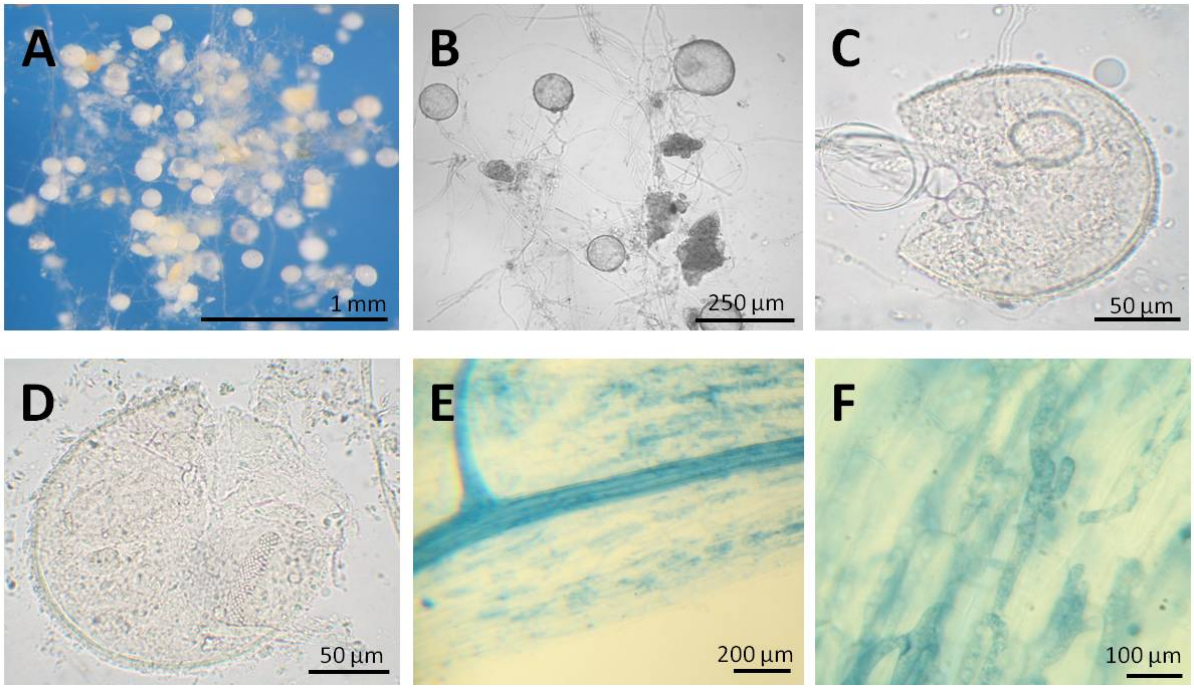


Fig. 4: AMF spores and mycorrhizal structures fungus in culture Att1449-5. A: Spores under the dissecting microscope, **B, D:** Spores in PVLG-Melzer's, **C:** Crushed spore, **E:** Arbuscules in *Plantago lanceolata* roots stained by methyl blue, **F:** Stained hyphae in roots.

3.1.1.2 *Claroideoglomus etunicatum*-like (Att1449-10, Att1451-6, Att1456-11)

A *Claroideoglomus etunicatum*-like AMF species was present in three cultures Att1449-10, Att1451-6 and 1456-11, forming spores strongly attached to large amounts of mycelium.

The culture Att1449-10 was derived from one spore and produced *Claroideoglomus etunicatum*-like spores mainly located close to the roots. Spores appeared single or in loose clusters. The spores are variable in colors, ranging from hyaline to yellow to reddish yellow to yellow-brownish to strong brown to rarely grayish olive. Young spores were observed with open pores (Fig. 5, D1). Spores are globose to subglobose to broad ellipsoidal to ovoid to obovoid to irregular, and are 115 µm (67 to 188 µm) long and 114 µm (67 to 203 µm) broad. A slow reaction of the outermost wall component of the spores in PVLG-Melzer's reagent was observed. No spores, only arbuscules, vesicles and hyphal coils were found in roots (Fig. 6).

The culture Att1451-6 containing *Claroideoglomus etunicatum*-like spores was a multispore culture initiated by application of 12 spores supposed to present one morphotype. The culture mainly contained *Cl. etunicatum*-like spores of yellow to light brown to white color. Spores are various in size and shape, appear single or form loose clusters. They are globose to subglobose to broad ellipsoidal, also pyriform and are 104 µm (32 to 166 µm) in length and 105 µm (35 to 165 µm) in breadth. Vesicles and hyphae were found in roots stained by

methyl blue. Spores showed no reaction to Melzer's reagent. Additionally, this multispore culture contained most probably a *Rhizophagus* sp. and an unknown glomoid AMF (see 3.1.1.8, Table 8).

The single spore culture Att1456-11 formed also *Cl. etunicatum*-like spores. Spores range in color from white to light gray to brownish-yellow and appear single or form loose clusters. Globose to broad ellipsoidal, also clavate spore shapes were observed. Spores were 124 µm (76 to 172 µm) long and 122 µm (80 to 181 µm) broad. No reaction in PVLG-Melzer's reagent could be observed. Arbuscules, hyphal coils and vesicles could be observed in roots.

All three cultures formed only few *Cl. etunicatum*-like spores. Spores of Att1451-6 and Att1456-11 did not react to Melzer's reagent, whereas the spores of Att1449-10 showed a slow pink reaction of the outermost wall component.

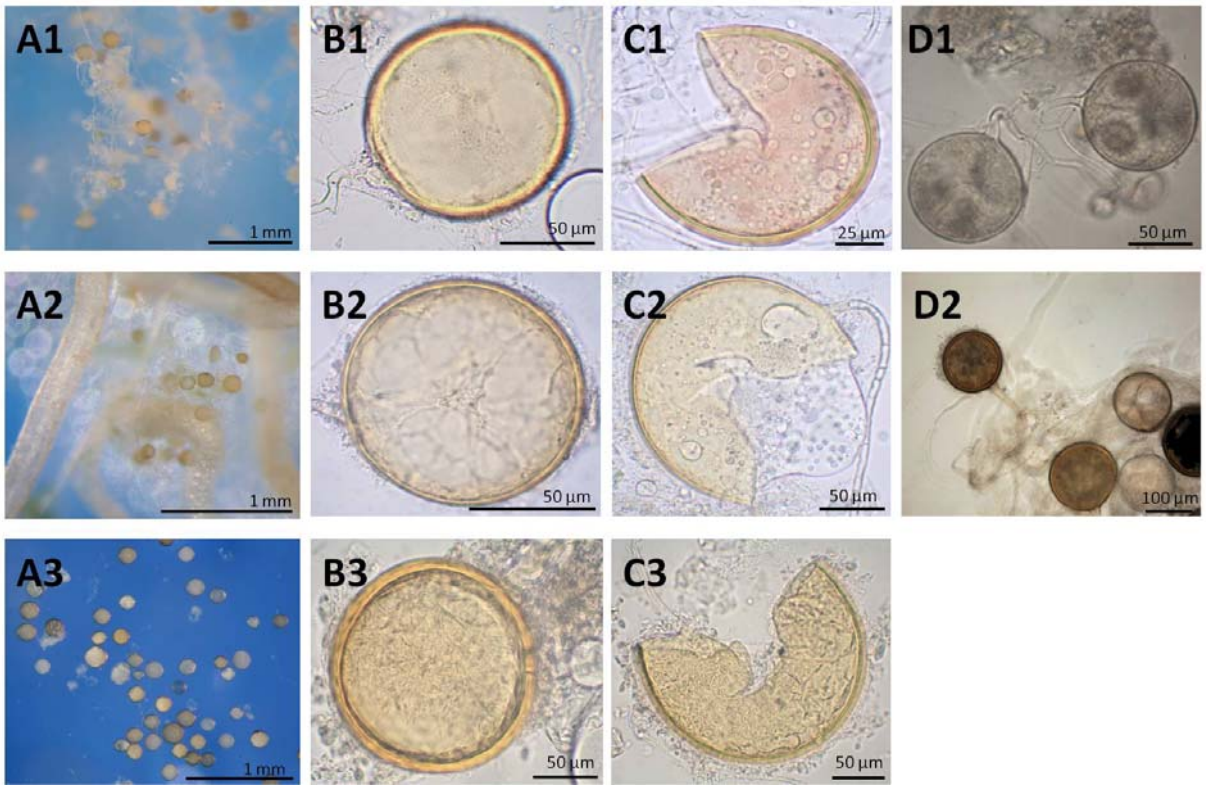


Fig. 5: AMF spores of the *Claroideoglomus etunicatum*-like fungus in cultures Att1449-10 (1), Att1451-6 (2) and Att1456-11 (3). A: Spores under the dissecting microscope, **B, D:** Spores in PVLG, **C:** Reaction of crushed spores in PVLG-Melzer's.

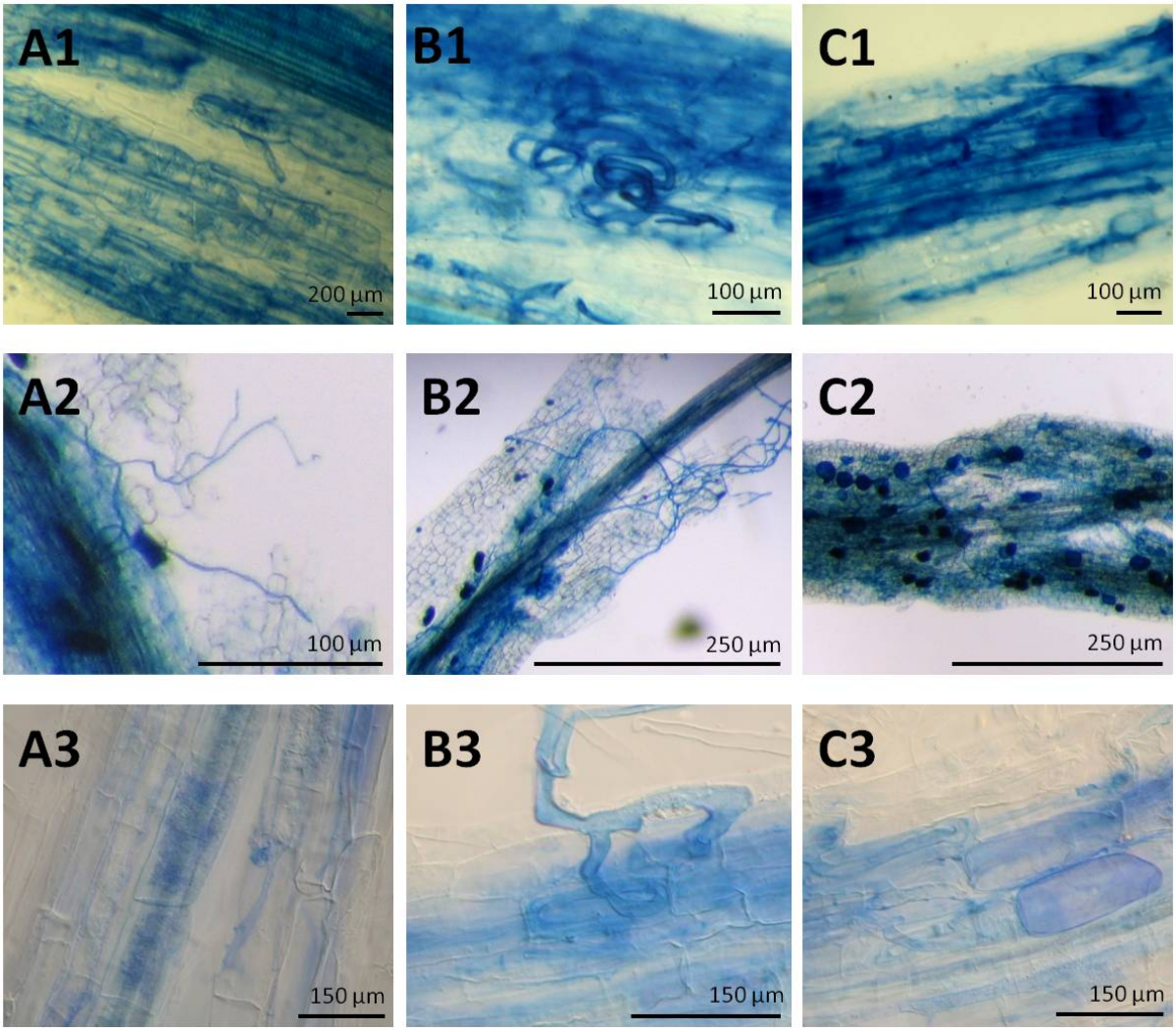


Fig. 6: Mycorrhizal structures of the *Claroideoglomus etunicatum*-like fungus in cultures Att1449-10 (1), Att1451-6 (2) and Att1456-11 (3), in roots of *Plantago lanceolata*. A1, A2: Arbuscules; **A2, B:** Hyphae or hyphal coils; **C:** Vesicles.

3.1.1.3 *Ambispora* sp. (Att1449-12)

Vesicle-like structures could be observed in stained roots of *Plantago lanceolata* (see Fig. 7), but no further AMF structures were noticed. Since no spores were found this AMF was provisionally named *Ambispora* sp. as sequences of this genus were achieved by molecular methods (see chapter 3.1.2). The culture was initially prepared as a single spore isolate. The application of the individual inoculum of this culture showed positive growth effects of the inoculated seedlings in the Nursery experiment No. 4 and No. 4A (see chapter 3.3).

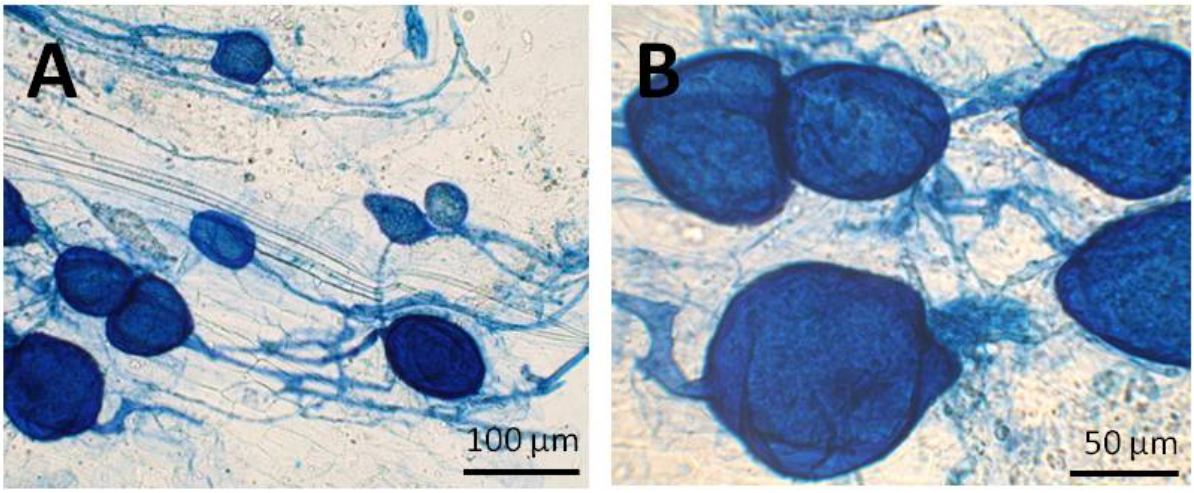


Fig. 7: Stained intraradical AMF structures of *Ambispora* sp. fungus in culture Att1449-12. A, B: Vesicle-like structures and hyphae in *Plantago lanceolata* roots, stained by methyl blue.

3.1.1.4 *Acaulospora* sp. nov. (Att1450-1)

The single spore culture (isolate) Att1450-1 contains orange-brownish acauloid spores, partly found with sacculum. A scar appears where the sacculum breaks, when the spores mature. Spores are single, globose to subglobose to broad ellipsoidal, also irregular and 191 µm (78 to 232 µm) in length and 194 µm (87 to 255 µm) in breadth. Two reactive spore wall components were identified when treated with PVLG-Melzer's reagent. The sacculum reacts also slightly red to Melzer's. Hyphae and vesicles in roots of *P. lanceolata* only stain faintly with methyl blue (Fig. 8).

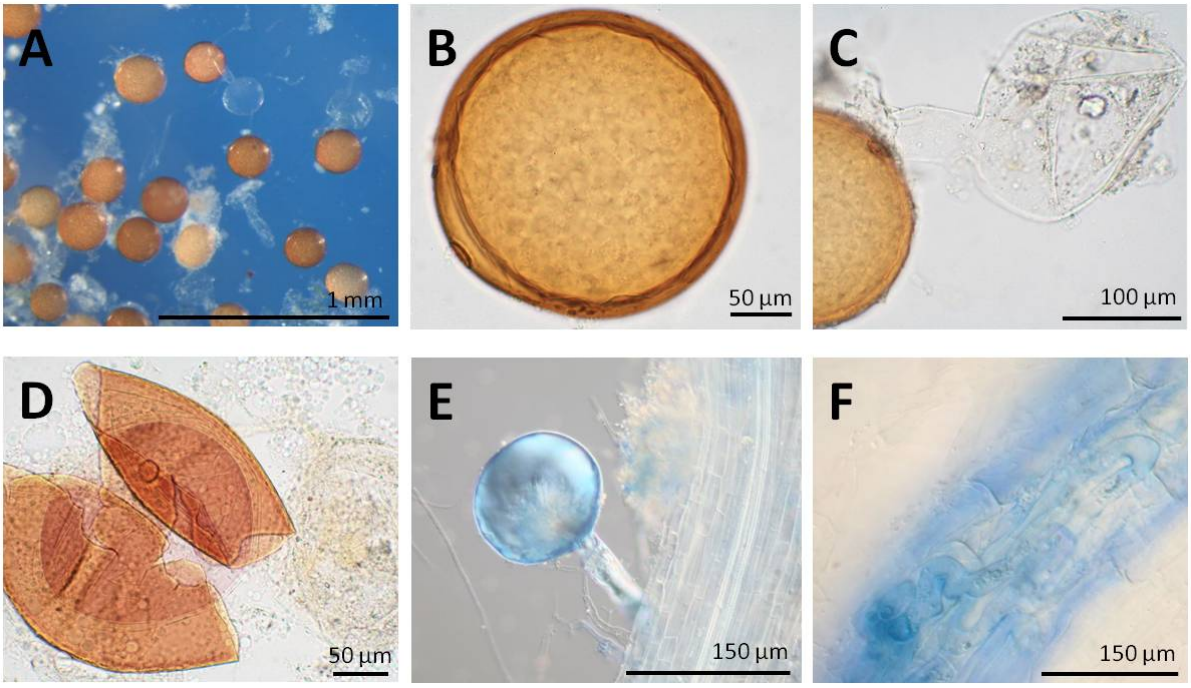


Fig. 8: AMF spores and mycorrhizal structures of *Acaulospora* sp. nov. fungus in culture Att1450-1. A: Spores with attached sacculum under the dissecting microscope, **B:** Spore with scar in PVLG, **C:** Spore with attached sacculum, **D:** Reaction of a crushed spore with attached sacculum in PVLG-Melzer's, **E, F:** Sacculum (**E**) and hyphal coils in *Plantago lanceolata* roots (**F**) stained by methyl blue.

3.1.1.5 *Rhizophagus* sp. (Att1451-8, Att1456-1)

An AMF species related to *Rhizophagus* was identified in the two culture attempts (Att) 1451-8 and 1456-1, forming spores of various shapes mostly in roots. Each culture was originally set up with one 1 cm root fragment placed on a *Plantago lanceolata* root, as no spores outside the roots could be observed.

Att1451-8 formed large amounts of mycelium around the roots of *P. lanceolata*. Hyaline tiny external spores are very rare. Spores in roots were tightly packed especially in darker roots. In young whitish roots only few spores were observed. The spores are hyaline to pale yellow in color, subglobose to broad ellipsoid to ellipsoidal to ovoid, also irregular, pyriform or obovoid, and 85 µm (37 to 160 µm) long to 57 µm (29 to 114 µm) broad. No reaction of the spore walls in PVLG-Melzer's reagent was found. Vesicles and hyphae in roots stained dark blue by methyl blue.

Att1456-1 contains spores in roots quite various in shape, globose to subglobose to broad ellipsoidal to ellipsoidal to ovoid, also irregular and obovoid. Spores are very pale yellow in color and 59 µm (39 to 109 µm) long to 48 µm (31 to 104 µm) broad. A slow slight purple

reaction with Melzer's reagent could be seen, varying in intensity. Arbuscules and vesicles could be observed in roots.

Interestingly the spores of Att1451-8 show no reaction to PVLG-Melzer's whereas Att1456-1 shows a light to heavy red reaction, although both cultures contained the same fungal species (Fig. 9, see also molecular analysis chapter 3.1.2).

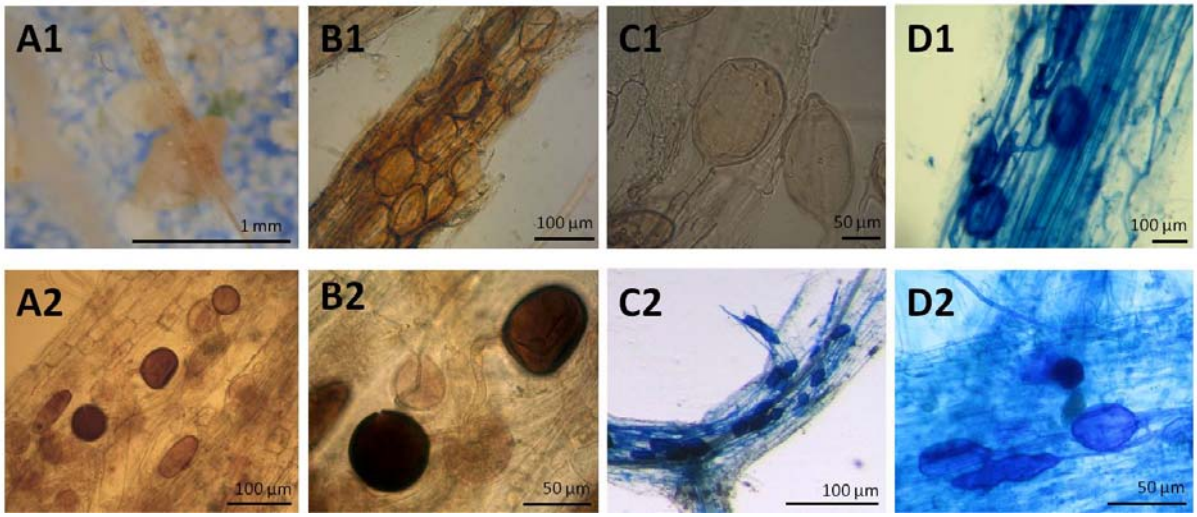


Fig. 9: AMF structures of the *Rhizophagus* sp. fungus in cultures Att1451-8 (1) and Att1456-1 (2). A1: Spores under the dissecting microscope, **A2, B, C1:** Spores in roots in PVLG/Melzer's, **C2, D:** Spores and hyphae in roots stained by methyl blue.

3.1.1.6 *Archaeospora trappei*-like (Att1452-6, Att1456-7)

This *Archaeospora* sp. was identified in two cultures forming tiny hyaline spores floating on the water surface and is therefore hard to handle. Spores with a sacculum were detected, a feature of *Archaeospora* species.

Culture Att1452-6 was initially started with 80 spores, designated as identical morphotype. The *Archaeospora*-like spores are small and hyaline in color, similar to *Ar. trappei*. The single appearing spores are globose to subglobose to broad ellipsoidal to ovoid to obovoid. Spores are 64 µm (51 to 78 µm) in length and 62 µm (51 to 77 µm) in breadth. No reaction in Melzer's reagent was observed.

The single spore culture (isolate) Att1456-7 also contained *Ar. trappei*-like spores. The single appearing spores are hyaline in color, globose to subglobose to broad ellipsoidal, also irregular to obovoid. The measured spores are 60 µm (43 to 69 µm) long and 60 µm (43 to 83 µm) broad and did not react to PVLG-Melzer's reagent. Arbuscules only stain weakly with methyl blue.

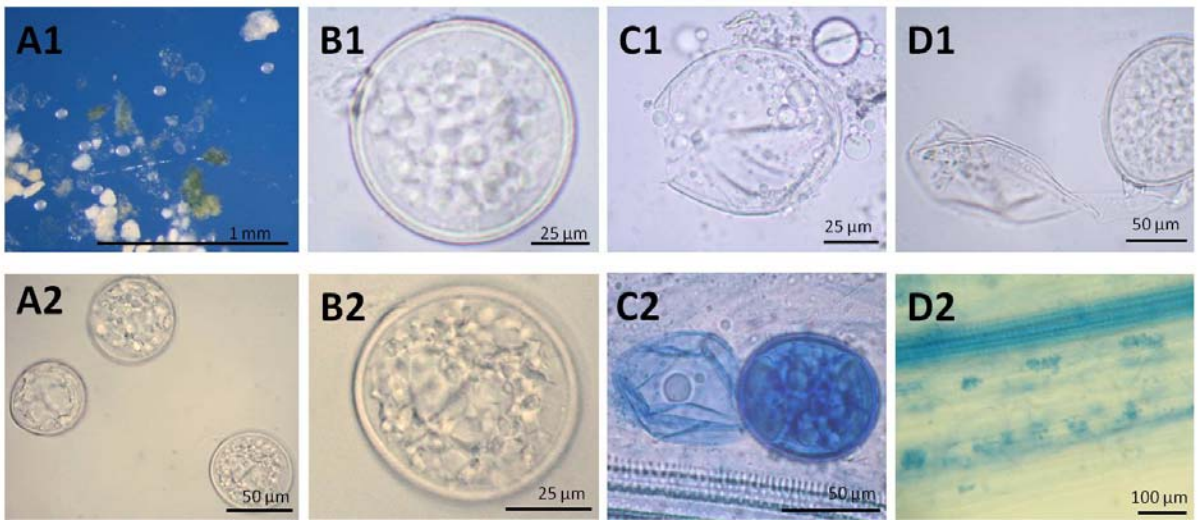


Fig. 10: *Archaeospora trappei*-like fungus in cultures Att1452-6 (1) and Att1456-7 (2). A1: Spores under the dissecting microscope; **A2, B1, B2, C1:** Spores in PVLG; **C2:** Spore with sacculum, after methyl blue staining; **D1:** Spore with sacculum; **D2:** Arbuscules stained by methyl blue.

3.1.1.7 *Dentiscutata savannicola* (Att1455-2)

Culture attempt 1455-2 originated from a single spore. The spores appear single and are globose to broad ellipsoidal to ellipsoidal to ovoid, also pyriform and obovoid. The spore color varies from white when young, becoming yellow to brownish yellow to dark yellowish brown when moribund. Measured from the base, spores are 321 µm (215 to 585 µm) long and 367 µm (235 to 510 µm) broad. The spore wall reacts to PVLG-Melzer's reagent. The outer spore wall shows a heavy blood red reaction, whereas the inner component shows a slow purple reaction. A germination shield (Fig. 11, C and D) and auxiliary cells (Fig. 11, G) were observed. Arbuscules and hyphae stain dark blue by methyl blue. However, some of the hyphae did not stain at all and remained brownish. Hyphae are up to 35 µm thick and form coils inside the roots without vesicles.

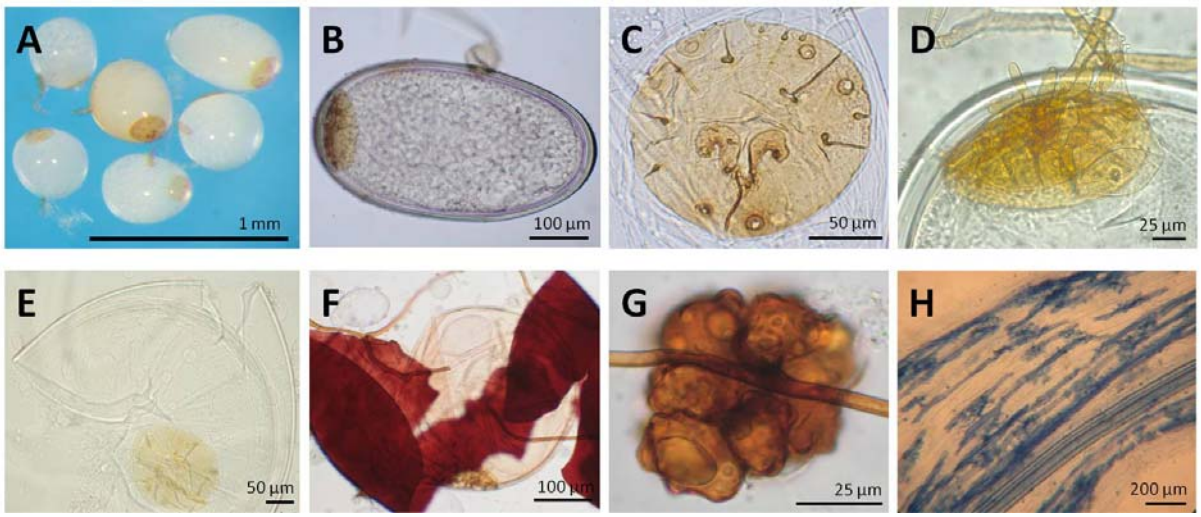


Fig. 11: AMF characteristics of *Dentiscutata savannicola* fungus in culture Att1455-2 . **A:** Spores under the dissecting microscope, **B:** One single spore with germination shield in PVLG, **C, D:** Closer look on the germination shield, **E:** Crushed spore in PVLG, **F:** Crushed spore in PVLG-Melzer's, **G:** Auxiliary cells, **H:** Hyphae and arbuscules in methyl blue stained roots.

3.1.1.8 Multispore culture Att1451-6

The multispore culture Att1451-6 was established by use of 12 spores which appeared to represent the same species. However, it turned out that this culture contained more than one AMF species. The main AMF observed in this culture was *Cl. etunicatum*-like. In addition, a species belonging to *Rhizophagus* and an unknown glomoid species was found (see Table 8 and Appendix Table A2).

Att-No.	Name	Voucher	Spore length × breadth (min. to max.) [µm]	Shape	Appearance	Color	Melzer's reaction	Muronym
1449-5	*Diversispora* sp.	W5349/ W5661	102 (25 to 180) × 102 (27 to 166)	Globose to subglobose to broad ellipsoidalal	Single or in loose clusters	10YR 7/1 - 8/2 - 8/4 (white to very pale brown) [MSC], colorless to white	No reaction	A(UL)
1449-10	*Claroideoglomus etunicatum*-like	W5333/ W5668	115 (67 to 188) × 114 (67 to 203)	Globose to subglobose to ovoid	Single or in loose clusters	7.5R 8/6-7/8-6/8-5/8 (reddish yellow to strong brown) [MSC], colorless to yellow to yellow-brown rarely grayish olive	Very slow pink reaction of outermost wall component	A(EL)
1449-12	*Ambispora* sp.	W5341		Globose to subglobose to irregular	Spores in roots			
1450-1	*Acaulospora* sp. nov.	W5350/ W5666	191 (78 to 232) × 194 (87 to 255)	Globose to subglobose to broad ellipsoidal	Single	7YR 5/6 (strong brown) [MSC], Peach to orange yellow to orange	Two reactive wall components	
1451-6	*Claroideoglomus etunicatum*-like	W5335/ W5554/ W5595/ W5667	104 (32 to 166) × 105 (35 to 165)	Globose to subglobose to broad ellipsoidal, also pyriform	Single or in loose clusters	Hyaline to 2.5Y 8/6 - 8/8 (yellow) [MPT], pale pinkish cream to ochre to sienna (4-9-11) [RBG], olivaceous, white to yellow to yellow brown	No reaction	A(EL) maybe A(ELU)

Att-No.	Name	Voucher	Spore length × breadth (min. to max.) [µm]	Shape	Appearance	Color	Melzer's reaction	Muronym
1451-6	*Rhizophagus vesiculiferum*-like	W5472/ W5555	50 (35 to 67) × 47 (34 to 66)	Globose to subglobose to broad ellipsoidal	In tight clusters	Colorless to pale yellowish cream (colorless - 3) [RBG],	Rapid blood red	A(EL)
1451-6	'Glomoid unknown'	W5553/ W5596	188 (166 to 202) × 187 (168 to 204)	Globose to subglobose	Single	White (1) [RBG], whitish	Evanescent component pink	A(EL)
1451-8	*Rhizophagus* sp.	W5338/ W5662	85 (37 to 160) × 57 (29 to 114)	Subglobose to ellipsoidal to ovoid	Spores in roots tightly packed together (juxtaposed)	Hyaline to pale yellow	No reaction	A(UL)?
1452-6	*Archaeospora trappei*-like	W5340/ W5670	64 (51 to 78) × 62 (51 to 77)	Globose to subglobose to broad ellipsoidal to ovoid to obovoid	Single	Hyaline	No reaction	
1456-1	*Rhizophagus* sp.	W5336/ W5664	59 (39 to 109) × 48 (31 to 104)	Globose to subglobose to broad ellipsoidal	Spores in roots	Very pale yellow	Various reactions from rapid blood red to slow light	

Att-No.	Name	Voucher	Spore length × breadth (min. to max.) [µm]	Shape	Appearance	Color	Melzer's reaction	Muronym
				to ovoid, also irregular			purple	
1456-7	*Archaeospora trappei*-like	W5337/ W5663	60 (43 to 69) × 60 (43 to 83)	Globose to subglobose to broad ellipsoidal	Single	Colorless, hyaline	No reaction	A(F)B(L)?
1456-11	*Claroideoglomus etunicatum*-like	W5348/ W5669	124 (76 to 172) × 122 (80 to 181)	Globose to broad ellipsoidal, also clavate	Single or in loose clusters	10YR 6/8 (brownish yellow) [MSC], white, light gray to brownish-yellow	No reaction	A(UL)?
1455-2	*Dentiscutata savannicola*	W5538/ W5893	321 (215 to 585) × 367 (235 to 510)	Globose to broad ellipsoidal to ovoid, also pyriform and obovoid	Single	10YR 8/1 becoming 8/4 to 7/8 (- 3/6 when moribund) white becoming yellow to brownish yellow to dark yellowish brown when dead) [MSC]	Rapid outer blood red. Inner slowly purple.	A(EL)B(F) C(***)

Table 8: Summarized voucher information of the Ecuadorian cultures. Different color charts were used: **[MSC]** Munsell Soil Chart, **[MPT]** Munsell plant tissue Chart and **[RBG]** Royal Botanical Garden Edinburgh chart. The mean spore size is given for length and breadth with measured minimum and maximum values in brackets. Muronym descriptions are used according to Walker (1986).

3.1.2 Molecular characterization of AMF from Ecuador

AMF sequences obtained from the described Ecuadorian AMF culture attempts, the Nursery experiment No. 1 and environmental samples of *Podocarpus oleifolius* roots with or without nodules. For further details on origin of the AMF sequences see Appendix Table A3.

The phylogenetic tree (Fig. 12) shows the clustering of all analyzed Ecuadorian samples in the phylum *Glomeromycota.* Up to three sequences per AMF species were used for better overview (taking the intraspecific variation into account). Clades were collapsed to genera for easier visualization and the Ecuadorian sequences illustrated in gray. Maximum likelihood trees were calculated with RAxML v7.2.8 (Stamatakis *et al.* 2008) using 1000 bootstraps. The phylogenetic trees were calculated using the approx. 3 kb (SSU_{full}-ITS-LSU_{part}) and the ≤ 1800 bp long SSU_{part}-ITS-LSU_{part} rDNA fragments, when no SSU rDNA fragment was available. The highly variable ITS rDNA region was excluded from calculation. The alignment, which was also used for the 454 analysis, will be provided as fasta-file on a CD together with this dissertation.

The Ecuadorian sequences clustered in the genera *Rhizophagus*, *Glomus*, *Claroideoglomus*, *Dentiscutata*, *Diversispora*, *Acaulospora*, *Archaeospora* and *Ambispora*. One sequence (CK060-3) clustered next to *Paraglomus* basal in the *Glomeromycota*.

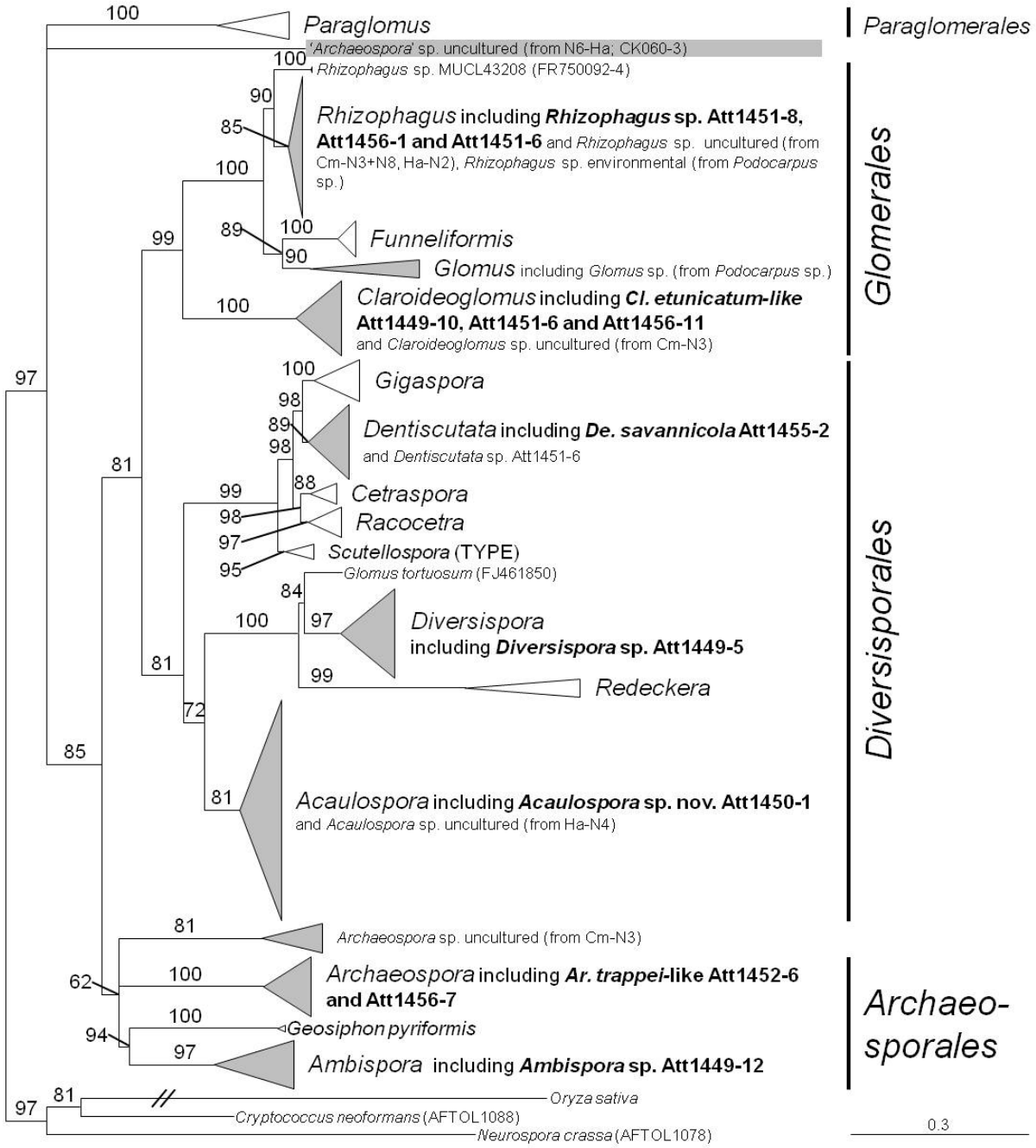


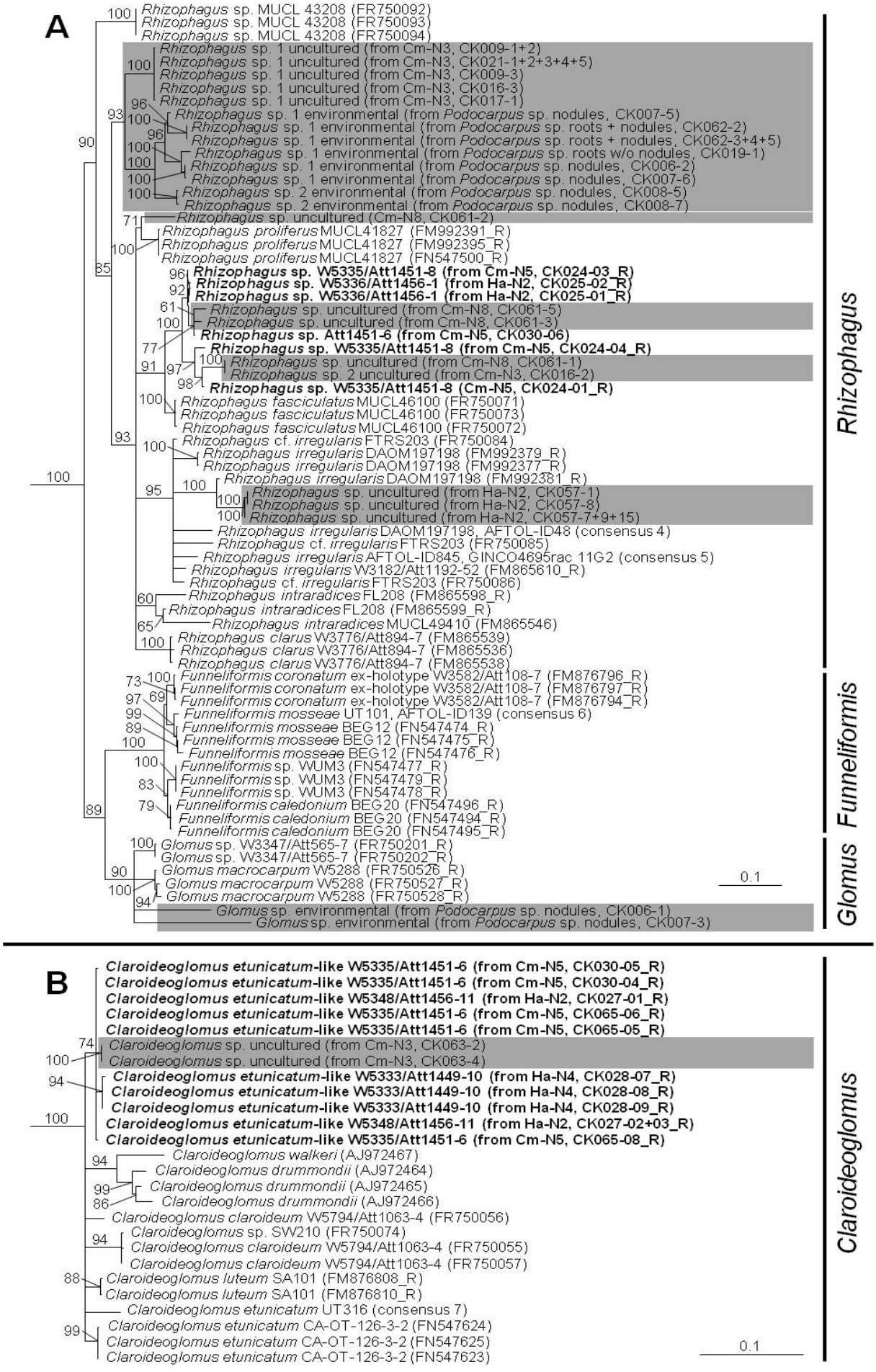
Fig. 12: Maximum likelihood phylogenetic tree of the *Glomeromycota* (SSU_{full}-ITS-LSU_{part}) including the Ecuadorian sequences. *Oryza sativa*, *Neurospora crassa* and *Cryptococcus neoformans* were used as outgroup. Clades including Ecuadorian AMF sequences are marked in gray and the Ecuadorian cultures used as inoculum in nursery experiments are written in bold. Branches with BS fewer than 60% were reduced to polytomies. Two diagonal slashed indicate a 50% reduced branch length. The scale bar shows the substitutions per site. Classification of the AMF follows Schüßler & Walker 2010 and Redecker *et al.* (2013).

3.1.2.1 *Glomerales – Rhizophagus*, *Funneliformis*, *Glomus* and *Claroideoglomus*

The analyses of the *Glomeraceae* showed a cluster with sequences from the Ecuadorian nursery and forest basal to *Rhizophagus*, supported by 93% BS (Fig. 13A). In this cluster three different *Rhizophagus* spp. separate from each other with BS of 100, 96 and 100%. uncultured *Rhizophagus* sp. 1, stemming from *Cedrela montana* roots of the Nursery experiment No. 1 (sample N3) separated with 100% from the remaining *Rhizophagus* sequences stemming from Ecuadorian samples. Environmental *Rhizophagus* sp. 1 and 2 originating from *Podocarpus* formed two distinct clades, one with sequences originated from roots with and without nodules or solely nodules, and a second one from *Podocarpus* nodules only. A single *Rhizophagus* sequence from *C. montana* (N8) clusters sister to *Rh. proliferus* MUCL41827. The *Rhizophagus* sequences from the cultures Att1451-8, Att1456-1 and Att1451-6 and further Ecuadorian *Rhizophagus* sequences from nursery root samples (N3, N8) form one cluster supported by 100% BS sister to the *Rh. fasciculatus* MUCL46100 clade. Further *Rhizophagus* sequences originating from nursery roots of *H. americanus* (N2) clustered within the *Rh. irregularis* clade. Two sequences stemming from *Podocarpus* root nodules (modified lateral roots) cluster within *Glomus* unresolved sister to *Gl. macrocarpum* W5288 and a *Glomus* sp. W3347/Att565-7.

All sequences received from the three *Cl. etunicatum*-like cultures (Att1449-10, Att1451-6 and Att1456-11) clustered in *Claroideoglomus* forming their own clade, also *Claroideoglomus* sequences stemming from Ecuadorian nursery roots of *C. montana* (N3) with BS of 74% (Fig. 13B). The sequences from the cultures showed few intraspecific variations. *Cl. etunicatum*-like Att1449-10 formed an own clade with 94% BS, whereas the remaining two cultures Att1451-6 and Att1456-11 were not phylogenetically separated from each other. The Ecuadorian sequences from nursery roots (*C. montana*, N3) clustered together within the Ecuadorian culture sequences with BS of 100%.

Fig. 13: Maximum likelihood phylogenetic tree of the *Glomerales*, including the Ecuadorian sequences. Sequences from Ecuadorian AMF cultures are written in bold, uncultured and environmental sequences are marked by a gray box. Branches with bootstrap support below 60% were reduced to polytomies. The scale bar shows the substitutions per site.

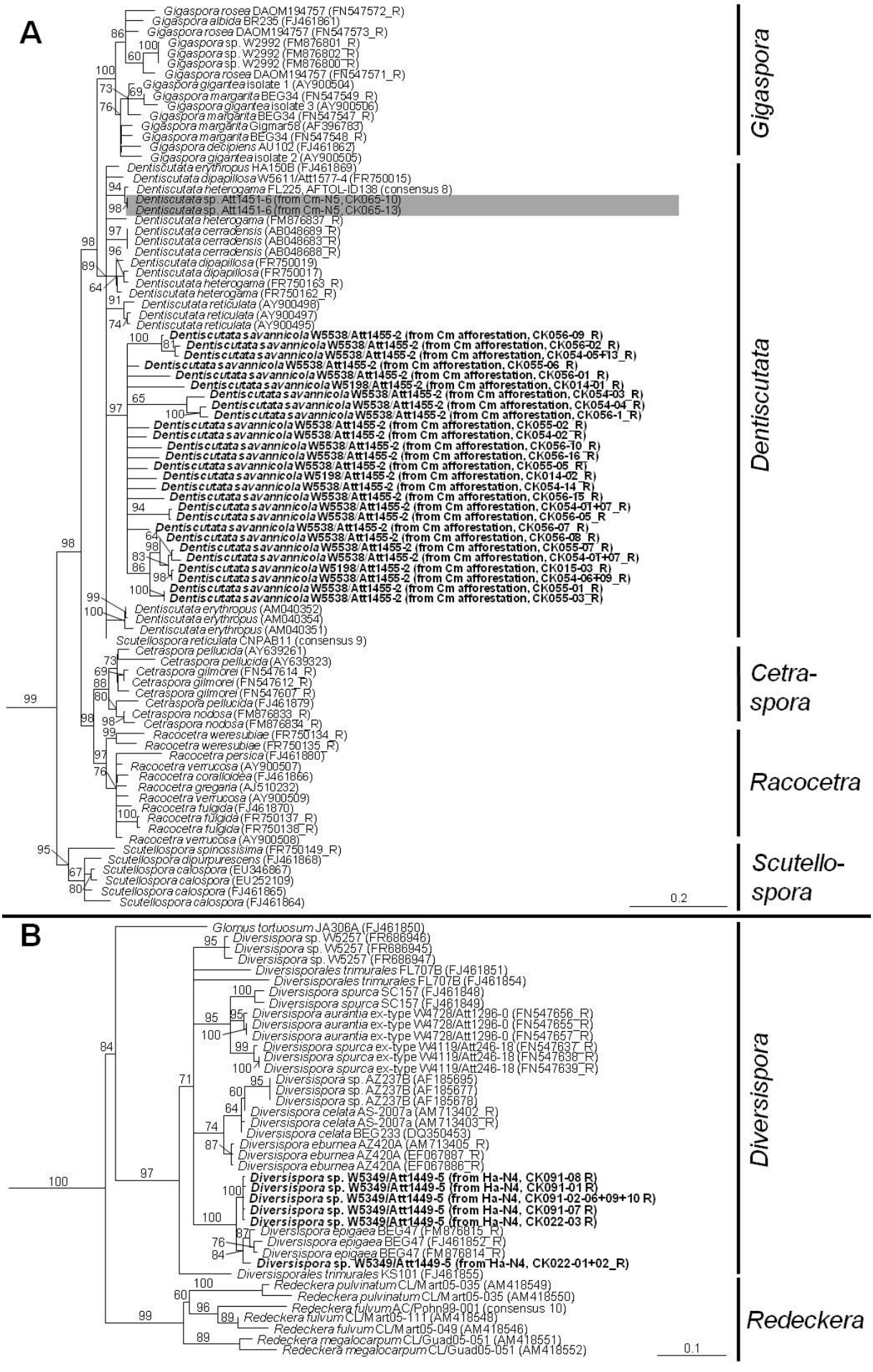

A
Rhizophagus sp. MUCL 43208 (FR750092)
Rhizophagus sp. MUCL 43208 (FR750093)
Rhizophagus sp. MUCL 43208 (FR750094)
Rhizophagus sp. 1 uncultured (from Cm-N3, CK009-1+2)
Rhizophagus sp. 1 uncultured (from Cm-N3, CK021-1+2+3+4+5)
Rhizophagus sp. 1 uncultured (from Cm-N3, CK009-3)
Rhizophagus sp. 1 uncultured (from Cm-N3, CK016-3)
Rhizophagus sp. 1 uncultured (from Cm-N3, CK017-1)
Rhizophagus sp. 1 environmental (from Podocarpus sp. nodules, CK007-5)
Rhizophagus sp. 1 environmental (from Podocarpus sp. roots + nodules, CK062-2)
Rhizophagus sp. 1 environmental (from Podocarpus sp. roots + nodules, CK062-3+4+5)
Rhizophagus sp. 1 environmental (from Podocarpus sp. roots w/o nodules, CK019-1)
Rhizophagus sp. 1 environmental (from Podocarpus sp. nodules, CK006-2)
Rhizophagus sp. 1 environmental (from Podocarpus sp. nodules, CK007-6)
Rhizophagus sp. 2 environmental (from Podocarpus sp. nodules, CK008-5)
Rhizophagus sp. 2 environmental (from Podocarpus sp. nodules, CK008-7)
Rhizophagus sp. uncultured (Cm-N8, CK061-2)
Rhizophagus proliferus MUCL41827 (FM992391_R)
Rhizophagus proliferus MUCL41827 (FM992395_R)
Rhizophagus proliferus MUCL41827 (FN547500_R)
Rhizophagus sp. W5335/Att1451-8 (from Cm-N5, CK024-03_R)
Rhizophagus sp. W5336/Att1456-1 (from Ha-N2, CK025-02_R)
Rhizophagus sp. W5336/Att1456-1 (from Ha-N2, CK025-01_R)
Rhizophagus sp. uncultured (from Cm-N8, CK061-5)
Rhizophagus sp. uncultured (from Cm-N8, CK061-3)
Rhizophagus sp. Att1451-6 (from Cm-N5, CK030-06)
Rhizophagus sp. W5335/Att1451-8 (from Cm-N5, CK024-04_R)
Rhizophagus sp. uncultured (from Cm-N8, CK061-1)
Rhizophagus sp. 2 uncultured (from Cm-N3, CK016-2)
Rhizophagus sp. W5335/Att1451-8 (Cm-N5, CK024-01_R)
Rhizophagus fasciculatus MUCL46100 (FR750071)
Rhizophagus fasciculatus MUCL46100 (FR750073)
Rhizophagus fasciculatus MUCL46100 (FR750072)
Rhizophagus cf. irregularis FTRS203 (FR750084)
Rhizophagus irregularis DAOM197198 (FM992379_R)
Rhizophagus irregularis DAOM197198 (FM992377_R)
Rhizophagus irregularis DAOM197198 (FM992381_R)
Rhizophagus sp. uncultured (from Ha-N2, CK057-1)
Rhizophagus sp. uncultured (from Ha-N2, CK057-8)
Rhizophagus sp. uncultured (from Ha-N2, CK057-7+9+15)
Rhizophagus irregularis DAOM197198, AFTOL-ID48 (consensus 4)
Rhizophagus cf. irregularis FTRS203 (FR750085)
Rhizophagus irregularis AFTOL-ID845, GINCO4695rac 11G2 (consensus 5)
Rhizophagus irregularis W3182/Att1192-52 (FM865610_R)
Rhizophagus cf. irregularis FTRS203 (FR750086)
Rhizophagus intraradices FL208 (FM865598_R)
Rhizophagus intraradices FL208 (FM865599_R)
Rhizophagus intraradices MUCL49410 (FM865546)
Rhizophagus clarus W3776/Att894-7 (FM865539)
Rhizophagus clarus W3776/Att894-7 (FM865536)
Rhizophagus clarus W3776/Att894-7 (FM865538)
Funneliformis coronatum ex-holotype W3582/Att108-7 (FM876796_R)
Funneliformis coronatum ex-holotype W3582/Att108-7 (FM876797_R)
Funneliformis coronatum ex-holotype W3582/Att108-7 (FM876794_R)
Funneliformis mosseae UT101, AFTOL-ID139 (consensus 6)
Funneliformis mosseae BEG12 (FN547474_R)
Funneliformis mosseae BEG12 (FN547475_R)
Funneliformis mosseae BEG12 (FN547476_R)
Funneliformis sp. WUM3 (FN547477_R)
Funneliformis sp. WUM3 (FN547479_R)
Funneliformis sp. WUM3 (FN547478_R)
Funneliformis caledonium BEG20 (FN547496_R)
Funneliformis caledonium BEG20 (FN547494_R)
Funneliformis caledonium BEG20 (FN547495_R)
Glomus sp. W3347/Att565-7 (FR750201_R)
Glomus sp. W3347/Att565-7 (FR750202_R)
Glomus macrocarpum W5288 (FR750526_R)
Glomus macrocarpum W5288 (FR750527_R)
Glomus macrocarpum W5288 (FR750528_R)
Glomus sp. environmental (from Podocarpus sp. nodules, CK006-1)
Glomus sp. environmental (from Podocarpus sp. nodules, CK007-3)
0.1
Rhizophagus
Funneliformis
Glomus
B
Claroideoglomus etunicatum-like W5335/Att1451-6 (from Cm-N5, CK030-05_R)
Claroideoglomus etunicatum-like W5335/Att1451-6 (from Cm-N5, CK030-04_R)
Claroideoglomus etunicatum-like W5348/Att1456-11 (from Ha-N2, CK027-01_R)
Claroideoglomus etunicatum-like W5335/Att1451-6 (from Cm-N5, CK065-06_R)
Claroideoglomus etunicatum-like W5335/Att1451-6 (from Cm-N5, CK065-05_R)
Claroideoglomus sp. uncultured (from Cm-N3, CK063-2)
Claroideoglomus sp. uncultured (from Cm-N3, CK063-4)
Claroideoglomus etunicatum-like W5333/Att1449-10 (from Ha-N4, CK028-07_R)
Claroideoglomus etunicatum-like W5333/Att1449-10 (from Ha-N4, CK028-08_R)
Claroideoglomus etunicatum-like W5333/Att1449-10 (from Ha-N4, CK028-09_R)
Claroideoglomus etunicatum-like W5348/Att1456-11 (from Ha-N2, CK027-02+03_R)
Claroideoglomus etunicatum-like W5335/Att1451-6 (from Cm-N5, CK065-08_R)
Claroideoglomus walkeri (AJ972467)
Claroideoglomus drummondii (AJ972464)
Claroideoglomus drummondii (AJ972465)
Claroideoglomus drummondii (AJ972466)
Claroideoglomus claroideum W5794/Att1063-4 (FR750056)
Claroideoglomus sp. SW210 (FR750074)
Claroideoglomus claroideum W5794/Att1063-4 (FR750055)
Claroideoglomus claroideum W5794/Att1063-4 (FR750057)
Claroideoglomus luteum SA101 (FM876808_R)
Claroideoglomus luteum SA101 (FM876810_R)
Claroideoglomus etunicatum UT316 (consensus 7)
Claroideoglomus etunicatum CA-OT-126-3-2 (FN547624)
Claroideoglomus etunicatum CA-OT-126-3-2 (FN547625)
Claroideoglomus etunicatum CA-OT-126-3-2 (FN547623)
0.1
Claroideoglomus

3.1.2.2 *Diversisporales* (without *Acaulospora*) – *Gigaspora*, *Scutellospora*, *Dentiscutata*, *Cetraspora*, *Racocetra* and *Diversispora*

Two sequences from roots of *Plantago lanceolata* of the multispore culture Att1451-6 clustered together with *De. heterogama* FL225 (94% BS). It is likely that these sequences are PCR contaminations, since no *Dentiscutata* sp. was observed morphologically in culture Att1451-6. This sequences (Att1451-6) further share a high similarity (99%) to another *De. heterogama* (Att1577-4) sequence also processed in the laboratory at the same time. The *De. savannicola* isolate Att1455-2 clustered within *Dentiscutata* forming an own clade well supported with 97% BS (Fig. 14A) showing a high intraspecific variability of the sequences. Unfortunately, a comparison with other *De. savannicola* sequences was impossible as no *De. savannicola* sequences or living culture is available.

The sequences of isolate Att1449-5 assigned as *Diversispora* sp. clusters within the *Diversisporaceae* (Fig. 17B) and show a high similarity to each other (≤ 98%). The *Diversispora* sp. Att1449-5 sequences clustered with *Di. epigaea* BEG47 in one clade supported by 100% BS. Only one sequence clusters basal in the clade of *Di. epigaea* BEG47. The *Di. epigaea* BEG47 sequences clustered in their own clade with BS of 87%. An additional maximum likelihood tree was computed, including the ITS region also showing Att1449-5 clustering sister to *Di. epigaea* BEG47, which formed its own clade supported by 63% within the Ecuadorian species (not shown).

Fig. 14: Maximum likelihood phylogenetic tree of *Diversisporales* (without *Acaulospora*) including the Ecuadorian sequences. Sequences from Ecuadorian AMF cultures are written in bold, uncultured and environmental sequences are marked by a gray box. Branches with bootstrap support below 60% were reduced to polytomies. The scale bar shows the substitutions per site.


A
Gigaspora rosea DAOM194757 (FN547572_R)
Gigaspora albida BR235 (FJ461861)
Gigaspora rosea DAOM194757 (FN547573_R)
Gigaspora sp. W2992 (FM876801_R)
Gigaspora sp. W2992 (FM876802_R)
Gigaspora sp. W2992 (FM876800_R)
Gigaspora rosea DAOM194757 (FN547571_R)
Gigaspora gigantea isolate 1 (AY900504)
Gigaspora margarita BEG34 (FN547549_R)
Gigaspora gigantea isolate 3 (AY900506)
Gigaspora margarita BEG34 (FN547547_R)
Gigaspora margarita Gigmar58 (AF396783)
Gigaspora margarita BEG34 (FN547548_R)
Gigaspora decipiens AU102 (FJ461862)
Gigaspora gigantea isolate 2 (AY900505)
Dentiscutata erythropus HA150B (FJ461869)
Dentiscutata dipapillosa W5611/Att1577-4 (FR750015)
Dentiscutata heterogama FL225, AFTOL-ID138 (consensus 8)
Dentiscutata sp. Att1451-6 (from Cm-N5, CK065-10)
Dentiscutata sp. Att1451-6 (from Cm-N5, CK065-13)
Dentiscutata heterogama (FM876837_R)
Dentiscutata cerradensis (AB048689_R)
Dentiscutata cerradensis (AB048683_R)
Dentiscutata cerradensis (AB048688_R)
Dentiscutata dipapillosa (FR750019)
Dentiscutata dipapillosa (FR750017)
Dentiscutata heterogama (FR750163_R)
Dentiscutata heterogama (FR750162_R)
Dentiscutata reticulata (AY900498)
Dentiscutata reticulata (AY900497)
Dentiscutata reticulata (AY900495)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-09_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-02_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-05+13_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-06_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-01_R)
Dentiscutata savannicola W5198/Att1455-2 (from Cm afforestation, CK014-01_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-03_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-04_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-1_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-02_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-02_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-10_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-16_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-05_R)
Dentiscutata savannicola W5198/Att1455-2 (from Cm afforestation, CK014-02_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-14_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-15_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-0T+07_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-05_R
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-07_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK056-08_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-07_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-0T+07_R)
Dentiscutata savannicola W5198/Att1455-2 (from Cm afforestation, CK015-03_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK054-06+09_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-01_R)
Dentiscutata savannicola W5538/Att1455-2 (from Cm afforestation, CK055-03_R)
Dentiscutata erythropus (AM040352)
Dentiscutata erythropus (AM040354)
Dentiscutata erythropus (AM040351)
Scutellospora reticulata CNPAB11 (consensus 9)
Cetraspora pellucida (AY639261)
Cetraspora pellucida (AY639323)
Cetraspora gilmorei (FN547614_R)
Cetraspora gilmorei (FN547612_R)
Cetraspora gilmorei (FN547607_R)
Cetraspora pellucida (FJ461879)
Cetraspora nodosa (FM876833_R)
Cetraspora nodosa (FM876834_R)
Racocetra weresubiae (FR750134_R)
Racocetra weresubiae (FR750135_R)
Racocetra persica (FJ461880)
Racocetra verrucosa (AY900507)
Racocetra coralloidea (FJ461866)
Racocetra gregaria (AJ510232)
Racocetra verrucosa (AY900509)
Racocetra fulgida (FJ461870)
Racocetra fulgida (FR750137_R)
Racocetra fulgida (FR750138_R)
Racocetra verrucosa (AY900508)
Scutellospora spinossisima (FR750149_R)
Scutellospora dipurpurescens (FJ461868)
Scutellospora calospora (EU346867)
Scutellospora calospora (EU252109)
Scutellospora calospora (FJ461865)
Scutellospora calospora (FJ461864)
0.2
Gigaspora
Dentiscutata
Cetra-spora
Racocetra
Scutello-spora
B
Glomus tortuosum JA306A (FJ461850)
Diversispora sp. W5257 (FR686946)
Diversispora sp. W5257 (FR686945)
Diversispora sp. W5257 (FR686947)
Diversisporales trimurales FL707B (FJ461851)
Diversisporales trimurales FL707B (FJ461854)
Diversispora spurca SC157 (FJ461848)
Diversispora spurca SC157 (FJ461849)
Diversispora aurantia ex-type W4728/Att1296-0 (FN547656_R)
Diversispora aurantia ex-type W4728/Att1296-0 (FN547655_R)
Diversispora aurantia ex-type W4728/Att1296-0 (FN547657_R)
Diversispora spurca ex-type W4119/Att246-18 (FN547637_R)
Diversispora spurca ex-type W4119/Att246-18 (FN547638_R)
Diversispora spurca ex-type W4119/Att246-18 (FN547639_R)
Diversispora sp. AZ237B (AF185695)
Diversispora sp. AZ237B (AF185677)
Diversispora sp. AZ237B (AF185678)
Diversispora celata AS-2007a (AM713402_R)
Diversispora celata AS-2007a (AM713403_R)
Diversispora celata BEG233 (DQ350453)
Diversispora eburnea AZ420A (AM713405_R)
Diversispora eburnea AZ420A (EF067887_R)
Diversispora eburnea AZ420A (EF067886_R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK091-08 R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK091-01 R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK091-02-06+09+10 R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK091-07 R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK022-03 R)
Diversispora epigaea BEG47 (FM876815_R)
Diversispora epigaea BEG47 (FJ461852_R)
Diversispora epigaea BEG47 (FM876814_R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK022-01+02_R)
Diversisporales trimurales KS101 (FJ461855)
Redeckera pulvinatum CL/Mart05-035 (AM418549)
Redeckera pulvinatum CL/Mart05-035 (AM418550)
Redeckera fulvum AC/Pohn99-001 (consensus 10)
Redeckera fulvum CL/Mart05-111 (AM418548)
Redeckera fulvum CL/Mart05-049 (AM418546)
Redeckera megalocarpum CL/Guad05-051 (AM418551)
Redeckera megalocarpum CL/Guad05-051 (AM418552)
0.1
Diversispora
Redeckera

3.1.2.3 *Acaulosporaceae*

Sequences of the *Acaulospora* sp. nov. isolate Att1450-1 clustered in *Acaulosporaceae*, as also some Ecuadorian sequences did, stemming from *Cedrela montana* or *Podocarpus oleifolius* (Fig. 15). Sequences of Att1450-1 cluster with 100% BS together with *Ac. koskei* WV786 (FJ461793). The *Ac. koskei* culture WV786 was not available at the INVAM and the only one sequence of this species present in the public database stems from a problematic sequence submission (mentioned in Krüger *et al.* 2012), the assignment to this species is questionable. The uncultured *Acaulospora* spp. from *C. montana* (N1, N3 and N5) grouped together in an own cluster with 100% BS, sister to *Ac. delicata* ML103. The environmental *Acaulospora* sequences from *P*. *oleifolius* formed their own cluster sister to *Ac. alpina* ST2700, *Ac. brasiliensis* W4699/Att1211-0 and *Ac. colliculosa* (91% BS). While all *Acaulospora* sequences stemming from *P. oleifolius* roots with nodules (lateral roots) cluster together in a clade (BS 95%), one *Acaulospora* sequence (CK018-1) stemming from *P. oleifolius* roots without nodules clusters basally to the other sequences.

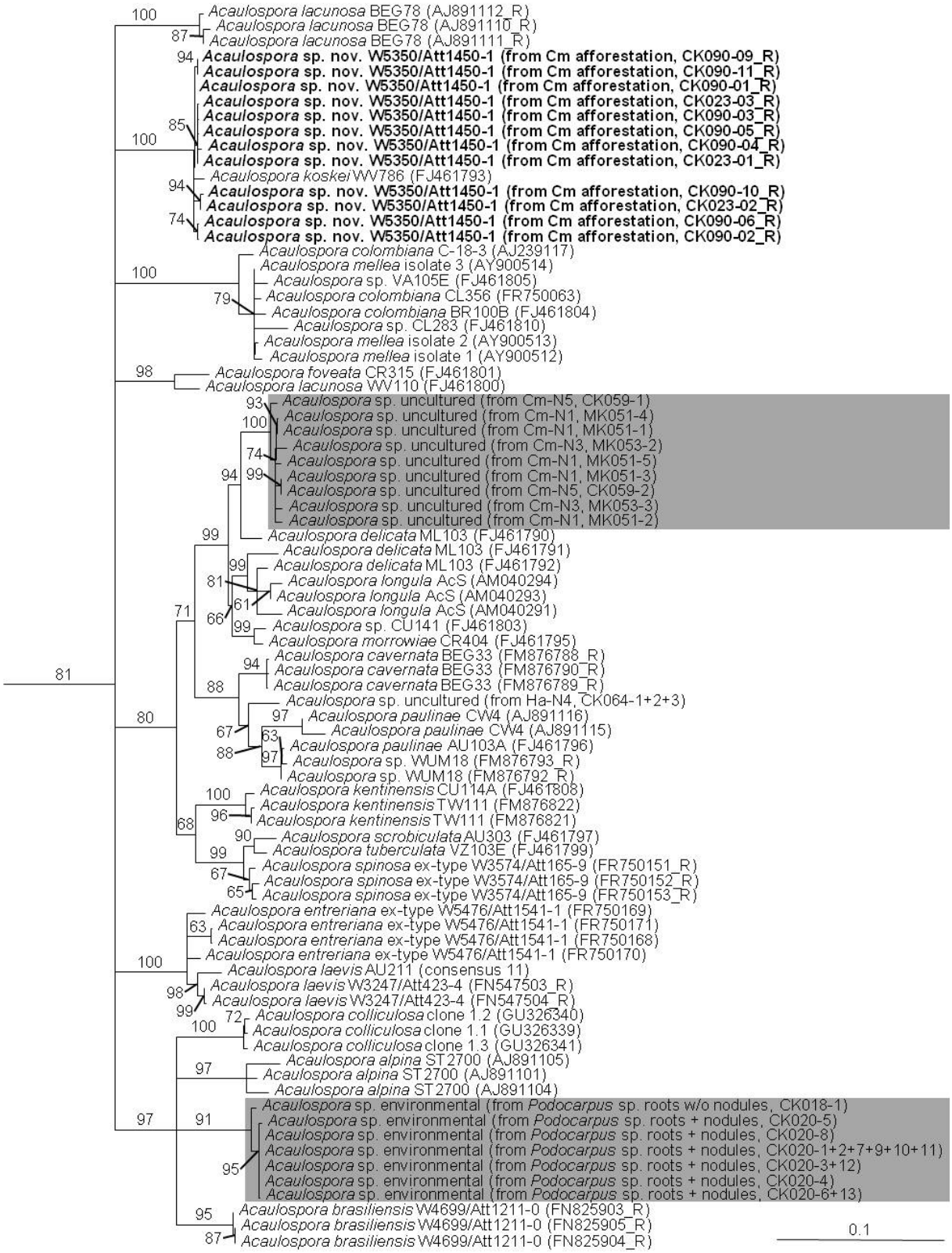


Fig. 15: Maximum likelihood phylogenetic tree of *Acaulosporaceae* including the Ecuadorian sequences. Sequences from Ecuadorian AMF cultures are written in bold, uncultured and environmental sequences are marked by a gray box. Branches with bootstrap support under 60% were reduced to polytomies. The scale bar shows the substitutions per site.

3.1.2.4 *Archaeosporales – Archaeospora* and *Ambispora*

The phylogenetic tree of *Archaeosporaceae* shows several sequences from *C. montana* (N3) and *H. americanus* (N2, N6) stemming from the nursery and sequences from the *Ar. trappei*-like isolates Att1452-6 and Att1456-7 (Fig. 16A). The *Archaeospora*-like sequences originating from *C. montana* N3 formed their own clade supported with 81% BS together with one *Ac. denticulata* sequence CL139-3 (AJ239115). As the *Ac. denticulata* CL139 culture was not available at INVAM and only one public sequence is available the assignment to this species is uncertain. One *Archaeospora* sequence (MK052-6) clusters sister to the remaining sequences, together with *Ac. denticulata* CL139 (AJ239115), whereas all sequences marked as CK011 form their own cluster well supported by 100% BS. At least three different Ecuadorian *Archaeospora* species were detected from nursery roots. The sequences from isolates Att1452-6 (N6) and those from Att1456-7 (N2) clustered together in one clade sister to the Ecuadorian *Archaeospora* sequences from sample N2. Both sequence types formed their own cluster supported with 72% (sequences from Att1456-7 and Att1452-6) and 75% (sequences from nursery roots) it may be that these sequences represent only one or possibly two AMF species. An additional *Archaeospora* sequence (CK012-2+3+4) also from nursery sample 2 (N2) clusters basal to the AMF cultures Att1452-6 and Att1456-7. Two other *Archaeospora* sequences from *H. americanus* (N6) clustered separately with 91% BS sister to a cluster of *Ar. trappei* AU219 and NB112, *Ar. schenkii* W5673/Att212-4 and *Archaeospora* sp. W5791/Att178-3.

The only sequences obtained from *Ambispora* sp. Att1449-12 clustered in an own clade in the *Ambisporaceaea* (BS 90%) unresolved together with *Am. callosa* MAFF520058, *Am. gerdemannii* AU215, *Am. appendicula* NC169-3 and *Am. leptoticha* NC176, FL130, MAFF520055 (Fig. 16B).

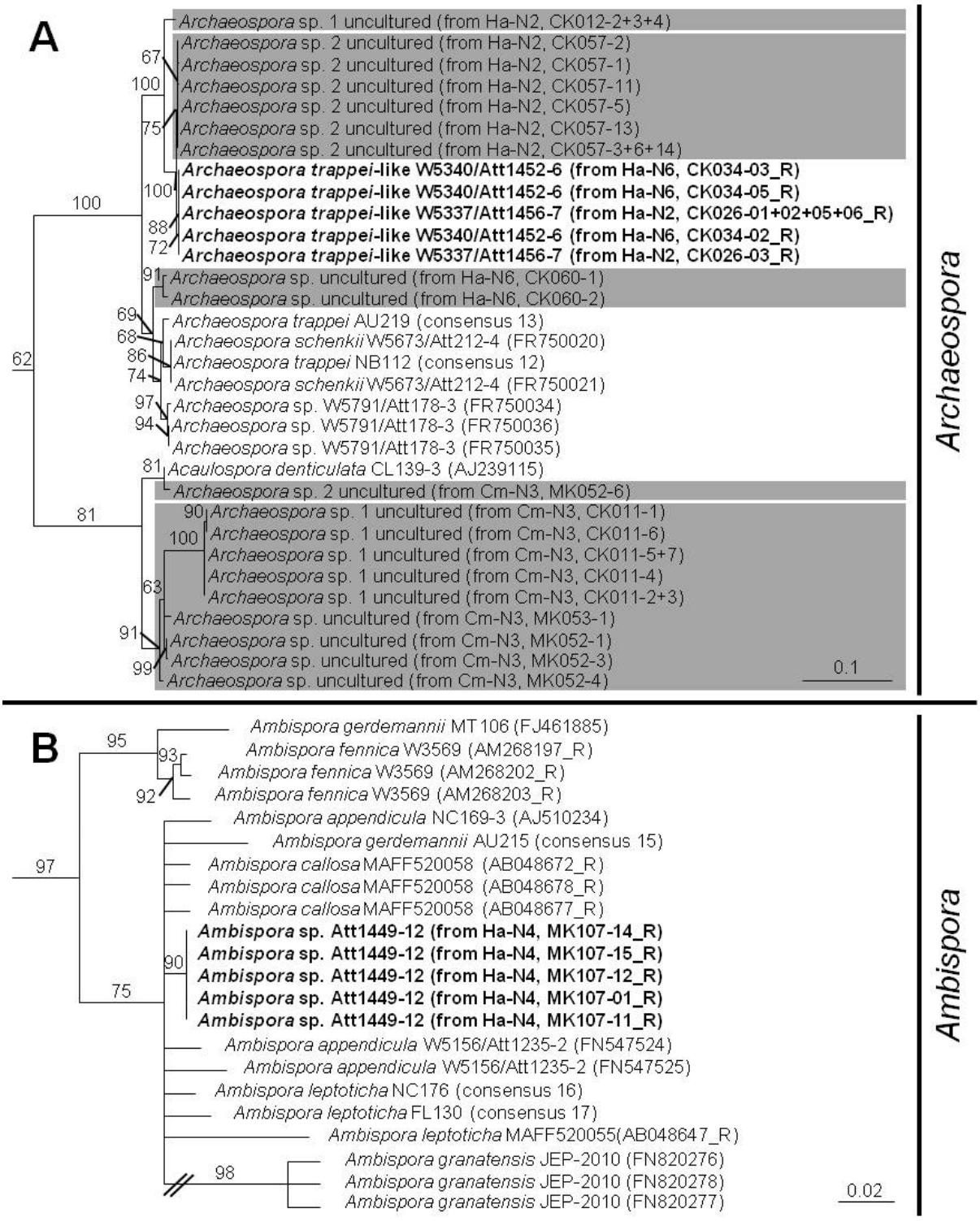


Fig. 16: Maximum likelihood phylogenetic tree of *Archaeosporales* including the Ecuadorian sequences. Sequences from Ecuadorian AMF cultures are written in bold, uncultured and environmental sequences are marked by a gray box. Branches with bootstrap support below 60% were reduced to polytomies. The scale bar shows the substitutions per site. Two diagonal slashes indicate a reduced branch of 50%.

Up to three BLAST hits (date 07.02.2012), which showed the highest similarity to the sequences from Ecuadorian AMF cultures achieved are summarized in Table 3, for further details see also Appendix Table A3. The individual accession numbers used to construct the consensus sequences marked with (consensus #) used in the phylogenetic trees of Fig. 13 - Fig. 16 are listed hereafter. **1:** DQ322629, AY997069, DQ273827. **2**: AJ006799, AJ012113. **3:** AJ012203, AJ012112. **4:** AY635831, AY997052, DQ273790. **5:** DQ322630, AY997054, DQ273828. **6:** AY635833, AY997053, DQ273793. **7:** Y16739, Z14008, AJ239125. **8:** AY635832, AY997088, DQ273792. **9:** AJ871270-73. **10:** AM418543-44. **11:** AJ250847, AJ242499, FJ461802. **12:** AJ006800, AJ243420. **13:** AJ006801, AJ243419. **14:** AM183923, AM183920, AM268204. **15:** AJ012111, AJ510233, AM743187. **16:** AJ301861, AJ006466, AJ006794-97, AJ012109-10. **17:** AJ006793, AJ012201.

			Closest BLAST hits (% similarity)	
Attempt No.	**Type**	**Morphospecies**	**SSU-ITS-LSU fragment (ca. 1.7 kb)**	**SSU fragment (ca. 1.7 kb)**
1449-5	ss	*Diversispora* sp.	*Di. epigaea* BEG47 (96%), *Di. eburnea* W4729 (94% LSU), *Di. aurantia* (93% LSU)	*Di. epigaea* BEG47 (98% SSU), *Diversispora* sp. W2423 (98% SSU)
1449-10	ss	*Claroideoglomus etunicatum*-like	*Cl. claroideum* (91%), uncultured *Claroideoglomus* clone Pa127 (94% SSU/ITS)	*Cl. etunicatum* isolate UFPE06 (99% SSU), *Cl. lamellosum* pWD116-1-2 (99% SSU), *Cl. claroideum* pKL4-2 (99% SSU)
1449-12	ss	*Ambispora* sp.	*Am. gerdemannii* (97% LSU), uncultured *Archaeospora* (96% LSU), *Am. fennica* (87% ITS)	n.d.
1450-1	ss	*Acaulospora* sp. nov.	*Ac. colossica* (99% ITS), uncultured *Acaulospora* sp. (90% LSU)	*Ac. mellea* (93% SSU), *Ac. spinosa* (93% SSU)
1451-6	ms*	*Claroideoglomus etunicatum*-like (prominent sp.)	*Cl. etunicatum* BEG 92 (99% LSU); uncultured *Glomus* clone Pa127 (98% LSU), *Cl. etunicatum* isolate SP208 (96% SSU/ITS)	*Cl. etunicatum* isolate UFPE06 (99% SSU), *Cl. lamellosum* (pWD 116-1-2, 99% SSU), *Cl. claroideum* (pKL4-2, 99% SSU)
			De. heterogama BEG35 (98%), *Scutellospora* sp. hr83 (97% LSU)	n.d.
			Glomus sp. WFVAM23 (95% LSU), *Glomus* sp. MUCL 43203 (93% LSU) (not shown)	n.d.

			Closest BLAST hits (% similarity)	
Attempt No.	**Type**	**Morphospecies**	**SSU-ITS-LSU fragment (ca. 1.7 kb)**	**SSU fragment (ca. 1.7 kb)**
1451-8	rf	*Rhizophagus* sp.	*Rhizophagus* sp. MUCL 43206 (94% LSU), *Rh. irregularis* AFTOL-ID845 (93% LSU), uncultured *Glomus* K142c2 (100% SSU)	Uncultured *Rhizophagus* from *Afrothismia foertheriana* (99% SSU), *Rhizophagus* sp. MUCL 43206 (98% SSU), *Rh. irregularis* pWD164-1-5 & AFTOL-ID845 (97% SSU)
1452-6	ms	*Archaeospora trappei*-like	Uncultured *Archaeospora* (87% SSU/ITS), *Ar. trappei* (85% SSU/ITS)	*Ar. trappei* Att186-1 (97% SSU), *Am. fennica* (93% SSU)
1456-1	rf	*Rhizophagus* sp.	*Rh. diaphanus* (86%), *Rhizophagus* sp. MUCL 43203 (93% LSU)	Uncultured *Rhizophagus* from *Afrothismia foertheriana* (98% SSU), *Rhizophagus* sp. MUCL 43206 (97% SSU), *Rh. irregularis* pWD164-1-5 & AFTOL-ID845 (97% SSU)
1456-7	ss	*Archaeospora trappei*-like	*Ar. trappei* (85% SSU/ITS), uncultured *Archaeospora* (97% SSU/ITS)	*Ar. trappei* Att186-1 (97% SSU), *Am. fennica* (93% SSU)
1456-11	ss	*Claroideoglomus etunicatum*-like	*Cl. claroideum* (95%), *Cl. etunicatum* BEG92 (98% LSU)	*Cl. etunicatum* isolate UFPE06 (99% SSU), *Cl. lamellosum* pWD 116-1-2 (99% SSU), *Cl. luteum* pWD141-1 (99% SSU)
1455-2	ss	*Dentiscutata savannicola*	*Dentiscutata* sp. hr83 (96% LSU), *De. heterogama* isolate AFTOL-ID138 (94% LSU)	*De. heterogama* isolate AFTOL-ID138 (99%), *De. reticulata* (99%), *De. cerradensis* (99%)

Table 9: Closest BLAST hits to the Ecuadorian inoculum culture sequences. ss: Single spore isolate. **ms:** Multispore culture. **rf:** Root fragment culture. **n.d.:** No sequence data. Att1451-6 is marked by*, because it contains at least 3 species. Similarities are given in brackets.

3.2 Nursery experiment No. 3

3.2.1 Efficiency of inoculum tested on *Plantago lanceolata* as host plant

Inoculation with the mixed 'AMF cocktail' increased growth of *P. lanceolata* as host in the Ecuadorian nursery for all inoculum concentrations and at the three different sampling dates (Table 10). The lowest amounts of inoculum (0.5 and 1 g) resulted in similar mycorrhization classes at the different sampling times. Application of higher amounts of AMF inoculum (2 and 4 g) resulted in higher mycorrhization classes. The highest mycorrhization class (III) was achieved by application of 4 g of the AMF inoculum mix. No visible growth differences of the *P. lanceolata* seedlings were observed among the inoculated plants.

Amount of applied inoculum [g]	AMF inoculum mix		
	8 week	10 week	12 week
0.5	I (< 1%)	I (< 1%)	II (< 10%)
1	I (< 1%)	I (< 1%)	II (< 10%)
2	II (< 10%)	II (< 10%)	II (< 10%)
4	II (< 10%)	II (< 10%)	III (< 50%)

Table 10: Average mycorrhization of *Plantago lanceolata* 'test'-cultures. Different amounts of inoculum were applied to *Plantago lanceolata* cultures and checked at three sampling dates. Means resulting from three replicates are given. Mycorrhization rates are given in classes after Trouvelot *et al.* (1986) with the according percentages in brackets.

3.2.2 Inoculum effect in the nursery

Inoculation with the mixed 'AMF cocktail' improved growth of the seedlings according to the tree species transplanted in the Ecuadorian nursery at the two sampling points. 1050 seedlings per each tree species were analyzed on their variance via one-way ANOVA using the Tukey's HSD test. This test was used as it reduces the total error rate (correcting and decreasing the type I errors – rejecting the null hypothesis) and therefore produces less false positives than the Fisher-LSD test. Results for both tests (Fisher and Tukey) are listed in Appendix Table A4.

A mite attack in the nursery after the 3 months sampling, resulted in infection and loss of leaves for almost all *Cedrela montana* and a part of the *Tabebuia chrysantha* seedlings, affecting the data and the statistical analyses. The mean data ± standard errors (SE) for each tree species are summarized in Table 17. In total 21 repetitions (7 replicates in 3 blocks) for each treatment and tree species were set up, standard errors were used to reduce the influence of outliers in the analyses.

3.2.2.1 *Cedrela montana*

During the nursery phase height, RCD and leaves number were significantly increased in all treatments, when compared to the control (Fig. 17). The number of leaves was drastically reduced in the 6 months sampling because of the above noted mite attack (in month 5), causing a heavy leaf fall of all seedlings. Therefore the level of significances for leaf number and area differed in the expected high range between the 3 and 6 months sampling. The 3 months sampling showed significantly increased leaf numbers for all treatments in comparison to the control, the -AMF + LF treatment showed the highest leaf numbers. The 6 months data only showed a significant higher leaf number in the two fertilization treatments (HF and –AMF + LF), whereas both AMF treatments showed the same significance level as the control. Neither fertilization nor AMF significantly increased leaf area. The mycorrhization rate increased significantly in the AMF treatments, but not in the non-AMF treatments. The mortality of the seedlings was significantly higher in the control and HF treatment than in the remaining (-AMF + LF, +AMF + LF and +AMF) treatments.

The *C. montana* seedlings showed no significant increase in fresh weight of the roots, shoot, leaves and root biomass across the treatments. The shoot and leaves biomass showed significant differences in the 6 months sampling only. The -AMF + LF treatment showed the highest significant biomass increase for both shoot and leaves, when compared to the control. The HF as well as both AMF treatments showed a significant shoot biomass increase. Leaves biomass was significantly higher for almost all treatments, except for the +AMF treatment, when compared to the control.

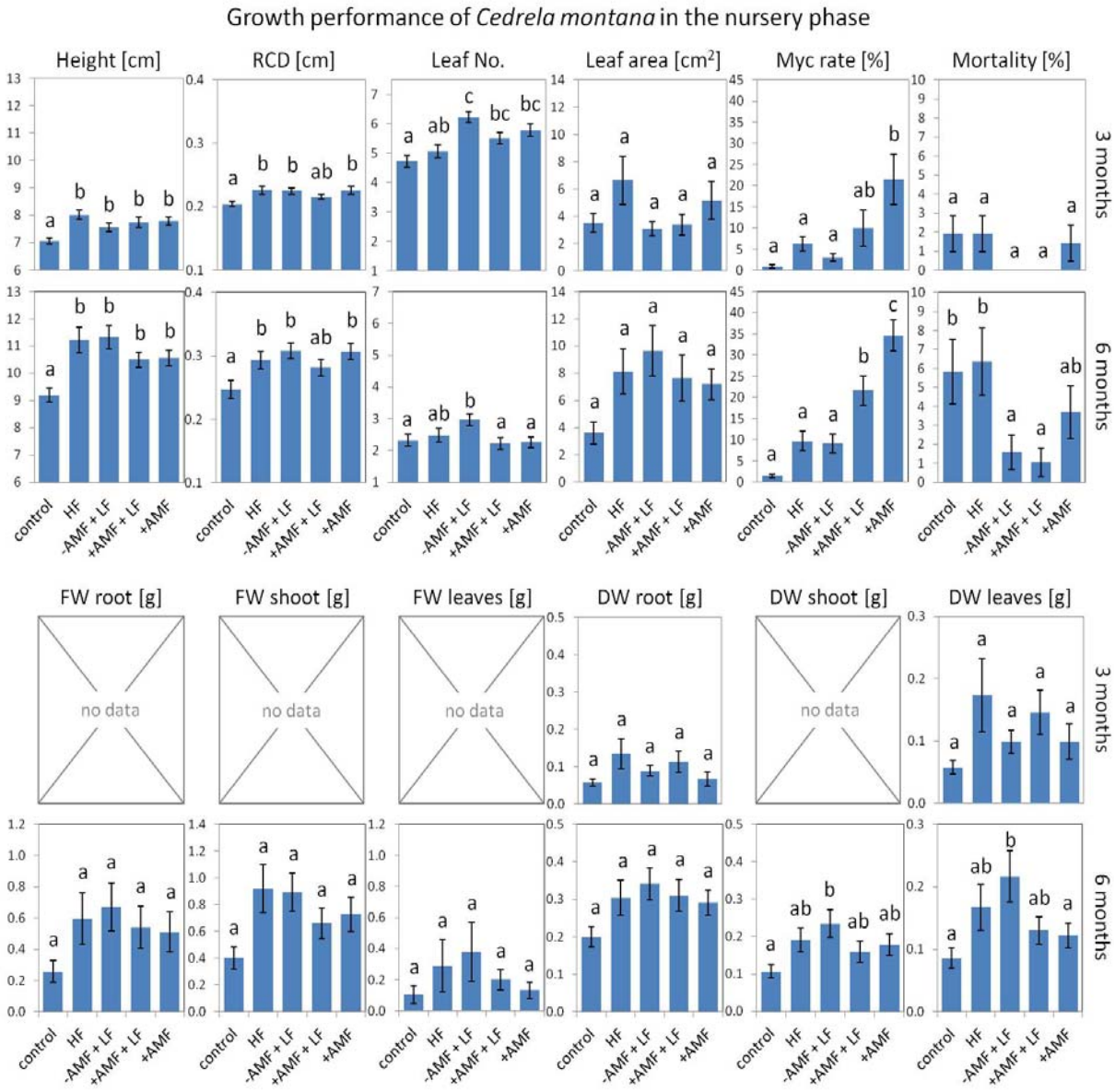


Fig. 17: Growth of *Cedrela montana* in the nursery phase. Units are given in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Means ± SE are illustrated by the scale bars, the letters indicate the level of significances at P<0.05 using the Tukey's-HSD test. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The multifactorial analysis of the two main factors AMF and fertilizer revealed different dependencies (Table 11). Plant height and RCD were dependent on AMF and fertilizer, whereas the leaf number was only dependent on AMF. Biomass of roots and leaves at 3 months, fresh weight and biomass of shoots at 6 months were fertilizer-dependent. AMF showed only relation to the root and leaves biomass after 6 months. Mycorrhization rate was of course dependent on AMF, but also on fertilization.

	AMF		Fertilizer		
Parameter	**3 months**	**6 months**	**3 months**	**6 months**	**Interaction (AMF × Fertilizer)**
Height	****	****	****	****	No output due to non factorial experimental setup.
RCD	***	****	****	****	
Leaf number	****	***	0.1234	0.7600	
Leaf area	0.6198	0.1467	0.1143	0.1046	
FW root	n.d.	0.3575	n.d.	0.2315	
FW shoot	n.d.	0.1229	n.d.	**	
FW leaves	n.d.	0.6060	n.d.	0.5522	
DW root	0.7924	0.2364	*	0.1686	
DW shoot	n.d.	*	n.d.	**	
DW leaves	0.4724	*	*	0.2814	
Mortality	0.8913	0.5300	0.3551	0.3788	
Myc rate	****	****	**	****	

Table 11: ANOVA table of P values of AMF and fertilizer effect on the different growth parameters of *Cedrela montana*. Significant differences marked by: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001). **n.d.:** no data.

The nutrient analysis of *C. montana* leaves showed increasing amounts for almost all nutrients, except for Al, Na, N and H compared to the control (Appendix Table A6). After 6 months, highest amounts of P was measured in the HF and the +AMF treatment (Table 12). Zn was the highest in the control and the HF treatment, but increased also in the +AMF treatment over time (3 to 6 months). In the leaves, phosphor values increased over time, except for the +AMF + LF treatment. Zinc increased in the control, the HF and the +AMF treatment, whereas Zn decreased in the LF treatments independent of inoculation by AMF. The control and the HF treatment showed the highest amounts of Zn after 6 months. The amount of N in the leaves decreased over time in the –AMF + LF and the two +AMF treatments, but increased in the control or stayed at the same level in the HF treatment. Control and +AMF treatments had the highest C values, interestingly the amount of C increased or stayed the same in the +AMF treatments.

In comparison to the control almost all nutrients increased in the seedling roots of *C. montana* during the nursery phase (Appendix Table A6). Al, Fe only increased in the +AMF treatment. The amount of P increased over time (Table 12), the highest values were measured in the HF and –AMF + LF treatments. Also the Zn values increased in all treatments over time, and were the highest in the HF treatment and the control. Nitrogen

amount in roots clearly increased in the HF and -AMF + LF treatment. One could assume that this effect was due to the applied fertilizer. However, the +AMF + LF treatment showed no increase in N. The +AMF treatments showed higher amounts of Ca, Mg, Al, Fe and B after 6 months than –AMF treatments. Furthermore, the amount of C in the +AMF seedling roots was lower than in the -AMF treatments. Increase of C was found in all seedling roots after 6 months independent of treatments.

		Leaves				Roots			
Time point	**Treatment**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**
1	control	1347	48.93	1.90	43.85	1141	150.48	1.58	33.84
1	HF	2322	56.59	3.35	43.34	1672	155.65	1.90	30.09
1	-AMF + LF	1734	54.67	2.69	43.20	1284	143.12	1.95	33.91
1	+AMF + LF	2191	68.67	3.03	44.97	1501	205.82	1.83	34.53
1	+AMF	1816	43.77	2.32	44.15	1595	150.56	1.82	36.26
2	control	2179	61.89	2.35	45.38	1551	377.81	1.63	41.14
2	HF	3035	66.33	3.35	44.84	2455	316.59	2.40	41.58
2	-AMF + LF	2275	47.74	2.06	40.87	2204	395.48	2.48	45.54
2	+AMF + LF	1877	46.87	2.14	45.10	1935	354.31	1.71	40.87
2	+AMF	2452	46.98	1.83	45.25	2091	344.75	1.72	39.09

Table 12: Nutrient analysis of *Cedrela montana* in the nursery phase. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 1 to 2, values in light gray stayed at equal or at a similar level.

3.2.2.2 *Heliocarpus americanus*

A part of the *H. americanus* seedlings (620 plants) was already transferred for hardening to the research station before reaching the age of 6 months in the nursery, due to the fixed time schedule of the forestry group. Therefore, only a reduced set of seedlings (63 plants, 3 in each replicate) per treatment remained in the nursery for the sampling and measurement at 6 months.

Height, RCD, leaf numbers and leaf area increased significantly in the fertilization treatments (HF, -AMF + LF and +AMF + LF) compared to the control (Fig. 18). Only leaf numbers at 3 months showed no significant differences when compared to the control. The +AMF treatment only increased RCD of the seedlings compared to the control. After 6 months leave number and biomass of shoot and root showed even lower values than the control. The +AMF + LF and the HF treatment showed the highest increase in height and RCD. Mycorrhization rate was significantly higher in the AMF than in the non-AMF treatments. No significant difference was observed in mortality.

When fertilized, fresh weight of roots, shoots and root biomass showed significant differences compared to the non-fertilization treatments at the 6 months, but not at the 3 months sampling. The three fertilization treatments showed higher values for all leave parameters (leaf number, area, fresh weight and biomass) than the remaining treatments.

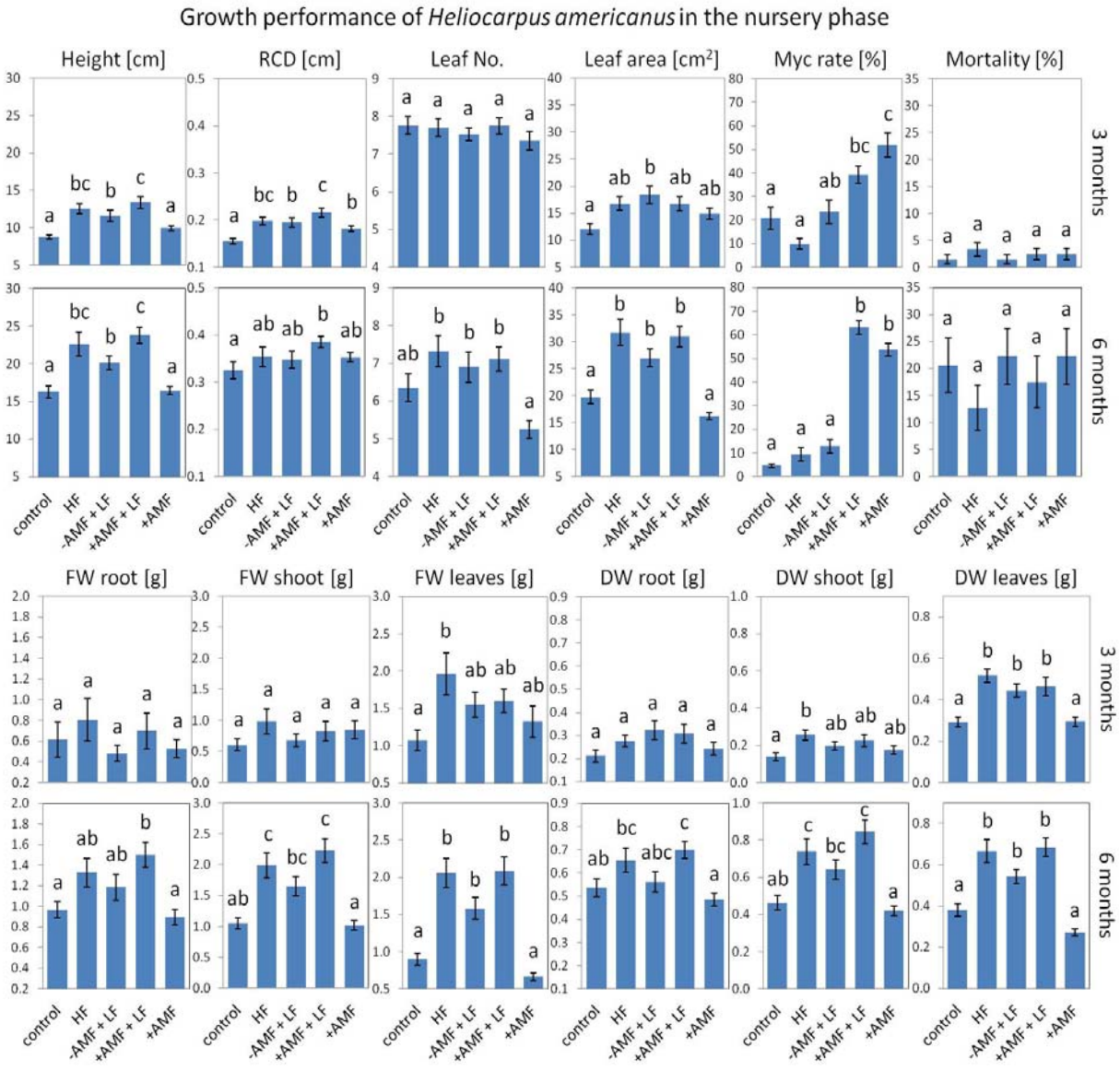


Fig. 18: Growth of *Heliocarpus americanus* in the nursery phase. Units in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Means ± SE are illustrated by the scale bars, the letters indicate the level of significances at P<0.05 using the Tukey's-HSD test. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The two-way ANOVA of the main factors AMF and fertilizer showed the following dependencies (Table 13). After 6 months, all growth parameters except root fresh weight

were AMF-dependent. Fertilization influenced all growth parameters except RCD at the 6 months sampling. Mycorrhization rate depended on AMF and fertilizer.

	AMF		Fertilizer		
Parameter	**3 months**	**6 months**	**3 months**	**6 months**	**Interaction (AMF × Fertilizer)**
Height	****	***	****	****	No output due to non-factorial experimental setup.
RCD	****	*	****	0.1281	
Leaf number	0.1574	*	0.2682	****	
Leaf area	0.2070	*	**	****	
FW root	0.5888	0.1359	0.5306	****	
FW shoot	0.4341	**	0.2338	****	
FW leaves	0.6889	**	**	****	
DW root	0.7621	**	0.1496	****	
DW shoot	0.4370	**	***	****	
DW leaves	0.9153	***	****	****	
Mortality	0.7667	*	0.4276	*	
Myc rate	****	****	**	**	

Table 13: ANOVA table with P values of the main factors AMF and fertilizer achieved via multifactorial analysis of variance for the different growth parameters of *Heliocarpus americanus*. Significant differences marked by * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001).

Nutrient analysis of leaves of the *H. americanus* seedlings showed only increases in Mn, Na and B in almost all treatments after 6 months, whereas other nutrients decreased over time. However, Ca, Mg, Al, Fe, Zn and N increased over time in the +AMF treatments (3 to 6 months, Appendix Table A7). The amount of P decreased over time in all treatments except in the control (Table 14). The +AMF treatments showed the highest values of P at the 3 months sampling. This changed in the 6 months sampling, where the control and the +AMF+ LF treatment showed the highest amount of P. The amount of Zn decreased in all fertilization treatments. After 6 months, the control and the +AMF treatment showed the highest Zn values. The highest values of N and C were measured in the fertilization treatments (HF, -AMF + LF and +AMF + LF), and the lowest in the control and the +AMF treatment.

Almost all nutrient values decreased in the roots of *H. americanus* over time (3 to 6 months, Appendix Table A7), except for the amount of B which increased in all treatments. Additionally, the amounts of Al, Cu, Na and S increased in the –AMF + LF treatment. The

control showed an increase of Cu and the HF treatment in Na. The highest amounts of P were found in the +AMF treatments. Zinc was high in the +AMF+ LF treatment and in the control. The amount of N was highest in the +AMF+ LF and the HF treatment, whereas the amount of C had its maximum in the –AMF + LF and the +AMF treatments (Table 14).

		Leaves				Roots			
Time point	**Treatment**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**
1	control	1843	154.19	3.07	43.33	1669	75.26	1.95	38.63
1	HF	2092	98.87	4.03	43.27	1449	72.11	2.44	36.35
1	-AMF + LF	1662	63.47	3.56	43.67	2331	99.18	1.91	40.26
1	+AMF + LF	2408	117.68	2.11	42.60	1896	77.04	1.89	39.27
1	+AMF	2506	79.45	3.40	43.08	2092	71.40	1.60	39.22
2	control	1866	144.50	2.29	40.81	1266	44.32	1.40	35.45
2	HF	1503	77.90	3.13	41.63	1277	35.37	1.60	35.14
2	-AMF + LF	1509	64.15	2.88	42.03	1158	29.55	1.54	38.42
2	+AMF + LF	1845	67.61	2.58	41.30	1671	45.95	1.64	35.84
2	+AMF	1669	117.50	1.89	39.85	1675	38.01	1.29	37.95

Table 14: Nutrient analysis of *Heliocarpus americanus* in the nursery phase. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 1 to 2, values in light gray stayed at equal or at a similar level.

3.2.2.3 *Tabebuia chrysantha*

Tabebuia chrysantha seedlings showed a significant increase in height, RCD, leaf number and leaf area in the fertilization treatments (HF, -AMF + LF and +AMF + LF), especially in the HF treatment at the 3 months sampling (Fig. 19). This changed after 6 months when the +AMF + LF treatment showed similar data for height and RCD as the HF treatment. The highest values of leaf number and leaf area were measured in the +AMF + LF treatment at the 6 months sampling. The mycorrhization rate was significantly higher in the AMF than in the non-AMF treatments. Mortality was significantly higher in the non-AMF treatments after 3 months, but not after 6 months. Fresh weight and biomass showed a significant increase in the fertilization treatments. The +AMF + LF treatment performed best or similar to the HF treatment, especially after 6 months.

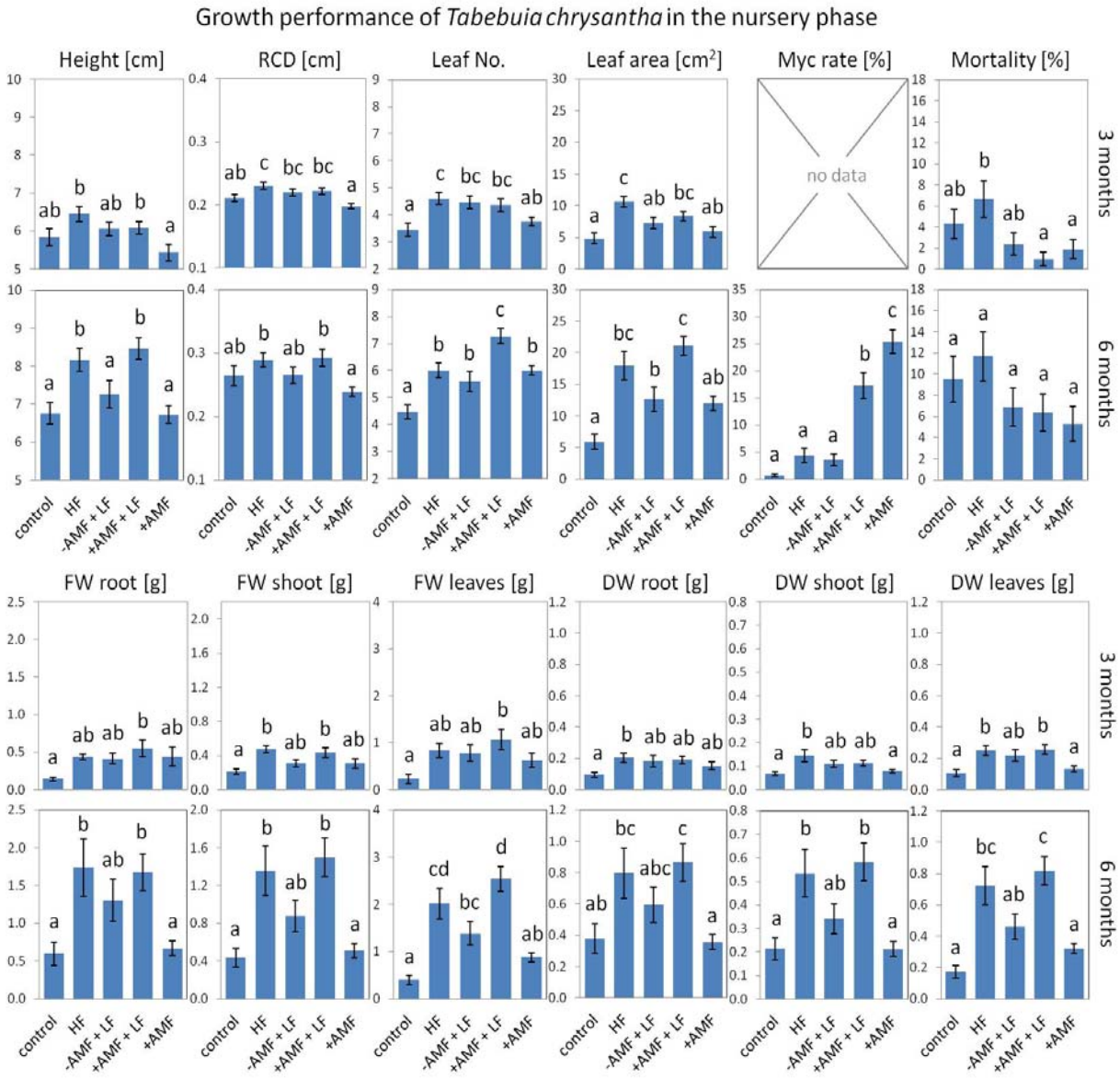


Fig. 19: Growth of *Tabebuia chrysantha* in the nursery phase. Units of the diagrams are written in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Means ± SE are illustrated by the scale bars, the letters indicate the level of significances at P<0.05 using the Tukey's-HSD test. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The multifactorial analysis of variance of the main factors AMF and fertilizer showed that *H. americanus* was more fertilizer-dependent than AMF-dependent, especially for root fresh weight and biomass (Table 15). No dependency was observed for mortality.

	AMF		Fertilizer		
Parameter	**3 months**	**6 months**	**3 months**	**6 months**	**Interaction (AMF × Fertilizer)**
Height	0.1413	***	****	****	No output due to non-factorial experimental setup.
RCD	*	**	****	****	
Leaf number	0.2184	****	****	****	
Leaf area	0.4505	****	****	****	
FW root	**	0.5683	*	****	
FW shoot	*	**	****	****	
FW leaves	0.1375	****	**	****	
DW root	0.3288	0.2509	**	****	
DW shoot	0.8782	**	****	****	
DW leaves	0.5299	***	****	****	
Mortality	0.2190	0.4967	0.3845	0.6288	
Myc rate	n.d.	****	n.d.	***	

Table 15: ANOVA table with P values of the main factors AMF and fertilizer achieved via multifactorial analysis of variance for the different growth parameters of *Tabebuia chrysantha*. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001). **n.d.:** no data.

Nutrient analysis of the leaves of *T. chrysantha* showed increased values of Ca, Mn, and B over time (3 to 6 months, Appendix Table A8) in almost all treatments. Additionally, increased values of Mg, Al, Fe and P were found in the +AMF treatments. The amount of P was higher in the fertilization treatments after 3 months, but increased in both +AMF treatments and showed the highest values at the second sampling (6 months, Table 16). The amount of Zn was the highest in the +AMF treatment during the whole nursery phase. N and C decreased over time. N showed the highest values in the HF and +AMF + LF treatments, whereas the percentage of C was the highest in the HF treatment and the control after 6 months.

A similar situation as in leaves occurred in the element analyses of the roots. Only Cu, B, S and C increased in all treatments. Amounts of Mg and Fe increased in the control treatment over time. The two +AMF treatments showed increases in Fe, Na, P and Zn over time (3 to 6 months, Appendix Table A8). The amount of phosphor increased in both +AMF treatments from the first to the second sampling, whereas P decreased in the -AMF treatments (Table 16). Although Zn increased only in the +AMF + LF treatment, the highest value was detected in the control treatment. The percentage of N decreased over time, whereas C

increased. The highest amount of N was detected in the HF treatment, the highest C in the –AMF + LF and HF treatment after 6 months.

		Leaves				Roots			
Time point	**Treatment**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**
1	control	1437	59.42	2.61	46.38	1006	156.19	1.55	34.64
1	HF	1566	44.03	2.94	45.44	1223	69.76	1.99	33.33
1	-AMF + LF	1539	43.91	2.73	45.63	1339	101.47	1.83	33.05
1	+AMF + LF	1550	46.98	2.58	45.49	1247	80.42	1.85	34.52
1	+AMF	1461	66.14	2.32	45.76	887	121.14	1.56	34.26
2	control	947	26.50	1.91	44.49	758	88.46	1.17	34.57
2	HF	1430	17.29	2.27	44.77	1122	54.31	1.60	35.62
2	-AMF + LF	1493	22.27	1.98	44.07	1287	56.03	1.47	37.06
2	+AMF + LF	1596	18.22	2.12	44.40	1503	87.66	1.51	30.21
2	+AMF	1542	27.34	2.02	44.31	1330	80.86	1.48	34.79

Table 16: Nutrient analysis of *Tabebuia chrysantha* in the nursery phase. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 1 to 2, values in light gray stayed at equal or at a similar level.

Cedrela montana

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
1	T0 - control	7.1 ± 0.11 a	0.20 ± 0.004 a	5 ± 0.19 a	3.53 ± 0.685 a	0.90 ± 0.359 a	1.90 ± 0.940 a
1	T1 - HF	8.0 ± 0.17 b	0.23 ± 0.006 b	5 ± 0.21 ab	6.64 ± 1.773 a	6.20 ± 1.690 a	1.90 ± 0.940 a
1	T2 - -AMF + LF	7.6 ± 0.16 b	0.22 ± 0.005 b	6 ± 0.18 c	3.09 ± 0.511 a	3.00 ± 0.800 a	0.00 ± 0.000 a
1	T3 - +AMF + LF	7.7 ± 0.17 b	0.22 ± 0.004 ab	6 ± 0.19 bc	3.37 ± 0.783 ab	10.00 ± 4.169 ab	0.00 ± 0.000 a
1	T4 - +AMF	7.8 ± 0.14 b	0.23 ± 0.006 b	6 ± 0.21 bc	5.16 ± 1.406 b	21.50 ± 5.886 b	1.43 ± 0.940 a
2	T0 - control	9.2 ± 0.24 a	0.25 ± 0.014 a	2 ± 0.19 a	3.63 ± 0.821 a	1.46 ± 0.431 a	5.82 ± 1.700 b
2	T1 - HF	11.2 ± 0.47 b	0.29 ± 0.014 b	2 ± 0.21 ab	8.13 ± 1.664 a	9.70 ± 2.326 a	6.35 ± 1.770 b
2	T2 - -AMF + LF	11.3 ± 0.42 b	0.31 ± 0.012 b	3 ± 0.19 bc	9.65 ± 1.839 a	9.09 ± 2.231 a	1.59 ± 0.910 a
2	T3 - +AMF + LF	10.5 ± 0.28 b	0.28 ± 0.013 ab	2 ± 0.18 a	7.66 ± 1.689 a	21.63 ± 3.508 b	1.06 ± 0.740 a
2	T4 - +AMF	10.6 ± 0.28 b	0.31 ± 0.013 b	2 ± 0.16 a	7.20 ± 1.140 a	34.63 ± 3.615 c	3.70 ± 1.370 ab
Sample time	**Treatment**	**FW root**	**FW shoot**	**FW leaves**	**DW root***	**DW shoot**	**DW leaves**
1	T0 - control	n.d.	n.d.	n.d.	0.06 ± 0.010 a	n.d.	0.06 ± 0.011 a
1	T1 - HF	n.d.	n.d.	n.d.	0.13 ± 0.041 a	n.d.	0.17 ± 0.059 a
1	T2 - -AMF + LF	n.d.	n.d.	n.d.	0.09 ± 0.014 a	n.d.	0.10 ± 0.019 a
1	T3 - +AMF + LF	n.d.	n.d.	n.d.	0.11 ± 0.029 a	n.d.	0.15 ± 0.035 a
1	T4 - +AMF	n.d.	n.d.	n.d.	0.07 ± 0.020 a	n.d.	0.10 ± 0.028 a
2	T0 - control	0.26 ± 0.069 a	0.40 ± 0.082 a	0.11 ± 0.058 a	0.20 ± 0.028 a	0.11 ± 0.018 a	0.09 ± 0.017 a
2	T1 - HF	0.60 ± 0.166 a	0.92 ± 0.179 a	0.29 ± 0.170 a	0.30 ± 0.047 a	0.19 ± 0.032 ab	0.17 ± 0.036 ab
2	T2 - -AMF + LF	0.67 ± 0.155 a	0.89 ± 0.141 a	0.38 ± 0.191 a	0.34 ± 0.042 a	0.23 ± 0.037 b	0.22 ± 0.041 b
2	T3 - +AMF + LF	0.54 ± 0.136 a	0.66 ± 0.112 a	0.20 ± 0.065 a	0.31 ± 0.043 a	0.16 ± 0.028 ab	0.13 ± 0.022 ab
2	T4 - +AMF	0.51 ± 0.128 a	0.73 ± 0.129 a	0.13 ± 0.051 a	0.29 ± 0.033 a	0.18 ± 0.029 ab	0.12 ± 0.020 a

Heliocarpus americanus

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
1	T0 - control	8.8 ± 0.28 a	0.15 ± 0.006 a	8 ± 0.24 a	12.04 ± 1.016 a	20.75 ± 4.583 a	1.43 ± 0.819 a
1	T1 - HF	12.5 ± 0.70 bc	0.20 ± 0.009 bc	8 ± 0.23 a	16.77 ± 1.259 ab	9.87 ± 2.245 a	3.33 ± 1.239 a

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
1	T2 - -AMF + LF	11.6 ± 0.75 b	0.19 ± 0.010 b	8 ± 0.17 a	18.38 ± 1.642 b	23.47 ± 4.899 ab	1.43 ± 0.819 a
1	T3 - +AMF + LF	13.3 ± 0.80 c	0.22 ± 0.010 c	8 ± 0.22 a	16.75 ± 1.271 ab	39.28 ± 3.719 bc	2.38 ± 1.052 a
1	T4 - +AMF	10.0 ± 0.35 a	0.18 ± 0.006 b	7 ± 0.25 a	14.88 ± 1.009 ab	51.98 ± 5.106 c	2.38 ± 1.052 a
2	T0 - control	16.3 ± 0.81 a	0.33 ± 0.018 a	6 ± 0.37 ab	19.74 ± 1.270 a	4.59 ± 0.694 a	20.63 ± 5.099 a
2	T1 - HF	22.6 ± 1.61 bc	0.35 ± 0.021 ab	7 ± 0.41 b	31.69 ± 2.454 b	9.32 ± 2.759 a	12.70 ± 4.195 a
2	T2 - -AMF + LF	20.1 ± 0.92 b	0.35 ± 0.018 ab	7 ± 0.40 b	26.98 ± 1.693 b	12.76 ± 2.888 a	22.22 ± 5.238 a
2	T3 - +AMF + LF	23.8 ± 1.09 c	0.39 ± 0.011 b	7 ± 0.33 b	30.99 ± 1.915 b	63.14 ± 2.917 b	17.46 ± 4.783 a
2	T4 - +AMF	16.4 ± 0.50 a	0.35 ± 0.010 ab	5 ± 0.23 a	16.11 ± 0.663 a	53.73 ± 2.699 b	22.22 ± 5.238 a
Sample time	**Treatment**	**FW root**	**FW shoot**	**FW leaves**	**DW root***	**DW shoot**	**DW leaves**
1	T0 - control	0.61 ± 0.167 a	0.61 ± 0.097 a	1.07 ± 0.143 a	0.21 ± 0.026 a	0.14 ± 0.020 a	0.29 ± 0.023 a
1	T1 - HF	0.81 ± 0.207 a	0.98 ± 0.201 a	1.96 ± 0.279 b	0.28 ± 0.024 a	0.26 ± 0.028 b	0.52 ± 0.032 b
1	T2 - -AMF + LF	0.48 ± 0.079 a	0.68 ± 0.097 a	1.55 ± 0.167 ab	0.32 ± 0.040 a	0.20 ± 0.022 ab	0.44 ± 0.033 b
1	T3 - +AMF + LF	0.70 ± 0.170 a	0.83 ± 0.154 a	1.60 ± 0.155 ab	0.31 ± 0.040 a	0.23 ± 0.031 ab	0.46 ± 0.044 b
1	T4 - +AMF	0.53 ± 0.087 a	0.85 ± 0.143 a	1.32 ± 0.209 ab	0.24 ± 0.027 a	0.18 ± 0.022 ab	0.29 ± 0.022 a
2	T0 - control	0.97 ± 0.079 a	1.05 ± 0.089 ab	0.90 ± 0.082 a	0.54 ± 0.040 ab	0.46 ± 0.039 ab	0.38 ± 0.030 a
2	T1 - HF	1.33 ± 0.140 ab	1.99 ± 0.203 c	2.06 ± 0.193 b	0.66 ± 0.053 bc	0.74 ± 0.068 c	0.66 ± 0.056 b
2	T2 - -AMF + LF	1.19 ± 0.126 ab	1.65 ± 0.155 bc	1.58 ± 0.142 b	0.56 ± 0.044 abc	0.64 ± 0.053 bc	0.54 ± 0.034 b
2	T3 - +AMF + LF	1.50 ± 0.121 b	2.22 ± 0.193 c	2.08 ± 0.191 b	0.70 ± 0.035 c	0.84 ± 0.064 c	0.69 ± 0.044 b
2	T4 - +AMF	0.90 ± 0.074 a	1.01 ± 0.080 a	0.66 ± 0.054 a	0.48 ± 0.026 a	0.42 ± 0.028 a	0.27 ± 0.017 a

Tabebuia chrysantha

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
1	T0 - control	5.8 ± 0.23 ab	0.21 ± 0.006 ab	3 ± 0.26 a	4.85 ± 0.825 a	n.d.	4.29 ± 1.398 ab
1	T1 - HF	6.5 ± 0.20 b	0.23 ± 0.006 c	5 ± 0.22 c	10.60 ± 0.820 c	n.d.	6.67 ± 1.721 b
1	T2 - -AMF + LF	6.1 ± 0.19 ab	0.22 ± 0.006 bc	4 ± 0.24 bc	7.24 ± 0.894 ab	n.d.	2.38 ± 1.052 ab
1	T3 - +AMF + LF	6.1 ± 0.17 b	0.22 ± 0.005 bc	4 ± 0.24 bc	8.36 ± 0.690 bc	n.d.	0.95 ± 0.670 a
1	T4 - +AMF	5.4 ± 0.22 a	0.20 ± 0.004 a	4 ± 0.16 ab	5.87 ± 0.871 ab	n.d.	1.90 ± 0.943 a

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
2	T0 - control	6.8 ± 0.28 a	0.26 ± 0.016 ab	4 ± 0.28 a	5.91 ± 1.171 a	0.69 ± 0.272 a	9.52 ± 2.135 a
2	T1 - HF	8.2 ± 0.30 b	0.29 ± 0.012 b	6 ± 0.28 b	17.95 ± 2.225 bc	4.33 ± 1.329 b	11.64 ± 2.333 a
2	T2 - -AMF + LF	7.3 ± 0.36 a	0.26 ± 0.013 ab	6 ± 0.37 b	12.66 ± 1.871 bc	3.55 ± 1.047 ab	6.88 ± 1.841 a
2	T3 - +AMF + LF	8.5 ± 0.28 b	0.29 ± 0.014 b	7 ± 0.28 c	21.08 ± 1.493 c	17.22 ± 2.390 ab	6.35 ± 1.774 a
2	T4 - +AMF	6.7 ± 0.24 a	0.24 ± 0.007 a	6 ± 0.18 b	12.02 ± 1.126 ab	25.32 ± 2.188 a	5.29 ± 1.628 a
Sample time	**Treatment**	**FW root**	**FW shoot**	**FW leaves**	**DW root***	**DW shoot**	**DW leaves**
1	T0 - control	0.14 ± 0.024 a	0.21 ± 0.034 a	0.23 ± 0.094 a	0.09 ± 0.015 a	0.07 ± 0.007 a	0.11 ± 0.024 a
1	T1 - HF	0.44 ± 0.040 ab	0.48 ± 0.041 b	0.84 ± 0.147 ab	0.20 ± 0.029 b	0.14 ± 0.025 b	0.25 ± 0.030 b
1	T2 - -AMF + LF	0.41 ± 0.073 ab	0.31 ± 0.041 ab	0.78 ± 0.174 ab	0.18 ± 0.037 ab	0.11 ± 0.015 ab	0.22 ± 0.036 ab
1	T3 - +AMF + LF	0.55 ± 0.113 b	0.43 ± 0.056 b	1.07 ± 0.215 b	0.19 ± 0.025 ab	0.11 ± 0.012 ab	0.26 ± 0.029 b
1	T4 - +AMF	0.44 ± 0.125 ab	0.31 ± 0.054 ab	0.63 ± 0.152 ab	0.15 ± 0.024 ab	0.08 ± 0.008 a	0.13 ± 0.020 a
2	T0 - control	0.60 ± 0.156 a	0.43 ± 0.101 a	0.40 ± 0.099 a	0.38 ± 0.096 ab	0.21 ± 0.046 a	0.17 ± 0.041 a
2	T1 - HF	1.74 ± 0.378 b	1.36 ± 0.263 b	2.02 ± 0.322 cd	0.80 ± 0.159 bc	0.53 ± 0.101 b	0.72 ± 0.121 bc
2	T2 - -AMF + LF	1.31 ± 0.274 ab	0.88 ± 0.162 ab	1.38 ± 0.249 bc	0.60 ± 0.114 abc	0.34 ± 0.062 ab	0.46 ± 0.082 ab
2	T3 - +AMF + LF	1.68 ± 0.243 b	1.50 ± 0.202 b	2.54 ± 0.267 d	0.86 ± 0.121 c	0.58 ± 0.079 b	0.82 ± 0.090 c
2	T4 - +AMF	0.67 ± 0.100 a	0.51 ± 0.077 a	0.88 ± 0.098 ab	0.36 ± 0.048 a	0.21 ± 0.031 a	0.32 ± 0.032 a

Table 17: Growth of *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* in the nursery phase. Mean ± SE are shown. Significantly differences between measured data across the treatments were tested via the Tukey's HSD test ($P<0.05$) and are marked with different letters. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. **n.d.:** no data.

3.2.3 Reforestation success by AMF inoculation

Due to the different transplanting dates and hardening times of the tree species all tree seedlings had different ages when out-planted (see Table 18).

	Tree species					
	Cedrela montana		*Heliocarpus americanus*		*Tabebuia chrysantha*	
Sampling date	**Age of seedlings**	**Time after out-planting**	**Age of seedlings**	**Time after out-planting**	**Age of seedlings**	**Time after out-planting**
Out-planting	12 mo.	0 mo.	7 mo.	0 mo.	7 mo.	0 mo.
Jun. 2009	15 mo.	3 mo.	13 mo.	6 mo.	12 mo.	5 mo.
Nov. 2009	18 mo.	6 mo.	16 mo.	9 mo.	15 mo.	8 mo.

Table 18: Age of seedlings (in months) after out-planting on the reforestation plots. Out-planting took place at different time points. *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* were out-planted in March 2009, December 2008 and January 2009.

Up to 20% 'background' mycorrhization of non-applied AMF was detected in all treatments during the nursery phase, which may affect data collected and resulting in less significant effects of plant performance.

3.2.3.1 *Cedrela montana*

Due to the low sampling size and the high variation of the measured data no significant differences in growth parameters via the Tukey's HSD test were obtained. Fisher-LSD test showed some significant differences for RCD and root fresh weight in June 2009 and for height and leaf area in November 2009 (for detailed statistical results see Appendix Table A4). Since the Fisher test is more error prone than the Tukey's HSD test, the results are treated as tendencies in plant performance of the tree seedlings. Following these trends, the seedlings in the -AMF + LF treatment showed the highest increase in height, RCD and leaf area (Fig. 20). The other treatments (HF, +AMF + LF and +AMF) showed also increase in these parameters when compared to the control. A different tendency was found for root fresh weight where the HF and both +AMF treatments showed an increase, and the -AMF + LF treatment was at same level as the control.

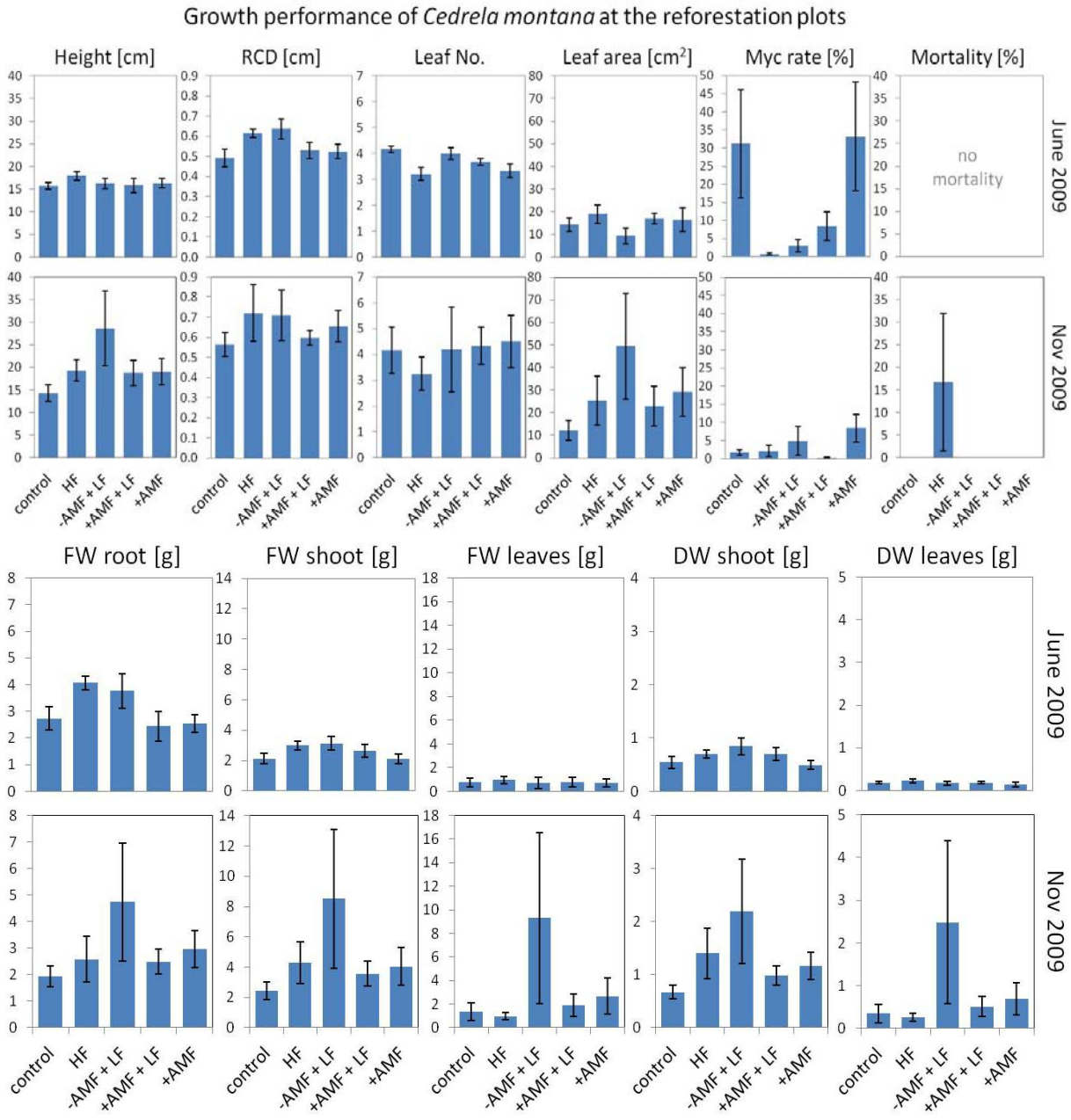


Fig. 20: Growth of *Cedrela montana* on the reforestation plots. Units are written in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Means ± SE are illustrated by the scale bars, the Tukey's HSD test showed no significances at P<0.05. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The multifactorial ANOVA showed dependency of mycorrhization rate on fertilizer at June 2009-sampling. No other dependencies were observed (Table 19).

	AMF		Fertilizer		
Parameter	**Jun 09**	**Nov 09**	**Jun 09**	**Nov 09**	**Interaction**
Height	0.9193	0.2954	0.4674	0.7783	No output due to non-factorial experimental setup.
RCD	0.2069	0.6092	0.1587	0.5475	
Leaf number	0.5942	0.8539	0.2303	0.6454	
Leaf area	0.8679	0.2175	0.7132	0.7777	
FW root	0.1983	0.3965	0.1996	0.9082	
FW shoot	0.7089	0.3512	0.2526	0.8727	
FW leaves	0.9586	0.2520	0.7855	0.9769	
DW shoot	0.6844	0.2759	0.3585	0.6663	
DW leaves	0.8569	0.2412	0.7120	0.9815	
Mortality	d.e.	1.0000	d.e.	0.3038	
Myc rate	0.9230	0.1731	*	0.1947	

Table 19: ANOVA table with P values of the main factors: AMF and fertilizer achieved via multifactorial analysis of variance for the different growth parameters of *Cedrela montana*. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001). **d.e.:** data values are equal, thus no dependency could be calculated.

The nutrient analysis of *C. montana* leaves in the reforestation phase showed increases of K, Ca, Mg, Al, Cu, Fe, P, Zn, B and C in almost all treatments (Appendix Table A6). The highest values of Mn were measured in the +AMF treatments. The amount of P was highest at the first sampling in the control and the +AMF treatments. Amount changed in the second sampling where the –AMF + LF treatment and the +AMF treatment showed the highest values (Table 20). Increase in Zn were only detected in the –AMF + LF and the two +AMF treatments, but the control treatment and the +AMF treatment showed the highest zinc values. The percentage of N increased only in the –AMF + LF treatment, whereas the amount of C increased in general, except for the +AMF + LF treatment. The highest percentage of N and C was measured in the –AMF + LF treatment after 6 months.

Time point	Treatment	P [µg/g]	Zn [µg/g]	N [%]	C [%]
3	control	2929	308.90	2.88	44.63
3	HF	2227	162.70	2.31	44.87
3	-AMF + LF	2453	81.56	2.13	44.72
3	+AMF + LF	2125	91.21	2.22	45.35
3	+AMF	3375	135.20	2.55	44.29
4	control	2555	213.80	2.20	45.15
4	HF	2548	108.60	1.82	45.52
4	-AMF + LF	4092	157.60	2.79	46.27
4	+AMF + LF	2239	135.90	2.19	44.92
4	+AMF	3069	259.00	2.50	45.92

Table 20: Nutrient analysis of *Cedrela montana* leaves in the reforestation phase, 3 and 6 months after out-planting. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 3 to 4, values in light gray stayed at equal or at a similar level.

3.2.3.2 *Heliocarpus americanus*

The seedlings of *H. americanus* showed only significant differences in mycorrhization rate at both samplings and in the shoot fresh weight in June 2009 (Fig. 21). The +AMF treatment had the highest mycorrhization rate in the June 2009-sampling, whereas the other treatments and the control showed no significant differences in mycorrhization rate. In November 2009 mycorrhization rate in the -AMF + LF treatment dropped and was even lower than the control. Shoot fresh weight showed the highest value in the HF treatment, also the seedlings of the other treatment performed significantly better than the control in November 2009. Due to the high variation and the low number of samples (4) in the +AMF treatments the statistical analysis showed no significant differences.

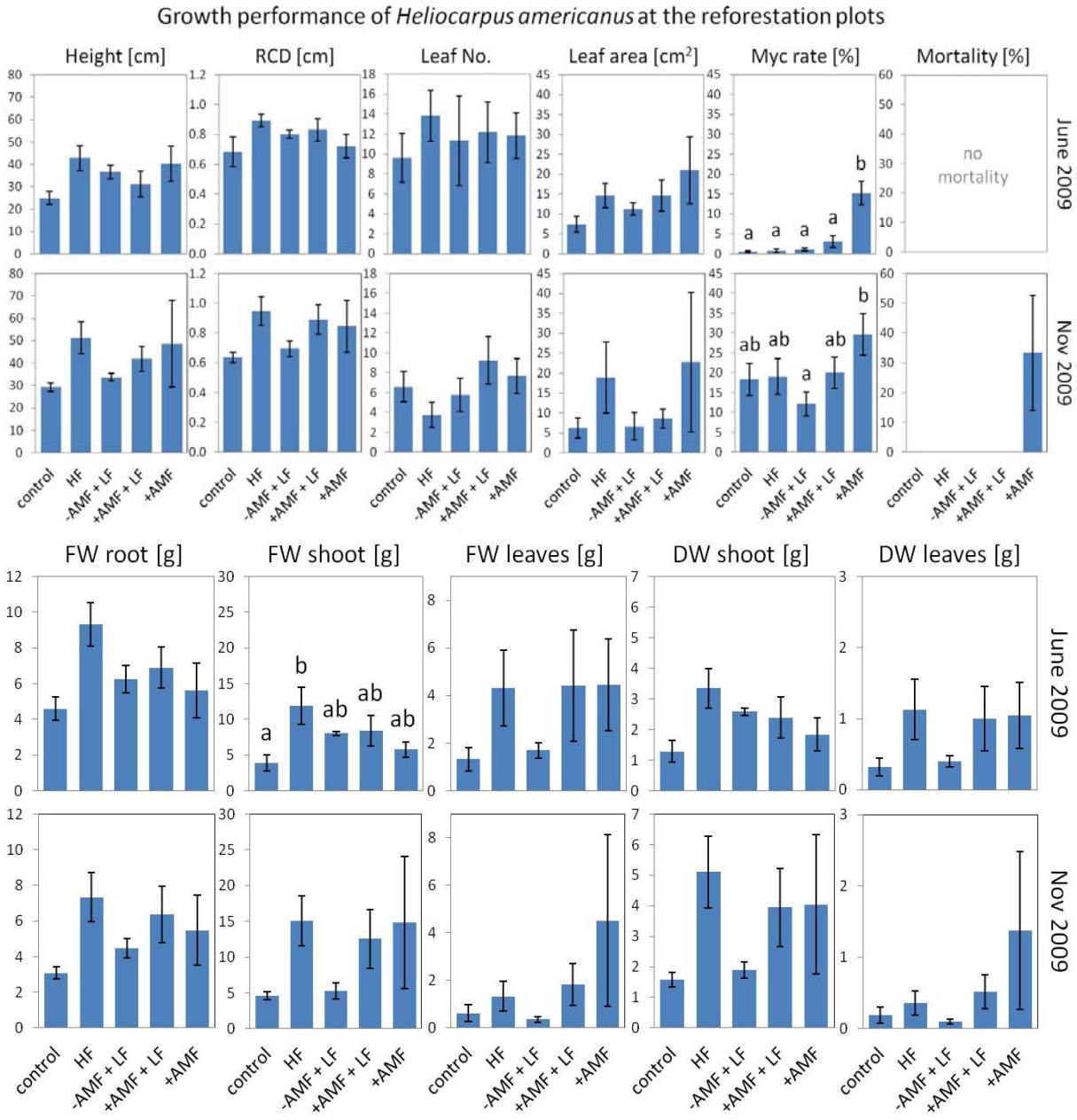


Fig. 21: Growth of *Heliocarpus americanus* on the reforestation plots. Units are written in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Means ± SE are illustrated by the scale bars, the letters indicate the level of significances at P<0.05 using the Tukey's HSD test. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The *H. americanus* seedlings showed significant dependency to the factor AMF only in mycorrhization rates for both samplings. The multifactorial analysis for fertilizer (as factor) demonstrated that *H. americanus* was more dependent on fertilizer than on AMF, as

revealed in dependent values in height, RCD, fresh weight of root and shoot, biomass of shoot and mortality (Table 21).

	AMF		Fertilizer		
Parameter	**Jun 09**	**Nov 09**	**Jun 09**	**Nov 09**	**Interaction**
Height	0.1652	0.2738	*	0.1647	No output due to non-factorial experimental setup.
RCD	0.9068	0.1633	0.1101	*	
Leaf number	0.7170	0.6999	0.4837	0.6855	
Leaf area	0.1116	0.4387	0.3280	0.3379	
FW root	0.7803	0.3157	**	*	
FW shoot	0.7603	0.1676	**	0.2228	
FW leaves	0.2213	0.2222	0.4215	0.4443	
DW shoot	0.7617	0.2265	**	0.1366	
DW leaves	0.2232	0.2201	0.3075	0.4166	
Mortality	d.e.	0.0615	d.e.	*	
Myc rate	**	**	**	0.1667	

Table 21: ANOVA table with P values of the main factors: AMF and fertilizer achieved via multifactorial analysis of variance for the different growth parameters of *Heliocarpus americanus*. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001). **d.e.:** data values are equal, thus no dependency could be calculated.

The nutrient analysis of the *H. americanus* leaves showed increases for almost all measured elements independent of the treatment, except for Al, Fe, Zn and N in comparison to the control (Appendix Table A7). The +AMF treatments showed the highest values of all treatments for K, Mg, Cu, Mn, P, B, S, N and C, at the last field sampling. The highest amounts of P were detected in the +AMF treatments at both samplings (Table 22). The value of Zn increased in the –AMF + LF treatments over time, however the highest measured amounts of Zn were found in the HF and the control treatment. Nitrogen increased over time only in the control and the +AMF treatment, whereas the percentage of C increased in all treatments reaching maximum in the HF and the +AMF treatments.

Time point	Treatment	P [µg/g]	Zn [µg/g]	N [%]	C [%]
3	control	1294	451.50	1.69	41.64
3	HF	1728	385.50	2.33	44.15
3	-AMF + LF	1645	258.10	1.99	44.00
3	+AMF + LF	2282	311.70	2.40	43.29
3	+AMF	1949	311.60	2.27	42.93
4	control	1892	263.70	1.86	45.59
4	HF	3706	315.20	2.17	46.64
4	-AMF + LF	2948	249.30	1.94	45.03
4	+AMF + LF	3316	220.20	2.12	46.47
4	+AMF	3696	144.70	3.25	46.48

Table 22: Nutrient analysis of *Heliocarpus americanus* leaves in the reforestation phase, 6 and 9 months after out-planting. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 3 to 4, values in light gray stayed at equal or at a similar level.

3.2.3.3 *Tabebuia chrysantha*

One half of the *Tabebuia chrysantha* seedlings were out-planted on shaded and the other half on unshaded plots.

Shaded plots

No significant differences were observed for the measured data of *T. chrysantha* on the shaded plots, neither when using the Tukey's HSD or the Fisher-LSD test (Fig. 22). This effect was mainly caused by the high variation in seedling performance and low sample number.

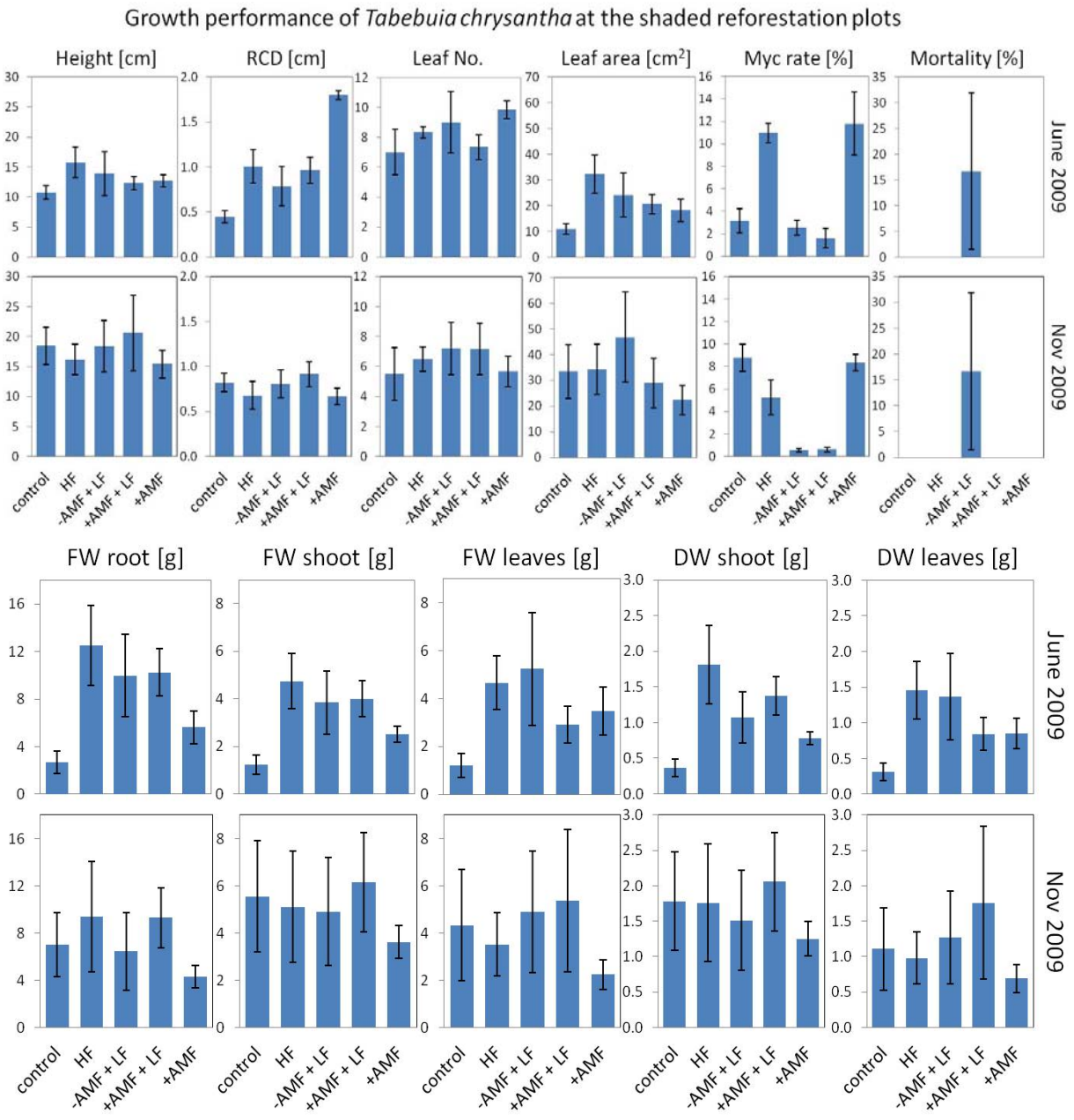


Fig. 22: Growth of *Tabebuia chrysantha* on the shaded reforestation plots. Units are written in brackets, **FW:** fresh weight, **DW:** biomass (dry weight). Mean ± SE are illustrated by the scale bars, the Tukey's HSD test showed no significances at P<0.05. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The two-way ANOVA of the factors AMF and fertilizer showed no dependencies among any of the growth parameters (Table 23).

	AMF		Fertilizer		
Parameter	**Jun 09**	**Nov 09**	**Jun 09**	**Nov 09**	**Interaction**
Height	0.7251	0.8550	0.3744	0.6632	No output due to non-factorial experimental setup.
RCD	0.7763	0.7017	0.7088	0.4001	
Leaf number	0.8719	0.9974	0.9486	0.7134	
Leaf area	0.6207	0.4937	0.3964	0.9199	
FW root	0.4448	0.7390	0.3347	0.5183	
FW shoot	0.5469	0.7885	0.1833	0.7018	
FW leaves	0.6745	0.8326	0.3524	0.6064	
DW shoot	0.5180	0.7769	0.2681	0.7202	
DW leaves	0.6304	0.8355	0.4137	0.5627	
Mortality	0.3038	0.1110	1.0000	0.1672	
Myc rate	0.4108	0.5194	0.6013	0.9204	

Table 23: ANOVA table with P values of the main factors: AMF and fertilizer, achieved via multifactorial analysis of variance for the different growth parameters of *Tabebuia chrysantha* shaded. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001).

The nutrient analysis of the *T. chrysantha* leaves of the shaded plots showed increase of almost all nutrients over time, except for Ca, Al and Fe (Appendix Table A8). Both +AMF treatments showed a decrease in Ca, Mn and Mg over time. The fertilization treatments showed decrease in Al and Fe, whereas the control and the +AMF treatment had increased values from the first to the second sampling. The amounts of P were the highest in the +AMF treatment for both sample dates (Table 24). Amount of Zn increased in all treatments except HF over time. The highest zinc values were measured in the +AMF + LF and the control treatment at the last sampling. The percentage of N and C increased in all treatments, but N stayed stable over time in the HF and the +AMF treatment. Higher values of N were measured in the LF, in the –AMF + LF and the +AMF treatment.

		Shaded plots			
Time point	**Treatment**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**
3	control	1676	220.60	1.66	44.26
3	HF	1643	582.50	1.60	43.91
3	-AMF + LF	1632	411.90	1.85	44.35
3	+AMF + LF	1419	257.30	1.57	43.41
3	+AMF	1881	67.99	1.61	44.30
4	control	1627	356.50	1.96	45.86
4	HF	1679	225.30	1.62	45.17
4	-AMF + LF	1637	514.00	2.04	47.86
4	+AMF + LF	1555	440.30	1.99	46.52
4	+AMF	1744	246.00	1.65	47.15

Table 24: Nutrient analysis of *Tabebuia chrysantha* leaves on the shaded reforestation plots, 5 and 8 months after out-planting. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 3 to 4, values in light gray stayed at equal or at a similar level.

Unshaded plots

Fisher-LSD test revealed some tendencies in the June 2009-sampling on the unshaded plots (Appendix Table A4). A better performance for full fertilized seedlings on the plots was reflected by increased RCD, leaf area, fresh weight of root and shoot and the biomass of shoot and leaves (Fig. 23). For RCD, fresh weight of roots and shoot the +AMF + LF treatment showed the similar values than HF. When treated with +AMF the seedlings showed higher leaf numbers than non-inoculated.

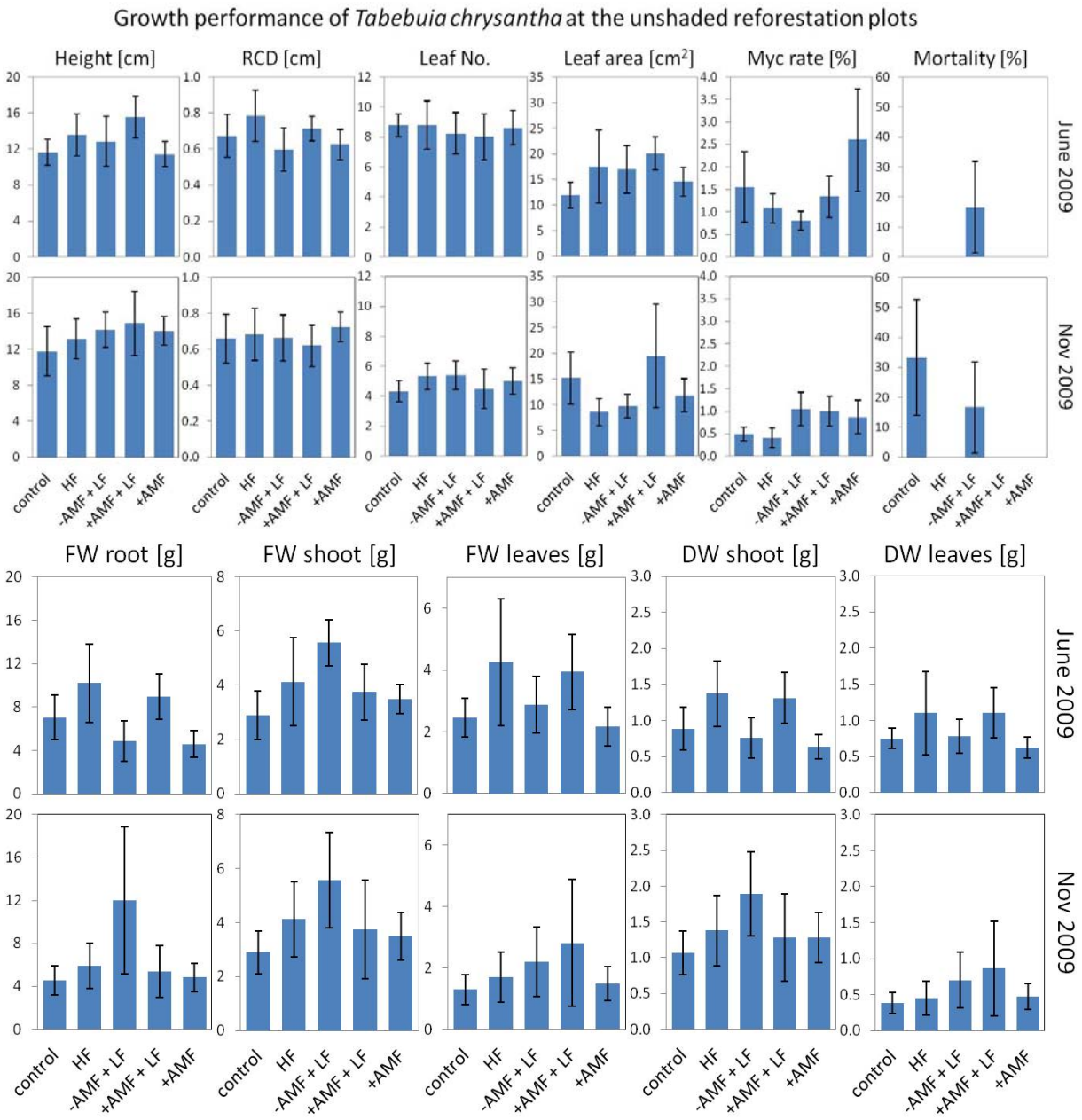


Fig. 23: Growth of *Tabebuia chrysantha* on the unshaded reforestation plots. Units are written in brackets. Mean ± SE are illustrated by the scale bars, no significances at P<0.05 using the Tukey's HSD test were observed. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The multifactorial analysis revealed only dependency in leaf numbers in the June 2009-sampling when using AMF as factor. Dependency on fertilization were observed by RCD, leaf area fresh weight of the root and shoot, and biomass of the shoot and the leaves in the first sampling (Table 25).

	AMF		Fertilizer		
Parameter	**Jun 09**	**Nov 09**	**Jun 09**	**Nov 09**	**Interaction**
Height	0.7399	0.8332	0.2923	0.9193	No output due to non-factorial experimental setup.
RCD	0.4481	0.9238	**	0.8587	
Leaf numbers	*	0.7656	0.1850	0.7538	
Leaf area	0.5293	0.5265	**	0.5230	
FW root	0.7276	0.4330	**	0.9557	
FW shoot	0.6195	0.6856	**	0.8370	
FW leaves	0.2205	0.9434	0.1585	0.7424	
DW shoot	0.6013	0.7025	**	0.9114	
DW leaves	0.3342	0.9520	*	0.7854	
Mortality	0.3038	0.3038	1.0000	1.0000	
Myc rate	0.6386	0.8722	0.3067	0.9760	

Table 25: ANOVA table with P values of the main factors: AMF and fertilizer, achieved via multifactorial analysis of variance for the different growth parameters of *Tabebuia chrysantha* unshaded. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001).

Nutrient analysis of the *T. chrysantha* leaves on the unshaded plots showed increases of all nutrients independent of the treatment over time (Appendix Table A8). The +AMF treatments showed decreases in Ca, Mn, Mg and additionally in Al and Fe. Nevertheless the +AMF treatment showed the highest values in P for both sample points (Table 26). Almost all treatments, except for the HF, increased the amount of Zn over time. High zinc values were measured in the +AMF + LF and the control treatment in the last sampling similar to the shaded plots. The percentage of C increased in all treatments. The amount of N stayed comparable over time in the control and the HF treatment, as also found in the shaded plots. High values of C were measured in the +AMF treatments. N was high in the +AMF treatment and in the control.

		Unshaded plots			
Time point	**Treatment**	**P [µg/g]**	**Zn [µg/g]**	**N [%]**	**C [%]**
3	control	1164	285.60	1.35	44.39
3	HF	1386	249.40	1.49	44.10
3	-AMF + LF	1533	316.20	1.54	43.86
3	+AMF + LF	1445	369.70	1.32	43.53
3	+AMF	1133	469.80	1.43	43.63
4	control	1768	465.00	1.43	47.12
4	HF	1814	531.30	1.54	46.45
4	-AMF + LF	1912	313.10	1.69	46.49
4	+AMF + LF	1708	429.90	2.19	47.02
4	+AMF	1820	302.60	1.95	48.08

Table 26: Nutrient analysis of *Tabebuia chrysantha* leaves on the unshaded reforestation plots, 5 and 8 months after out-planting. Amounts of phosphorus (**P**), zinc (**Zn**), nitrogen (**N**) and carbon (**C**) are shown in the table. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. Values marked in dark gray increased from sampling point 3 to 4, values in light gray stayed at equal or at a similar level.

3.2.3.4 Mortality of tree seedlings on the reforestation plots

In October 2009 and March 2011 an additional survey on all reforestation plots was carried out collecting mortality rate of all out-planted seedlings. Results obtained by Fisher-LSD and Tukey's HSD test are summarized in Appendix Table A5.

The seedlings of *C. montana* and *H. americanus* showed no significant differences in mortality when the Tukey's HSD test was used (Fig. 24). By applying the Fisher-LSD test *H. americanus* seedlings showed a tendency for reduced mortality when HF was applied in the nursery (Appendix Table A5). *T. chrysantha* seedlings showed the lowest mortality on shaded and unshaded plots when treated with AMF in the nursery phase. Results are supported by significance in the Tukey's HSD test. Mortality rate was also reduced in the HF and –AMF + LF treatment in comparison to the control, which showed the highest mortality rates.

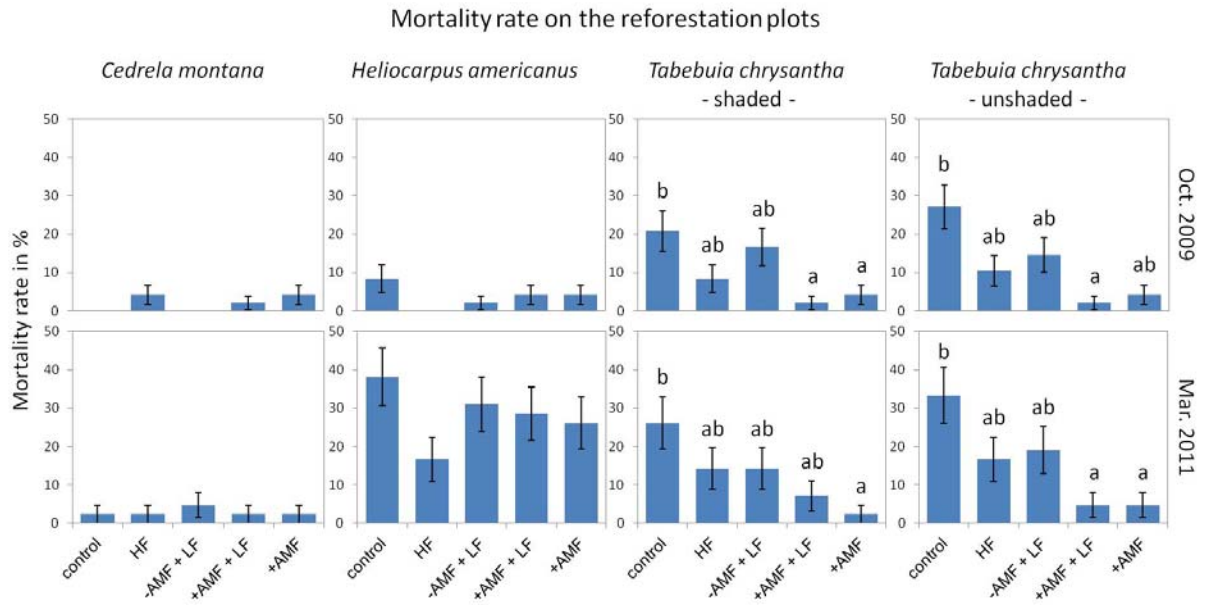


Fig. 24: Mortality rates of C*edrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* on the reforestation plots (shaded and unshaded) in Oct 2009 and Mar 2011. Means ± SE are illustrated by the scale bars, the letters indicate the level of significances at P<0.05 using the Tukey's HSD test. The treatments are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The multifactorial analysis on mortality showed no reaction of *C. montana* seedlings neither on AMF nor on fertilizer. *H. americanus* seedlings showed dependency to AMF but not on fertilizer in March 2011, almost 2 years after planting to the field. *T. chrysantha* seedlings depend on AMF, but also on fertilizer as applied during the nursery phase (Table 27).

	AMF		**Fertilizer**		
Parameter	**Oct 2009**	**Mar 2011**	**Oct 2009**	**Mar 2011**	**Interaction**
Cedrela montana	0.2823	0.8107	0.2823	1.0000	No output due to non-factorial experimental setup.
Heliocarpus americanus	0.4870	0.4661	0.1016	*	
Tabebuia chrysantha - shaded	****	***	**	0.2051	
Tabebuia chrysantha - unshaded	***	****	0.1198	*	

Table 27: ANOVA table with P values of the main factors AMF and fertilizer achieved via multifactorial analysis of variance for mortality of *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* shaded and unshaded. Significant data was marked with the according symbols: * (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001).

Cedrela montana

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
3	T0 - control	15.7 ± 0.82	0.49 ± 0.045	4 ± 0.76	14.25 ± 2.901	31.19 ± 14.949	0.00 ± 0.000
3	T1 - HF	17.9 ± 0.93	0.62 ± 0.021	3 ± 0.77	18.97 ± 4.171	0.81 ± 0.349	0.00 ± 0.000
3	T2 - -AMF + LF	16.3 ± 1.17	0.64 ± 0.049	4 ± 0.24	9.40 ± 3.440	3.01 ± 1.704	0.00 ± 0.000
3	T3 - +AMF + LF	15.8 ± 1.61	0.53 ± 0.041	4 ± 0.19	17.12 ± 2.407	8.51 ± 3.957	0.00 ± 0.000
3	T4 - +AMF	16.3 ± 1.12	0.52 ± 0.036	3 ± 0.51	16.43 ± 5.313	33.14 ± 14.982	0.00 ± 0.000
4	T0 - control	14.3 ± 1.78	0.56 ± 0.060	4 ± 0.89	12.20 ± 4.334	1.69 ± 0.685	0.00 ± 0.000
4	T1 - HF	19.3 ± 2.38	0.72 ± 0.141	3 ± 0.65	25.42 ± 10.928	2.17 ± 1.639	16.67 ± 15.215
4	T2 - -AMF + LF	28.6 ± 8.29	0.71 ± 0.124	4 ± 1.63	49.46 ± 23.391	4.96 ± 3.965	0.00 ± 0.000
4	T3 - +AMF + LF	18.8 ± 2.78	0.60 ± 0.038	4 ± 0.73	23.04 ± 8.727	0.28 ± 0.082	0.00 ± 0.000
4	T4 - +AMF	19.1 ± 2.91	0.65 ± 0.078	5 ± 1.02	29.15 ± 10.617	8.44 ± 3.873	0.00 ± 0.000

Sample time	Treatment	FW root	FW shoot	FW leaves	DW shoot	DW leaves
3	T0 - control	2.73 ± 0.440	2.14 ± 0.357	0.75 ± 0.122	0.54 ± 0.117	0.18 ± 0.028
3	T1 - HF	4.07 ± 0.263	2.99 ± 0.298	0.96 ± 0.252	0.70 ± 0.073	0.23 ± 0.049
3	T2 - -AMF + LF	3.77 ± 0.651	3.13 ± 0.447	0.71 ± 0.223	0.84 ± 0.153	0.17 ± 0.055
3	T3 - +AMF + LF	2.44 ± 0.545	2.64 ± 0.427	0.78 ± 0.125	0.70 ± 0.122	0.18 ± 0.031
3	T4 - +AMF	2.54 ± 0.328	2.12 ± 0.338	0.70 ± 0.267	0.49 ± 0.083	0.15 ± 0.055
4	T0 - control	1.94 ± 0.404	2.43 ± 0.567	1.37 ± 0.768	0.67 ± 0.134	0.35 ± 0.214
4	T1 - HF	2.58 ± 0.865	4.29 ± 1.361	0.97 ± 0.317	1.40 ± 0.479	0.27 ± 0.097
4	T2 - -AMF + LF	4.73 ± 2.231	8.51 ± 4.568	9.32 ± 7.242	2.19 ± 0.990	2.49 ± 1.902
4	T3 - +AMF + LF	2.48 ± 0.468	3.57 ± 0.838	1.89 ± 0.932	0.98 ± 0.179	0.51 ± 0.239
4	T4 - +AMF	2.97 ± 0.701	4.05 ± 1.226	2.67 ± 1.542	1.16 ± 0.260	0.70 ± 0.380

Heliocarpus americanus

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
3	T0 - control	24.9 ± 2.82	0.69 ± 0.099	10 ± 2.46	7.42 ± 1.985	0.50 ± 0.252 a	0.00 ± 0.000
3	T1 - HF	42.8 ± 5.65	0.89 ± 0.045	14 ± 2.53	14.68 ± 3.061	0.79 ± 0.450 a	0.00 ± 0.000

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
3	T2 - -AMF + LF	36.6 ± 3.10	0.80 ± 0.026	11 ± 4.50	11.25 ± 1.480	1.09 ± 0.354 a	0.00 ± 0.000
3	T3 - +AMF + LF	31.1 ± 5.71	0.83 ± 0.074	12 ± 3.03	14.65 ± 3.889	3.05 ± 1.401 a	0.00 ± 0.000
3	T4 - +AMF	40.2 ± 7.76	0.72 ± 0.078	12 ± 2.31	21.05 ± 8.389	15.18 ± 2.938 b	0.00 ± 0.000
4	T0 - control	29.3 ± 1.96	0.64 ± 0.033	7 ± 1.54	6.22 ± 2.497	18.28 ± 4.008 ab	0.00 ± 0.000
4	T1 - HF	51.3 ± 7.20	0.95 ± 0.096	4 ± 1.24	18.87 ± 8.930	19.04 ± 4.519 ab	0.00 ± 0.000
4	T2 - -AMF + LF	33.7 ± 1.56	0.69 ± 0.050	6 ± 1.67	6.63 ± 3.390	12.14 ± 2.985 a	0.00 ± 0.000
4	T3 - +AMF + LF	41.9 ± 5.57	0.89 ± 0.098	9 ± 2.41	8.64 ± 2.378	20.00 ± 3.902 ab	0.00 ± 0.000
4	T4 - +AMF	48.6 ± 19.30	0.84 ± 0.172	8 ± 1.78	22.73 ± 17.525	29.64 ± 5.201 b	33.33 ± 19.245

Sample time	Treatment	FW root	FW shoot	FW leaves	DW shoot	DW leaves
3	T0 - control	4.59 ± 0.664	3.91 ± 1.098 a	1.32 ± 0.477	1.29 ± 0.349	0.32 ± 0.127
3	T1 - HF	9.32 ± 1.223	11.85 ± 2.596 b	4.32 ± 1.599	3.35 ± 0.651	1.13 ± 0.429
3	T2 - -AMF + LF	6.26 ± 0.778	8.01 ± 0.328 ab	1.70 ± 0.328	2.59 ± 0.121	0.40 ± 0.076
3	T3 - +AMF + LF	6.89 ± 1.146	8.37 ± 2.130 ab	4.42 ± 2.340	2.39 ± 0.661	1.00 ± 0.455
3	T4 - +AMF	5.64 ± 1.536	5.79 ± 1.083 ab	4.44 ± 1.930	1.84 ± 0.544	1.05 ± 0.467
4	T0 - control	3.08 ± 0.333	4.56 ± 0.548	0.62 ± 0.352	1.59 ± 0.230	0.19 ± 0.113
4	T1 - HF	7.34 ± 1.370	15.07 ± 3.527	1.33 ± 0.639	5.11 ± 1.178	0.36 ± 0.166
4	T2 - -AMF + LF	4.48 ± 0.533	5.27 ± 1.110	0.36 ± 0.116	1.90 ± 0.276	0.10 ± 0.035
4	T3 - +AMF + LF	6.38 ± 1.590	12.56 ± 4.084	1.81 ± 0.881	3.95 ± 1.279	0.52 ± 0.238
4	T4 - +AMF	5.49 ± 1.986	14.88 ± 9.241	4.52 ± 3.606	4.04 ± 2.282	1.38 ± 1.105

***Tabebuia chrysantha* - shaded**

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
3	T0 - control	10.8 ± 1.11	0.45 ± 0.067	7 ± 1.52	10.87 ± 2.143	3.17 ± 1.090	0.00 ± 0.00
3	T1 - HF	15.8 ± 2.53	1.01 ± 0.182	8 ± 0.38	32.19 ± 7.361	10.96 ± 0.871	0.00 ± 0.00
3	T2 - -AMF + LF	13.9 ± 3.66	0.79 ± 0.215	9 ± 2.06	24.20 ± 8.623	2.55 ± 0.659	16.67 ± 15.21
3	T3 - +AMF + LF	12.3 ± 1.07	0.96 ± 0.145	7 ± 0.84	20.57 ± 3.800	1.60 ± 0.867	0.00 ± 0.00
3	T4 - +AMF	12.7 ± 1.02	1.80 ± 0.048	10 ± 0.60	18.16 ± 4.359	11.78 ± 2.801	0.00 ± 0.00

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
4	T0 - control	18.5 ± 3.09	0.82 ± 0.101	6 ± 1.79	33.39 ± 10.409	8.76 ± 1.196	0.00 ± 0.00
4	T1 - HF	16.2 ± 2.52	0.68 ± 0.156	7 ± 0.81	34.34 ± 9.758	5.25 ± 1.543	0.00 ± 0.00
4	T2 - -AMF + LF	18.4 ± 4.27	0.81 ± 0.154	7 ± 1.73	46.74 ± 17.563	0.56 ± 0.138	16.67 ± 15.21
4	T3 - +AMF + LF	20.6 ± 6.27	0.91 ± 0.141	7 ± 1.72	28.98 ± 9.638	0.62 ± 0.224	0.00 ± 0.00
4	T4 - +AMF	15.4 ± 2.30	0.67 ± 0.087	6 ± 1.02	22.39 ± 5.662	8.37 ± 0.739	0.00 ± 0.00

Sample time	Treatment	FW root	FW shoot	FW leaves	DW shoot	DW leaves
3	T0 - control	2.71 ± 0.940	1.24 ± 0.399	1.22 ± 0.502	0.37 ± 0.121	0.31 ± 0.124
3	T1 - HF	12.53 ± 3.364	4.74 ± 1.155	4.67 ± 1.124	1.82 ± 0.550	1.46 ± 0.403
3	T2 - -AMF + LF	9.98 ± 3.492	3.84 ± 1.318	5.24 ± 2.347	1.07 ± 0.362	1.37 ± 0.604
3	T3 - +AMF + LF	10.23 ± 1.978	4.01 ± 0.765	2.90 ± 0.762	1.38 ± 0.269	0.84 ± 0.227
3	T4 - +AMF	5.62 ± 1.364	2.52 ± 0.342	3.49 ± 0.995	0.78 ± 0.087	0.85 ± 0.210
4	T0 - control	7.03 ± 2.705	5.55 ± 2.363	4.33 ± 2.357	1.78 ± 0.693	1.11 ± 0.577
4	T1 - HF	9.38 ± 4.663	5.12 ± 2.354	3.52 ± 1.340	1.76 ± 0.832	0.98 ± 0.366
4	T2 - -AMF + LF	6.45 ± 3.275	4.91 ± 2.305	4.90 ± 2.573	1.51 ± 0.707	1.27 ± 0.658
4	T3 - +AMF + LF	9.30 ± 2.563	6.14 ± 2.096	5.36 ± 3.020	2.06 ± 0.697	1.76 ± 1.080
4	T4 - +AMF	4.29 ± 0.946	3.62 ± 0.698	2.24 ± 0.638	1.25 ± 0.245	0.69 ± 0.202

***Tabebuia chrysantha* - unshaded**

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
3	T0 - control	11.6 ± 1.43	0.67 ± 0.118	9 ± 0.77	11.99 ± 2.512	1.56 ± 0.790	0.00 ± 0.000
3	T1 - HF	13.6 ± 2.33	0.78 ± 0.144	9 ± 1.58	17.47 ± 7.130	1.08 ± 0.320	0.00 ± 0.000
3	T2 - -AMF + LF	12.8 ± 2.74	0.60 ± 0.121	8 ± 1.39	17.00 ± 4.598	0.80 ± 0.212	16.67 ± 15.215
3	T3 - +AMF + LF	15.5 ± 2.37	0.71 ± 0.068	8 ± 1.55	20.05 ± 3.273	1.34 ± 0.461	0.00 ± 0.000
3	T4 - +AMF	11.4 ± 1.42	0.62 ± 0.084	9 ± 1.15	14.54 ± 2.856	2.60 ± 1.135	0.00 ± 0.000
4	T0 - control	11.8 ± 2.76	0.66 ± 0.135	4 ± 0.69	15.19 ± 5.065	0.48 ± 0.152	33.33 ± 19.245
4	T1 - HF	13.2 ± 2.19	0.68 ± 0.146	5 ± 0.87	8.58 ± 2.590	0.40 ± 0.213	0.00 ± 0.000
4	T2 - -AMF + LF	14.2 ± 1.98	0.66 ± 0.127	5 ± 0.96	9.70 ± 2.326	1.05 ± 0.371	16.67 ± 15.215

Sample time	Treatment	Height [cm]	RCD [cm]	Leaf No.	Leaf area [cm^2]	Mycorrhization rate [%]	Mortality [%]
4	T3 - +AMF + LF	14.9 ± 3.59	0.62 ± 0.115	5 ± 1.31	19.45 ± 10.101	1.00 ± 0.331	0.00 ± 0.000
4	T4 - +AMF	14.1 ± 1.63	0.72 ± 0.082	5 ± 0.88	11.84 ± 3.186	0.87 ± 0.370	0.00 ± 0.000

Sample time	Treatment	FW root	FW shoot	FW leaves	DW shoot	DW leaves
3	T0 - control	7.02 ± 2.054	2.74 ± 0.887	2.47 ± 0.623	0.89 ± 0.296	0.75 ± 0.138
3	T1 - HF	10.19 ± 3.618	4.97 ± 1.626	4.25 ± 2.062	1.37 ± 0.455	1.10 ± 0.575
3	T2 - -AMF + LF	4.85 ± 1.840	2.37 ± 0.854	2.88 ± 0.916	0.76 ± 0.280	0.78 ± 0.236
3	T3 - +AMF + LF	8.96 ± 2.056	4.04 ± 1.033	3.94 ± 1.213	1.31 ± 0.352	1.10 ± 0.349
3	T4 - +AMF	4.58 ± 1.244	1.88 ± 0.546	2.17 ± 0.617	0.63 ± 0.169	0.62 ± 0.146
4	T0 - control	4.55 ± 1.329	2.89 ± 0.807	1.30 ± 0.491	1.06 ± 0.304	0.38 ± 0.143
4	T1 - HF	5.89 ± 2.097	4.13 ± 1.390	1.70 ± 0.829	1.38 ± 0.488	0.45 ± 0.235
4	T2 - -AMF + LF	12.01 ± 6.866	5.57 ± 1.757	2.20 ± 1.127	1.89 ± 0.586	0.70 ± 0.388
4	T3 - +AMF + LF	5.36 ± 2.410	3.75 ± 1.825	2.82 ± 2.083	1.28 ± 0.608	0.86 ± 0.657
4	T4 - +AMF	4.82 ± 1.304	3.49 ± 0.893	1.48 ± 0.556	1.28 ± 0.350	0.47 ± 0.178

Table 28: Growth of *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* on the reforestation plots. Means ± SE are shown. Significantly differences (found only for *Heliocarpus americanus*) between measured data across the treatments were tested via the Tukey's HSD test ($P<0.05$) and are marked with different letters. Treatment description are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

3.2.4 Tracing of AMF by 454 GS FLX sequencing

There were three cases in which the sequences of the Ecuadorian AMF cultures clustered together with AMF species stemming from the nursery samples of the Nursery experiment No. 1. The sequences from the nursery AMF occurring in the same clusters as the one used for inoculum were treated as being the same species. Therefore the following AMF stemming from the nursery roots were equated with the according AMF culture used for inoculum production shown in Table 29.

AMF originating from nursery roots	**Synonymous with**
Rhizophagus sp. (from Ha –N8)	*Rhizophagus* sp. Att1451-8 and Att1456-1
Rhizophagus sp. 2 (from Cm-N3)	
Claroideoglomus sp.	*Cl. etunicatum*-like Att1449-10, Att1451-6 and Att1456-11
Archaeospora sp. (from Ha-N2)	*Ar. trappei*-like Att1452-6 and Att1456-7

Table 29: Sequences from Nursery experiment No. 3 treated as same AMF species. Origin of the AMF species, concerning tree species and nursery sample code are written in brackets. **Ha:** *Heliocarpus americanus*, **Cm:** *Cedrela montana*.

Ambispora sp. Att1449-12 was excluded from the analysis, as for this AMF the origin is doubtful and an unambiguous morphological and molecular characterization was not possibly. Only two times sequence reads from an undefined *Ambispora* sp. appeared in a total of 31 reads in the nursery samplings. Thereof 23 reads occurred in the 6 months sampling of *Heliocarpus americanus* and 8 reads in the 3 months sampling of *Tabebuia chrysantha*. AMF were identified in each treatment including the control, as shown by the mycorrhization rates. The AMF applied as inoculum were also found in the control, most likely caused by watering practice (splash water) and the rearrangement of the plastic bags in the replicates during the nursery phase by students working in the tree nursery.

3.2.4.1 Molecular identification of AMF persisting in roots

The phylogenetic analysis of the 454 sequence data is exemplified in the following paragraph by both AMF treatments (+AMF + LF and +AMF) at the 3 months samplings in the nursery of *T. chrysantha.* Results of all treatments and all tree species are summarized in Appendix Table A9 to A11. Since tracing of the introduced AMF was the major aim of this approach only results of the AMF treatments are shown in detail here (for non-AMF treatments, see Appendix Table A9-A11). Results are shown in percentage including the reads achieved per

run in brackets as the 454 analysis of each tree species in the different treatments provided different numbers of sequence reads. All phylogenetic trees including the short 454 sequences are provided on a CD together with this dissertation.

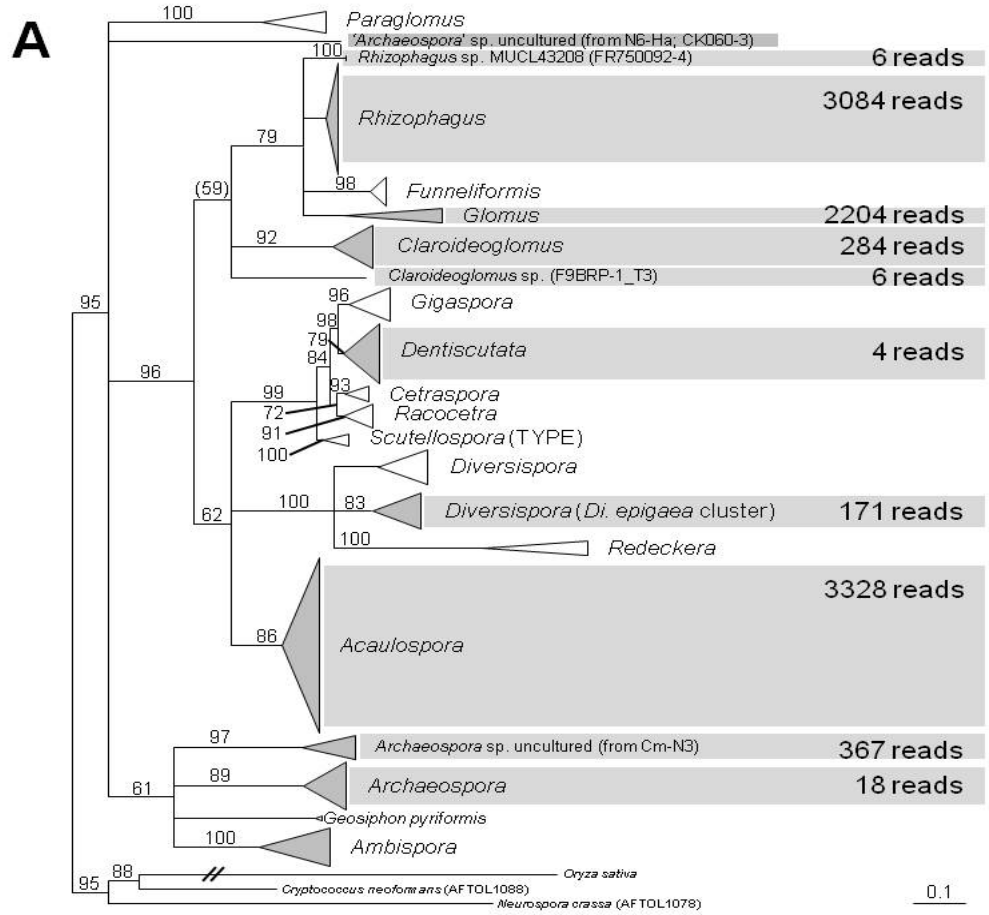
3.2.4.1.1 Detailed phylogenetic analysis of 454 sequence reads, exemplified by the first nursery sampling of *Tabebuia chrysantha* (3 months)

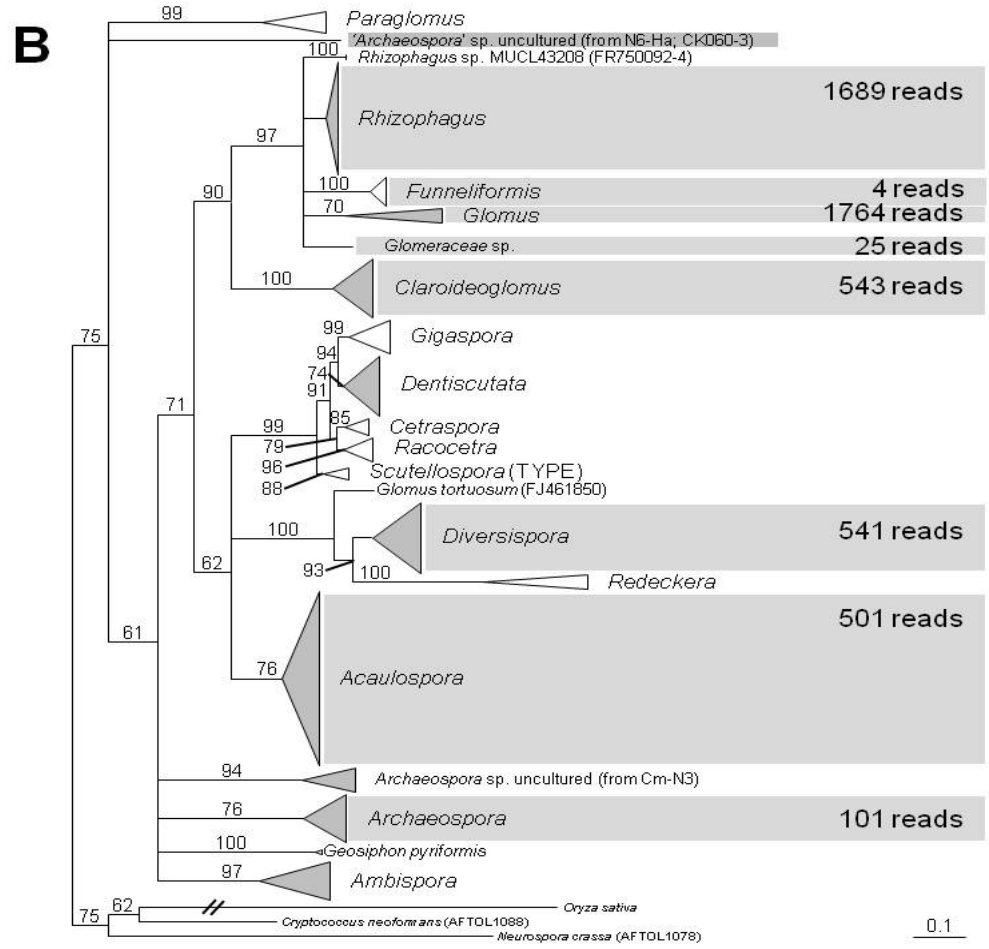
The phylogenetic results of all treatments on *T. chrysantha* of the first sampling at 3 months in the nursery are summarized in tables. The analysis method is exemplified on phylogenetic trees of both AMF treatments, firstly illustrated by phylogenetic trees with collapsed branches including the different read cluster in the *Glomeromycota* (Fig. 25) and in detailed phylogenetic clades with the according 454 reads (Fig. 26-29). For better visualization only the clades of a genus the 454 sequence felt in are shown. All detailed phylogenetic trees including the 454 sequence reads are provided on the CD and can be visualized with FigTree v1.2.1. The consensus sequences marked with (consensus #) used in the phylogenetic trees of Fig. 26-29 are as follows: **4:** AY635831, AY997052, DQ273790; **5:** DQ322630, AY997054, DQ273828; **7:** Y16739, Z14008, AJ239125. **8:** AY635832, AY997088, DQ273792. **9:** AJ871270-73. **10:** AM418543-44; **12:** AJ006800, AJ243420. **13:** AJ006801, AJ243419 and **18:** AY635833, AY997053, DQ273793.

AMF treatments (+AMF + LF, +AMF)

For better visualisation of the read clustering two phylogenetic trees were made showing an overview of the *Glomeromycota*, for each of the two AMF treatments. The topology and bootstrap supports (BS) changed between the two treatments according to clustering and amount of the 454 sequence reads (Fig. 25).

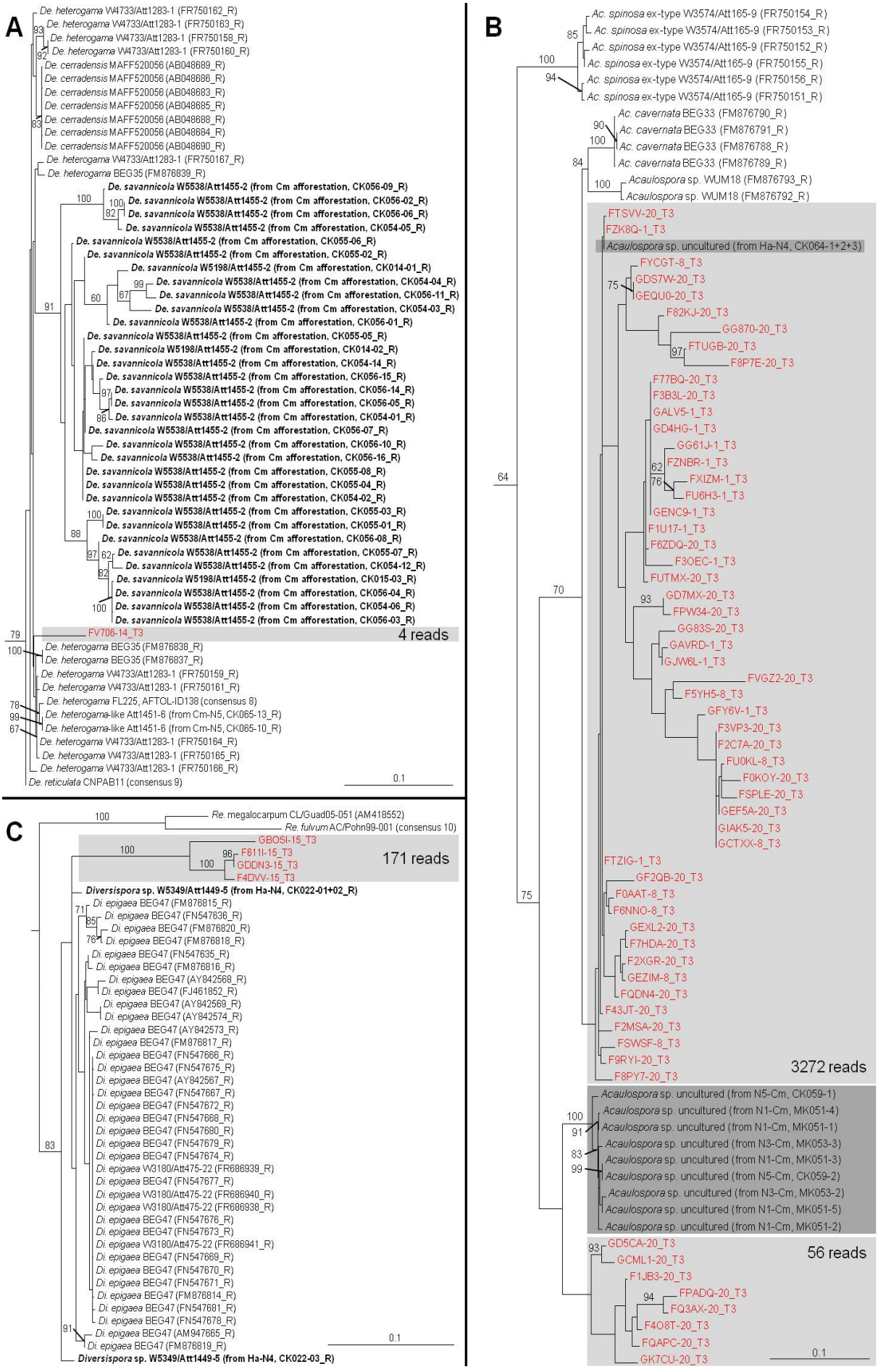
Fig. 25: Maximum likelihood phylogenetic tree of the 454 sequence reads clustering within the *Glomeromycota*, of *Tabebuia chrysantha* after 3 months in the nursery. A: +AMF + LF treatment, **B:** +AMF treatment. Clades including Ecuadorian AMF sequences are marked in dark gray, 454 sequence clusters are indicated by a light gray box, including the according read numbers. The scale bar shows the substitutions per site. Two diagonal slashed indicate a 50% reduced branch length. Classification of the AMF follows Schüßler & Walker 2010 and Redecker *et al.* (2013).


A
Paraglomus
'Archaeospora' sp. uncultured (from N6-Ha; CK060-3)
Rhizophagus sp. MUCL43208 (FR750092-4)
6 reads
Rhizophagus
3084 reads
Funneliformis
Glomus
2204 reads
Claroideoglomus
284 reads
Claroideoglomus sp. (F9BRP-1_T3)
6 reads
Gigaspora
Dentiscutata
4 reads
Cetraspora
Racocetra
Scutellospora (TYPE)
Diversispora
Diversispora (Di. epigaea cluster)
171 reads
Redeckera
3328 reads
Acaulospora
Archaeospora sp. uncultured (from Cm-N3)
367 reads
Archaeospora
18 reads
Geosiphon pyriformis
Ambispora
Oryza sativa
Cryptococcus neoformans (AFTOL1088)
Neurospora crassa (AFTOL1078)
0.1


B
Paraglomus
'Archaeospora' sp. uncultured (from N6-Ha; CK060-3)
Rhizophagus sp. MUCL43208 (FR750092-4)
1689 reads
Rhizophagus
Funneliformis
4 reads
Glomus
1764 reads
Glomeraceae sp.
25 reads
Claroideoglomus
543 reads
Gigaspora
Dentiscutata
Cetraspora
Racocetra
Scutellospora (TYPE)
Glomus tortuosum (FJ461850)
Diversispora
541 reads
Redeckera
501 reads
Acaulospora
Archaeospora sp. uncultured (from Cm-N3)
Archaeospora
101 reads
Geosiphon pyriformis
Ambispora
Oryza sativa
Cryptococcus neoformans (AFTOL1088)
Neurospora crassa (AFTOL1078)
0.1

9,466 sequence reads were obtained resulting in 159 unique reads after clustering from the +AMF + LF treatment (Fig. 26-28). The sequences clustered in *Dentiscutata*, *Acaulospora*, *Diversispora*, *Claroideoglomus*, *Rhizophagus*, *Glomus* and *Archaeospora*. One sequence cluster represented by the reference sequence (FV706-14_T3) clusters in *Dentiscutata,* next to *De. heterogama* (Fig. 26A). The main sequences cluster (3,272 sequence reads) appeared in *Acaulospora* (Fig. 26B), one part clusters together with the nursery-AMF *Acaulospora* sp. from *Heliocarpus americanus* (Ha) - N4 (Urgiles *et al.* 2009) and the other part next to the sequences of *Acaulospora* sp. from *Cedrela montana* (Cm) - N1/N3/N5. A smaller amount of sequences clustered in *Diversispora* next to *Di*. *epigaea* BEG47 and the *Diversispora* sp. Att1449-5 species from Ecuador (Fig. 26C). Therefore this cluster of sequences was labeled as *Diversispora* sp. Att1449-5. Further 454 sequences clustered basally and within *Claroideoglomus* (Fig. 27C). 278 sequence reads clustered next to sequences from the isolate *Cl. etunicatum*-like Att1449-10, Att1451-6, Att1456-11 and were named according to this AMF. A single reference sequence F9BRP-1_T3 clustering basal to *Claroideoglomus* is labeled as *Claroideoglomus* species. Another main sequence cluster (3.010 sequence reads) fell in *Rhizophagus* (Fig. 27B). They cluster together with the environmental *Rhizophagus* sp. (from *P. oleifolius* roots), uncultured *Rhizophagus* sp. 2 (Cm – N3), *Rhizophagus* sp. Att1451-8, Att1456-1 or *Rhizophagus* sp. MUCL43208. The second largest sequence amount was detected within *Glomus* (Fig. 27A). The main part of these sequences clustered together with *Gl*. *macrocarpum* and one reference sequence clustered together with the *Glomus* sp. from *P. oleifolius* roots. The remaining sequences clustered within *Archaeospora* (Fig. 27D) together with two uncultured *Archaeospora* spp., either stemming from Cm – N3 or Ha - N6.

Fig. 26: Maximum likelihood phylogenetic tree of *Diversisporales* clades including 454 sequence reads, of *Tabebuia chrysantha* after 3 months in the nursery (+AMF + LF treatment). The Ecuadorian AMF cultures are written in bold, uncultured and environmental AMF are marked with a dark gray box, 454 sequence reads are red and clusters are indicated by a light gray box, including the according read numbers. Bootstrap supports below 60% are not shown. The scale bar shows the substitutions per site.


A
De. heterogama W4733/Att1283-1 (FR750162_R)
De. heterogama W4733/Att1283-1 (FR750163_R)
De. heterogama W4733/Att1283-1 (FR750158_R)
De. heterogama W4733/Att1283-1 (FR750160_R)
De. cerradensis MAFF520056 (AB048689_R)
De. cerradensis MAFF520056 (AB048686_R)
De. cerradensis MAFF520056 (AB048683_R)
De. cerradensis MAFF520056 (AB048685_R)
De. cerradensis MAFF520056 (AB048688_R)
De. cerradensis MAFF520056 (AB048684_R)
De. cerradensis MAFF520056 (AB048690_R)
De. heterogama W4733/Att1283-1 (FR750167_R)
De. heterogama BEG35 (FM876839_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-09_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-02_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-06_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-05_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-06_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-02_R)
De. savannicola W5198/Att1455-2 (from Cm afforestation, CK014-01_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-04_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-11_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-03_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-01_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-05_R)
De. savannicola W5198/Att1455-2 (from Cm afforestation, CK014-02_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-14_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-15_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-14_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-05_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-01_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-07_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-10_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-16_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-08_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-04_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-02_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-03_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-01_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-08_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK055-07_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-12_R)
De. savannicola W5198/Att1455-2 (from Cm afforestation, CK015-03_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-04_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK054-06_R)
De. savannicola W5538/Att1455-2 (from Cm afforestation, CK056-03_R)
FV706-14_T3
4 reads
De. heterogama BEG35 (FM876838_R)
De. heterogama BEG35 (FM876837_R)
De. heterogama W4733/Att1283-1 (FR750159_R)
De. heterogama W4733/Att1283-1 (FR750161_R)
De. heterogama FL225, AFTOL-ID138 (consensus 8)
De. heterogama-like Att1451-6 (from Cm-N5, CK065-13_R)
De. heterogama-like Att1451-6 (from Cm-N5, CK065-10_R)
De. heterogama W4733/Att1283-1 (FR750164_R)
De. heterogama W4733/Att1283-1 (FR750165_R)
De. heterogama W4733/Att1283-1 (FR750166_R)
De. reticulata CNPAB11 (consensus 9)
0.1
B
Ac. spinosa ex-type W3574/Att165-9 (FR750154_R)
Ac. spinosa ex-type W3574/Att165-9 (FR750153_R)
Ac. spinosa ex-type W3574/Att165-9 (FR750152_R)
Ac. spinosa ex-type W3574/Att165-9 (FR750155_R)
Ac. spinosa ex-type W3574/Att165-9 (FR750156_R)
Ac. spinosa ex-type W3574/Att165-9 (FR750151_R)
Ac. cavernata BEG33 (FM876790_R)
Ac. cavernata BEG33 (FM876791_R)
Ac. cavernata BEG33 (FM876788_R)
Ac. cavernata BEG33 (FM876789_R)
Acaulospora sp. WUM18 (FM876793_R)
Acaulospora sp. WUM18 (FM876792_R)
FTSVV-20_T3
FZK8Q-1_T3
Acaulospora sp. uncultured (from Ha-N4, CK064-1+2+3)
FYCGT-8_T3
GDS7W-20_T3
GEQU0-20_T3
F82KJ-20_T3
GG870-20_T3
FTUGB-20_T3
F8P7E-20_T3
F77BQ-20_T3
F3B3L-20_T3
GALV5-1_T3
GD4HG-1_T3
GG61J-1_T3
FZNBR-1_T3
FXIZM-1_T3
FU6H3-1_T3
GENC9-1_T3
F1U17-1_T3
F6ZDQ-20_T3
F3OEC-1_T3
FUTMX-20_T3
GD7MX-20_T3
FPW34-20_T3
GG83S-20_T3
GAVRD-1_T3
GJW6L-1_T3
FVGZ2-20_T3
F5YH5-8_T3
GFY6V-1_T3
F3VP3-20_T3
F2C7A-20_T3
FU0KL-8_T3
F0KOY-20_T3
FSPLE-20_T3
GEF5A-20_T3
GIAK5-20_T3
GCTXX-8_T3
FTZIG-1_T3
GF2QB-20_T3
F0AAT-8_T3
F6NNO-8_T3
GEXL2-20_T3
F7HDA-20_T3
F2XGR-20_T3
GEZIM-8_T3
FQDN4-20_T3
F43JT-20_T3
F2MSA-20_T3
FSWSF-8_T3
F9RYI-20_T3
F8PY7-20_T3
3272 reads
Acaulospora sp. uncultured (from N5-Cm, CK059-1)
Acaulospora sp. uncultured (from N1-Cm, MK051-4)
Acaulospora sp. uncultured (from N1-Cm, MK051-1)
Acaulospora sp. uncultured (from N3-Cm, MK053-3)
Acaulospora sp. uncultured (from N1-Cm, MK051-3)
Acaulospora sp. uncultured (from N5-Cm, CK059-2)
Acaulospora sp. uncultured (from N3-Cm, MK053-2)
Acaulospora sp. uncultured (from N1-Cm, MK051-5)
Acaulospora sp. uncultured (from N1-Cm, MK051-2)
GD5CA-20_T3
GCML1-20_T3
F1JB3-20_T3
FPADQ-20_T3
FQ3AX-20_T3
F4O8T-20_T3
FQAPC-20_T3
GK7CU-20_T3
56 reads
0.1
C
Re. megalocarpum CL/Guad05-051 (AM418552)
Re. fulvum AC/Pohn99-001 (consensus 10)
GBOSI-15_T3
F811I-15_T3
GDDN3-15_T3
F4DVV-15_T3
171 reads
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK022-01+02_R)
Di. epigaea BEG47 (FM876815_R)
Di. epigaea BEG47 (FN547636_R)
Di. epigaea BEG47 (FM876820_R)
Di. epigaea BEG47 (FM876818_R)
Di. epigaea BEG47 (FN547635_R)
Di. epigaea BEG47 (FM876816_R)
Di. epigaea BEG47 (AY842568_R)
Di. epigaea BEG47 (FJ461852_R)
Di. epigaea BEG47 (AY842569_R)
Di. epigaea BEG47 (AY842574_R)
Di. epigaea BEG47 (AY842573_R)
Di. epigaea BEG47 (FM876817_R)
Di. epigaea BEG47 (FN547666_R)
Di. epigaea BEG47 (FN547675_R)
Di. epigaea BEG47 (AY842567_R)
Di. epigaea BEG47 (FN547667_R)
Di. epigaea BEG47 (FN547672_R)
Di. epigaea BEG47 (FN547668_R)
Di. epigaea BEG47 (FN547680_R)
Di. epigaea BEG47 (FN547679_R)
Di. epigaea BEG47 (FN547674_R)
Di. epigaea W3180/Att475-22 (FR686939_R)
Di. epigaea BEG47 (FN547677_R)
Di. epigaea W3180/Att475-22 (FR686940_R)
Di. epigaea W3180/Att475-22 (FR686938_R)
Di. epigaea BEG47 (FN547676_R)
Di. epigaea BEG47 (FN547673_R)
Di. epigaea W3180/Att475-22 (FR686941_R)
Di. epigaea BEG47 (FN547669_R)
Di. epigaea BEG47 (FN547670_R)
Di. epigaea BEG47 (FN547671_R)
Di. epigaea BEG47 (FM876814_R)
Di. epigaea BEG47 (FN547681_R)
Di. epigaea BEG47 (FN547678_R)
Di. epigaea BEG47 (AM947665_R)
Di. epigaea BEG47 (FM876819_R)
Diversispora sp. W5349/Att1449-5 (from Ha-N4, CK022-03_R)
0.1

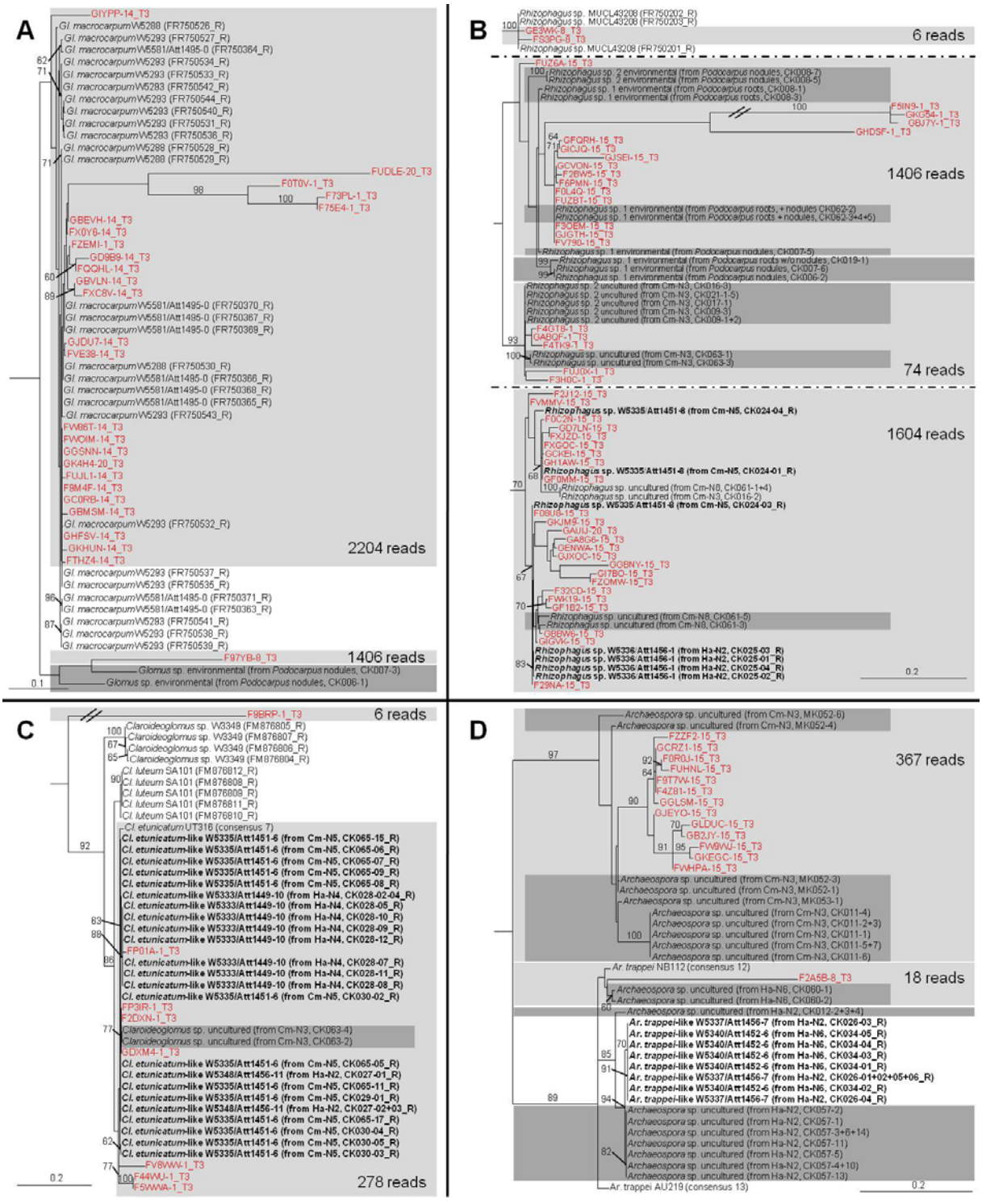
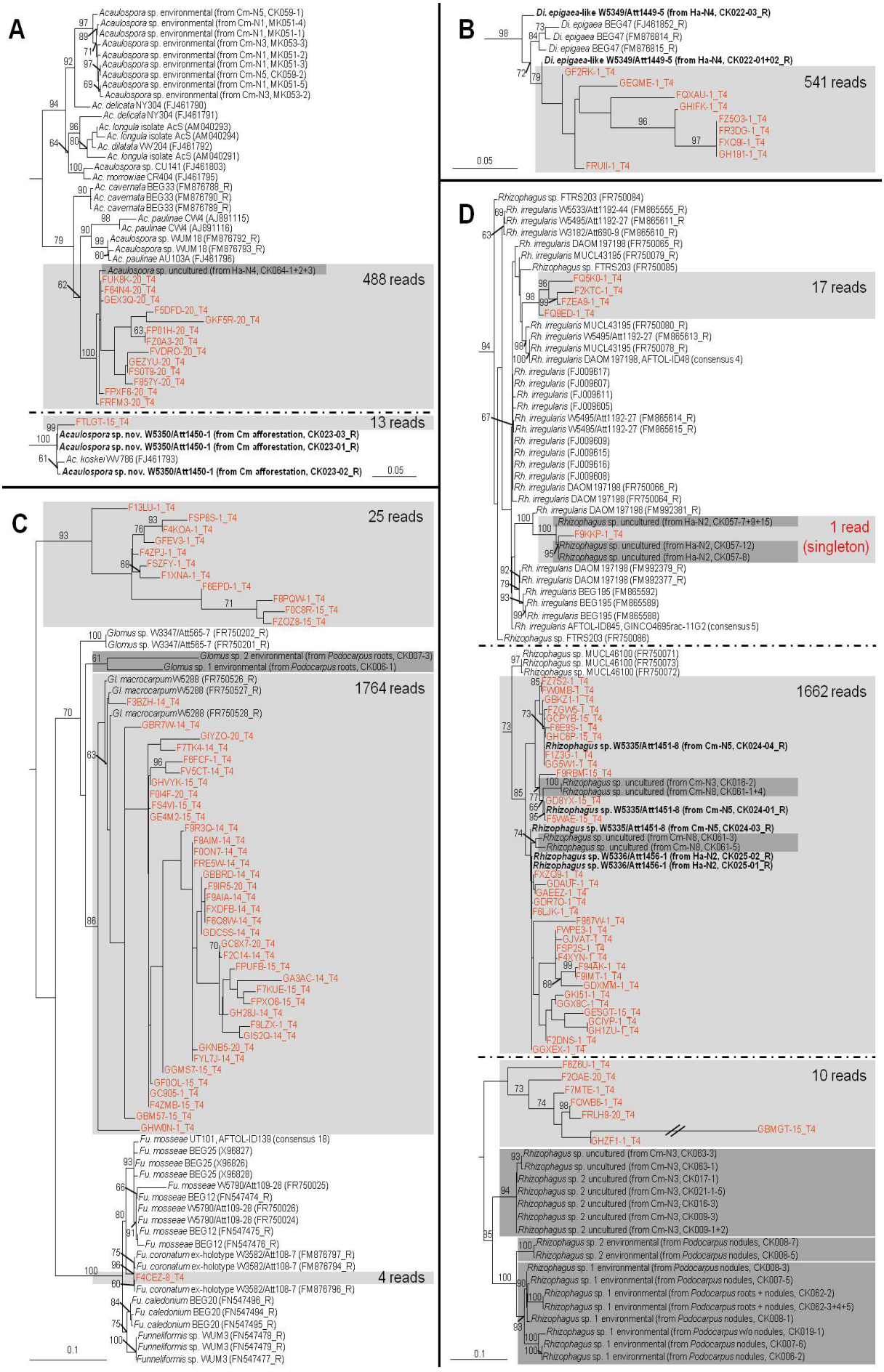


Fig. 27: Maximum likelihood phylogenetic tree of *Glomerales* (A-C) and *Archaeosporales* (D) clades including 454 sequence reads, of *Tabebuia chrysantha* after 3 months in the nursery (+AMF + LF treatment). The Ecuadorian AMF cultures are written in bold, uncultured and environmental AMF are marked with a dark gray box, 454 sequence reads are red and clusters are indicated by a light gray box, including the according read numbers. Bootstrap supports below 60% are not shown. The dot-and-dash line indicates different clusters within a genus. The scale bar shows the substitutions per site. Two diagonal slashes indicate a branch shortened by 50%.

5,168 sequence reads were obtained resulting in 131 unique reads after clustering from the +AMF treatment (Fig. 28, 29). The sequences clustered in *Acaulospora*, *Diversispora*, *Claroideoglomus*, *Funneliformis*, *Rhizophagus*, *Glomus* and *Archaeospora*. Two separate sequence cluster were found in the genus *Acaulospora* (Fig. 28A), the first falls in a clade together with the uncultured *Acaulospora* sp. (Ha-N4, 100% BS) and the second part with *Acaulospora* sp. nov. Att1450-1 (100% BS). A part of the 454 sequences cluster within *Diversispora* (Fig. 28B) together with the *Diversispora* sp. Att1449-5 and *Di. epigaea* BEG47 in one clade supported by 98% BS. Some of the 454 sequences named as *Glomeraceae* spp. cluster with 93% BS basal to the *Glomus* and *Funneliformis* clade. The main part of 454 sequences (1764 reads) falls within *Glomus* (Fig. 28C) and clusters together in a clade with *Gl. macrocarpum* W5288 supported by 86%. Only 4 sequence reads clustered in *Funneliformis* (Fig. 28C), together with *Fu. coronatum* W3582/Att108-7 with 96% BS. Four different clusters of 454 sequence reads were found in *Rhizophagus* (Fig. 28D). The first group of sequences clustered together with *Rh. irregularis* species. One single read (singleton) fall in a clade together with the uncultured *Rhizophagus* sp. from Ha-N2 and was excluded from analysis. The second largest 454 sequence cluster of the +AMF treatment falls into a clade together with the *Rhizophagus* sp. Att1451-8, Att1456-1 with 85% BS. The last 454 reads in the genus clustered only with low BS (47%) in their own clade and therefore were only named as *Rhizophagus* sp. Further 454 sequences cluster within *Claroideoglomus* (Fig. 29A), together with *Cl. etunicatum*-like Att1449-10, Att1451-6, Att1456-11. The last part of the 454 sequence reads clustered within *Archaeospora* (Fig. 29B), together with *Ar. trappei* NB112 and *Ar. schenkii* W5673/Att212-4 with 97% BS.

Fig. 28: Maximum likelihood phylogenetic tree of *Acaulospora* (A), *Diversispora* (B) and *Glomerales* (C, D) clades including 454 sequence reads, of *Tabebuia chrysantha* after 3 months in the nursery (+AMF treatment). The Ecuadorian AMF cultures are written in bold, uncultured and environmental AMF are marked with a dark gray box, 454 sequence reads are red and clusters are indicated by a light gray box, including the according read numbers. Bootstrap supports below 60% are not shown. The dot-and-dash line indicates different clusters within a genus. The scale bar shows the substitutions per site. Two diagonal slashes indicate a branch shortened by 50%.


A
488 reads
13 reads
Acaulospora sp. uncultured (from Ha-N4, CK064-1+2+3)
Acaulospora sp. nov. W5350/Att1450-1 (from Cm afforestation, CK023-03_R)
Acaulospora sp. nov. W5350/Att1450-1 (from Cm afforestation, CK023-01_R)
Acaulospora sp. nov. W5350/Att1450-1 (from Cm afforestation, CK023-02_R)
B
541 reads
Di. epigaea-like W5349/Att1449-5 (from Ha-N4, CK022-03_R)
Di. epigaea-like W5349/Att1449-5 (from Ha-N4, CK022-01+02_R)
C
25 reads
1764 reads
4 reads
D
17 reads
1 read (singleton)
1662 reads
10 reads
Rhizophagus sp. W5335/Att1451-8 (from Cm-N5, CK024-04_R)
Rhizophagus sp. W5335/Att1451-8 (from Cm-N5, CK024-01_R)
Rhizophagus sp. W5335/Att1451-8 (from Cm-N5, CK024-03_R)
Rhizophagus sp. W5336/Att1456-1 (from Ha-N2, CK025-02_R)
Rhizophagus sp. W5336/Att1456-1 (from Ha-N2, CK025-01_R)

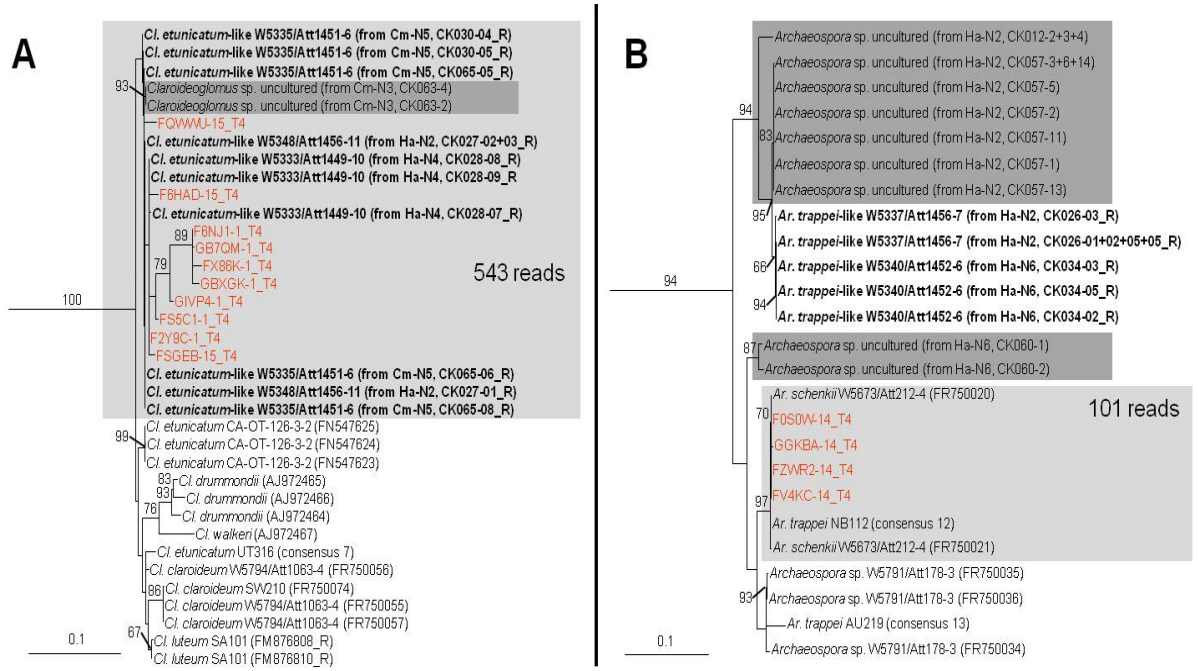


Fig. 29: Maximum likelihood phylogenetic tree of *Claroideoglomus* (A) and *Archaeospora* (B) clades including 454 sequence reads, of *Tabebuia chrysantha* after 3 months in the nursery (+AMF treatment). The Ecuadorian AMF cultures are written in bold, uncultured and environmental AMF are marked with a dark gray box, 454 sequence reads are red and clusters are indicated by a light gray box, including the according read numbers. Bootstrap supports under 60% are not shown. The scale bar shows the substitutions per site.

In summary the introduced AMF are detectable in the roots of *T. chrysantha* (see Table 30). *Cl*. *etunicatum* Att1449-10, Att1451-6, Att1456-11, *Diversispora* sp. Att1449-5 and *Rhizophagus* sp. Att1451-8, Att1456-1 colonized the roots in both AMF treatments. *Acaulospora* sp. nov. Att1450-1 was only detected in the +AMF treatment. Additionally, AMF detected in roots stemming from Nursery experiment No.1, especially *Acaulospora* spp., *Rhizophagus* sp. 1, *Gl*. *macrocarpum* and *Rhizophagus* spp. were found. The roots of *T. chrysantha* were mainly colonized by *Acaulospora* spp., *Gl*. *macrocarpum*, *Diversispora* sp. Att1449-5 and *Rhizophagus* spp. (Table 30). The +AMF + LF treatment contained more sequence reads of uncultured AMF, previously identified by Sanger sequencing within the nursery roots (see chapter 3.1.2), than the +AMF treatment.

Treat-ment	Number of replicate	AMF species	Reference sequence	No. of reads	Percent-age	Total reads*
+AMF+LF	20	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	GCML1-20	56	0.59%	9466
+AMF+LF	1/8/20	*Acaulospora* sp. uncultured (N4-Ha)	GDS7W-20	3272	34.57%	9466
+AMF+LF	15	*Archaeospora* sp. uncultured (N3-Cm)	F9T7W-15	367	3.88%	9466
+AMF+LF	1/8/14/20	*Archaeospora* sp. uncultured (N6-Ha)	F2A5B-8	18	0.19%	9466
+AMF+LF	1/8	*Cl. etunicatum*-like Att1449-10 (Ha-N4); Att1451-6 (Cm-N5); Att1456-11 (Ha-N2)	F2DXN-1	278	2.94%	9466
+AMF+LF	1	*Claroideoglomus* sp.	F9BRP-1	6	0.06%	9466
+AMF+LF	15	*Diversispora* sp. Att1449-5 (Ha-N4)	F4DVV-15	171	1.81%	9466
+AMF+LF	1/8/14/20	*Gl. macrocarpum*	GBVLN-14	2204	23.28%	9466
+AMF+LF	8	*Rhizophagus* sp.	FS3PG-8	6	0.06%	9466
+AMF+LF	15/20	*Rhizophagus* sp. Att1451-8 (Cm-N5); Att1456-1 (Ha-N2)	FWK19-15	1604	16.94%	9466
+AMF+LF	1/15	*Rhizophagus* sp. environmental (from *P. oleifolius* root nodules)	F0L4Q-15	1406	14.85%	9466
+AMF+LF	1/14	*Rhizophagus* sp. 1 uncultured (N3-Cm)	F3H0C-1	74	0.78%	9466
+AMF+LF	14	*De. heterogama*-like	FV706-14	4	0.04%	9466
+AMF	15	*Acaulospora* sp. nov. Att1450-1 (Cm-Rhizosphere afforestation)	FTLGT-15	13	0.25%	5168
+AMF	15/20	*Acaulospora* sp. uncultured (N4-Ha)	FP01H-20	488	9.44%	5168
+AMF	14/15/20	*Ar. schenkii*-like	FZWR2-14	101	1.95%	5168
+AMF	1/8/15	*Cl. etunicatum*-like Att1449-10 (Ha-N4); Att1451-6 (Cm-N5); Att1456-11 (Ha-N2)	F6HAD-15	543	10.51%	5168
+AMF	1/20	*Diversispora* sp. Att1449-5 (Ha-N4)	GF2RK-1	541	10.47%	5168
+AMF	8	*Fu. coronatum*-like	F4CEZ-8	4	0.08%	5168
+AMF	1/15	*Glomeraceae* sp.	F4ZPJ-1	25	0.48%	5168
+AMF	1/8/14/15/20	*Gl. macrocarpum*	GC8X7-20	1764	34.13%	5168
+AMF	1/15	*Rhizophagus* sp. Att1451-8 (Cm-N5); Att1456-1 (Ha-N2)	GESGT-15	1662	32.16%	5168
+AMF	1	*Rh. irregularis*	GHUYK-1	17	0.33%	5168
+AMF	1/15/20	*Rhizophagus* sp.	F2OAE-20	10	0.19%	5168

Table 30: 454 sequence reads of the AMF treatments occurring in roots of *Tabebuia chrysantha*, at 3 months in the nursery. *: Singletons and doubletons were excluded from the analysis. 454 sequence reads are listed after the related AMF species. Ecuadorian AMF-applied by inoculation are marked in dark gray, nursery-AMF detected in Nursery experiment No. 1 in light gray, sequences of *Podocarpus oleifolius* in medium gray. **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

Non-AMF treatments (control, HF, -AMF + LF)

Results of the phylogenetic analyses of the remaining 454 reads in the non-AMF treatments are shown in Table 31 (see also Appendix Table A11). The quality check of control, HF and –AMF + LF treatments resulted in 102, 121 and 64 unique reads. The control and HF treatment showed no AMF applied by the inoculum, but high percentages of the nursery-AMF as sequenced from mycorrhizal roots of Nursery experiment No. 1. However, *Diversispora* sp. Att1449-5 was found in the –AMF + LF treatment, which may be due to an incomplete heat inactivation of the inoculum. Only the control showed an additional AMF which was also found in the roots of *P. oleifolius*. *Glomus macrocarpum* was found in high percentages in all treatments. In the non-AMF treatments the roots of *T. chrysantha* were mainly colonized by *Acaulospora* spp., *Gl. macrocarpum* and *Archaeospora* spp. (Table 31).

Treat-ment	Number of replicate	AMF species	Reference sequence	No. of reads	Percent-age	Total reads*
control	15	*Ac. brasiliensis*	GHYD6-15	95	1.58%	6002
control	8/15	*Acaulospora* sp.	F1WYE-15	9	0.15%	6002
control	1/8/14/15	*Acaulospora* sp. uncultured (N4-Ha)	FTVIZ-15	2393	39.87%	6002
control	1/8/20	*Archaeospora* sp. uncultured (N6-Ha)	F0QMQ-20	813	13.55%	6002
control	1/8/14/15/20	*Gl. macrocarpum*	GG6DA-8	2682	44.69%	6002
control	8/20	*Rhizophagus* sp. environmental (from *P. oleifolius* roots w/o nodules)	GIU7N-8	7	0.12%	6002
control	8	*De. heterogama*	GJY31-8	3	0.05%	6002
HF	1/3	*Ac. brasiliensis*-like	FVVSK-1	35	0.43%	8069
HF	15	*Ac. laevis*-like	FR016-15	34	0.42%	8069
HF	1/3/8/15/20	*Acaulospora* sp. uncultured (N4-Ha)	F14TF-1	5933	73.53%	8069
HF	15/20	*Archaeospora* sp. uncultured (N2-Ha; N3-Cm)	F4OAV-20	14	0.17%	8069
HF	1/8/20	*Ar. trappei*-like	FSAV8-8	44	0.55%	8069
HF	1/8/15	*Glomeraceae* sp.	FW29H-8	57	0.71%	8069
HF	1/8/15/20	*Gl. macrocarpum*	GI98J-8	1952	24.19%	8069
-AMF +LF	1/8/14/15	*Acaulospora* sp. uncultured (N4-Ha)	F88MZ-1	2482	63.09%	3934
-AMF +LF	1	*Ambispora* sp.	F6NM1-1	8	0.20%	3934
-AMF +LF	1/14/15	*Ar. schenkii*-like	FQUWX-14	73	1.86%	3934
-AMF +LF	1	*Diversispora* sp. Att1449-5 (Ha-N4)	GBY5Q-1	6	0.15%	3934
-AMF +LF	1/8/14/15/20	*Gl. macrocarpum*	F0KA0-8	1346	34.21%	3934
-AMF +LF	8/15/20	*Rhizophagus* sp.	FYFP2-15	8	0.20%	3934
-AMF +LF	1/14	*Rhizophagus* sp. 1 uncultured (N3-Cm)	GEVB6-14	7	0.18%	3934
-AMF +LF	1/8	*De. dipapillosa*-like	FQ54Z-1	4	0.10%	3934

Table 31: 454 sequence reads of *Tabebuia chrysantha* at the first sampling (3 months) in the nursery, non-AMF treatments. *: Singletons and doubletons were excluded from the analysis. 454 sequence reads are listed after the related AMF species. Ecuadorian AMF-applied by inoculation are marked in dark gray, nursery-AMF detected in Nursery experiment No. 1 in light gray, sequences of *Podocarpus oleifolius* in medium gray. **Control:** control treatment, **HF:** High fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization.

3.2.4.2 *Cedrela montana*

Due to reduced space in the sequencing runs it was decided to exclude the control treatment of *C. montana* in the nursery phase from analysis. Therefore 90 instead of 100 samples were analyzed by 454 GS FLX sequencing. In total 227,576 sequence reads (> 300 bp) were achieved for *C. montana*, resulting in 11,366 clustered sequences. All results for *C. montana* are summarized in Appendix Table A9.

The two AMF treatments showed different AMF species distribution for *C. montana* (Table 32). Neither *Diversispora* sp. Att1449-5 nor *Cl. etunicatum*-like Att1449-10, Att1451-6, Att1456-11 sequence reads were detectable in the roots of the seedlings treated with +AMF + LF, while both appeared in the AMF-only treatment. *Acaulospora* sp. nov. Att1450-1 and *Rhizophagus* sp. Att1451-8, Att1456-1 reads were found in both AMF treatments. *Acaulospora* sp. Att1450-1 reads appeared constantly during the nursery and field phase. The percentage of *Rhizophagus* sp. Att1451-8, Att1456-1 detected in the roots of the out-planted tree seedlings declined over time. The nursery AMF from the Nursery experiment No. 1 (Urgiles *et al.* 2009) were identified in the seedlings roots in the nursery and in the field samples (Table 32). Sequence reads of *Acaulospora* spp. (nursery) could be detected in high percentage in the early phase of the seedlings (3 months nursery), but decreased clearly over time in both AMF treatments. The uncultured *Rhizophagus* spp. reads showed an increasing percentage over time for both AMF treatments. Additional AMF sequence reads were detected in both AMF treatments originating neither from the inoculum nor from the Ecuadorian nursery AMF identified before by Sanger-sequencing, such as *Gl. macrocarpum*, *Ac. brasiliensis* and *Ac. scrobiculata*. In the AMF-only treatment these AMF sequence reads increased over time. The comparison of the introduced (AMF applied by inoculation) and non-introduced AMF (uncultured and environmental AMF) sequence reads showed a high level of non-introduced AMF (ca. 4/5) in the +AMF + LF treatment. In the AMF-only treatment the number of introduced and non-introduced sequence reads reached similar percentage during the whole experimental phase (nursery and reforestation).

The non-AMF treatments also contained sequence reads from the inoculum, a high amount of nursery AMF (especially *Acaulospora* and *Rhizophagus* spp.) and few AMF newly

detected by 454 sequencing (such as *Claroideoglomus* and *Diversispora* spp.) in the nursery phase. A similar situation occurs in the field sampling, but a slight shift to increased unspecified AMF sequences could be seen (Appendix Table A9).

		¼ fertilization + AMF (T3)				AMF-only (T4)			
		Nursery phase		Field		Nursery phase		Field	
		3 mo.	6 mo.	15 mo.	18 mo.	3 mo.	6 mo.	15 mo.	18 mo.
AMF applied as mixed inoculum	*Diversispora* sp. Att1449-5					0.25% (33)		2.10% (241)	
	Cl. etunicatum-like Att1449-10, Att1451-6, Att1456-11							28.70% (3,296)	1.80% (254)
	Acaulospora sp. nov. Att1450-1	4.71% (568)		8.29% (1,143)	6.93% (1,398)	5.52% (726)	41.39% (1,775)	0.71% (82)	7.15% (1,007)
	Rhizophagus sp. Att1451-8, Att1456-1	14.15% (1,708)	1.08% (105)	12.29% (1,694)		42.01% (5,521)		23.78% (2,731)	12.88% (1,815)
	Ar. trappei-like Att1452-6, Att1456-7				0.91% (184)			0.05% (6)	
Nursery AMF	Uncultured *Acaulospora* spp.	60.16% (7,262)	25.12% (2,437)	21.47% (2,959)	5.21% (1,052)	39.86% (5,239)	33.63% (1,442)	18.21% (2,092)	4.98% (702)
	Uncultured *Archaeospora* spp.	0.15% (18)	73.26% (7,106)	0.07% (9)	0.09% (19)	0.10% (13)		0.53% (61)	0.16% (22)
	Uncultured *Rhizophagus* spp.	20.77% (2507)		41.02% (5,652)	80.87% (16,320)	5.98% (786)	24.98% (1,071)		33.76% (4,757)
Environ-mental AMF	Environmental *Rhizophagus* spp. [P]								0.06% (8)
	Environmental *Glomus* spp. [P]								
	AMF newly detected by 454	0.06% (8)	0.40% (39)	18.31% (2,310)	5.96% (1,202)	6.28% (825)		12.58% (1,445)	39.23% (5,526)
	Contamination (non-AMF)		0.13% (13)						
	Introduced AMF species	18.86% (2,276)	1.08% (105)	20.58% (2,837)	7.84% (1,582)	47.78% (6,280)	41.39% (1,775)	55.34% (3,625)	21.83% (3,076)
	None introduced AMF species	81.14% (9,795)	98.79% (9,582)	79.42% (10,943)	92.16% (18,600)	52.22% (7,863)	58.61% (2,513)	44.66% (7,860)	78.17% (11,015)
	Number of total reads	**12,071**	**9,700**	**13,780**	**20,182**	**13,143**	**4,288**	**11,485**	**14,091**

Table 32: AMF species detected via 454 GS FLX sequencing of *Cedrela montana* in the in the Nursery experiment No. 3 (nursery and field phase). The percentage of sequence reads per AMF species are given, the according number of reads in brackets. Trends are marked with a gray triangle in the according direction. ***P*:** environmental sequences first sequenced from *Podocarpus oleifolius* roots and nodules. **Nursery AMF:** AMF detected in nursery roots by Sanger sequencing, **introduced AMF species:** AMF applied as inoculum mix, **non-introduced AMF species:** all AMF not introduced by the inoculum mixture, **AMF newly detected by 454:** AMF sequences not identified in any Ecuadorian sample before by Sanger-sequencing.

3.2.4.3 *Heliocarpus americanus*

During sample preparation of sample point 4 (field phase, Nov 2009) only from 3 out of 6 samples DNA could be successfully amplified and therefore sampling consists of 3 representatives only. The additional space was filled with 2 samples of time point 3 (field

phase, Jun 2009), which therefore consists of 7 representatives. In total 180,138 sequences (> 300 bp) were gained and 14,227 reads were analyzed after clustering. Detailed results for all treatments, including plot or replicate numbers and the according AMF species, are summarized in Appendix Table A10.

No *Ar. trappei*-like Att1452-6, Att1456-7 was found in the AMF treatments (Table 33). Both treatments showed a decreasing proportion for the *Cl. etunicatum*-like Att1449-10, Att1451-6, Att1456-11 and the *Acaulospora* sp. nov. Att1450-1 over time. These AMF, especially the *Claroideoglomus*, appeared in the early nursery phase, but then dropped below detection level in the latest field phase. *Diversispora* sp. Att1449-5 only appeared in one field sampling for the +AMF + LF treatment, but twice in the nursery samplings in the AMF-only treatment. The *Rhizophagus* sp. Att1452-6, Att1456-7 was traced back in both treatments and stayed approximately at the same level. The nursery-AMF, such as the *Acaulospora* spp. decreased over time with fertilization. This process seemed to be more rapid in the +AMF + LF than in AMF-only treatment. Interestingly, the uncultured *Archaeospora* spp. did not appear in the +AMF but in the +AMF + LF treatment. The *Rhizophagus* spp. was detectable over the whole sampling period in both treatments. The AMF newly detected by 454 sequencing increased dramatically over time. Especially in the +AMF treatment they represented nearly 97% of all sequence reads in the last field sampling. The introduced AMF species decreased over sampling time and according to this the non-introduced AMF increased.

In the non-AMF treatments also sequence reads from the applied AMF-inoculum could be detected. A large amount of uncultured *Acaulospora* and *Rhizophagus* spp. identified in nursery roots before and AMF newly detected by 454 belonging to e.g. *Claroideoglomus*, *Archaeospora*, *Cetraspora* and *Glomus* were observed (for further details see Appendix Table A10).

		¼ fertilization + AMF (T3)				AMF-only (T4)			
		Nursery phase		Field		Nursery phase		Field	
		3 mo.	6 mo.	13 mo.	16 mo.	3 mo.	6 mo.	13 mo.	16 mo.
AMF applied as mixed inoculum	*Diversispora* sp. Att1449-5			0.12% (13)		4.34% (347)	3.30% (171)		
	Cl. etunicatum-like Att1449-10, Att1451-6, Att1456-11	59.98% (2,292)	15.28% (841)	1.99% (212)		30.39% (2,429)	23.42% (1,212)	19.44% (3,220)	
	Acaulospora sp. nov. Att1450-1	5.91% (226)	0.62% (34)			0.40% (32)		0.05% (8)	0.02% (3)
	Rhizophagus sp. Att1451-8, Att1456-1		6.56% (361)	2.45% (261)	18.71% (2,385)	1.86% (149)	22.45% (1,162)	2.37% (393)	3.42% (576)
	Ar. trappei-like Att1452-6, Att1456-7								
Nursery AMF	Uncultured *Acaulospora* spp.	11.67% (446)	5.80% (319)			23.42% (1,872)	24.15% (1,250)	0.04% (7)	0.02% (3)
	Uncultured *Archaeospora* spp.	22.43% (857)	37.32% (2,054)	0.39% (42)	0.65% (83)				
	Uncultured *Rhizophagus* spp.	0.13% (5)	27.03% (1,488)	57.65% (6,140)	38.67% (4,930)	9.06% (724)	0.06% (3)	60.41% (10,005)	0.02% (3)
Environ-mental AMF	Environmental *Rhizophagus* spp. [P]								
	Environmental *Glomus* spp. [P]								
	AMF newly detected by 454	0.18% (7)	7.40% (407)	37.39% (3,982)	41.97% (5,351)	30.52% (2,439)	26.63% (1,378)	17.69% (2,929)	96.52% (16,245)
	Contamination (non-AMF)								
	Introduced AMF species	65.89% (2,518)	22.46% (1,236)	4.56% (486)	18.71% (2,385)	36.99% (2,957)	49.17% (2,545)	21.86% (3,621)	3.44% (579)
	None introduced AMF species	34.11% (1,303)	77.54% (4,268)	95.44% (10,164)	81.29% (10,364)	63.01% (5,035)	50.83% (2,631)	78.14% (12,941)	96.56% (16,252)
	Number of total reads	**3,821**	**5,504**	**10,650**	**12,749**	**7,992**	**5,176**	**16,562**	**16,831**

Table 33: AMF species detected via 454 GS FLX sequencing of *Heliocarpus americanus* in the Nursery experiment No. 3 (nursery and field phase). The percentage of sequence reads per AMF species are given, the according number of reads in brackets. Trends are marked with a gray triangle in the according direction. ***P*:** environmental sequences first sequenced from *Podocarpus oleifolius* roots and nodules. **Nursery AMF:** AMF detected in nursery roots by Sanger sequencing, **introduced AMF species:** AMF applied as inoculum mix, **non-introduced AMF species:** all AMF not introduced by the inoculum mixture, **AMF newly detected by 454:** AMF sequences not identified in any Ecuadorian sample before by Sanger-sequencing.

3.2.4.4 *Tabebuia chrysantha*

As the reforestation data showed no significant differences between the shaded and the unshaded plots (see chapter 3.2.3.3, also Palomeque 2012), it was decided to do the 454 analysis only for the shaded plots as this represented a more forest-like condition of the seedlings on the pastures. In total 89,660 sequences with more than 300 bp in length were received from 454 GS FLX sequencing, resulting in 2,370 clustered single sequence reads for analysis.

All introduced AMF were detected, but the *Ar. trappei*-like Att1452-5, Att1456-7 appeared only once in all samplings (Table 34). The percentages of the AMF stayed relatively constant in the +AMF treatment. *Diversispora* sp. Att1449-5 AMF decreased over time, as did *Rhizophagus* sp. Att1451-8, Att1456-1. The *Cl. etunicatum*-like Att1449-10, Att1451-6, Att1456-11 showed no clear tendency for decrease or increase. Also AMF previously identified in seedling roots from the Nursery experiment No. 1 were found. *Acaulospora* spp. was the predominant species. Interestingly, the uncultured *Rhizophagus* spp. were predominant at the 3 months sampling in the nursery. Both AMF species found in *P. oleifolius* roots were also found in the roots of *T. chrysantha*. Similar to *H. americanus* AMF newly detected by 454 and non-introduced AMF increased over time, whereas the introduced AMF decreased.

The analysis of the *T. chrysantha* samples showed only few sequence reads of the AMF used for inoculation in the non-AMF treatments. Similar to *C. montana* and *H. americanus* the nursery *Acaulospora* spp. was detected in high amounts. AMF newly detected in the 454 sequence reads mainly belonged to *Glomus*, *Acaulospora* and *Archaeospora* species were also identified (see Appendix Table A11).

		¼ fertilization + AMF (T3)				AMF-only (T4)			
		Nursery phase		Field		Nursery phase		Field	
		3 mo.	6 mo.	12 mo.	15 mo.	3 mo.	6 mo.	12 mo.	15 mo.
AMF applied as mixed inoculum	*Diversispora* sp. Att1449-5	1.81% (171)	0.66% (39)	0.22% (9)	0.16% (3)	10.47% (541)	2.91% (168)	4.78% (341)	1.19% (8)
	Cl. etunicatum-like Att1449-10, Att1451-6, Att1456-11	2.94% (278)	0.14% (8)	20.56% (852)	7.13% (134)	10.51% (543)	0.42% (24)	1.43% (102)	4.61% (31)
	Acaulospora sp. nov. Att1450-1			0.58% (24)		0.25% (13)	7.61% (439)	0.15% (11)	4.02% (27)
	Rhizophagus sp. Att1451-8, Att1456-1	16.94% (1,604)	1.84% (109)	1.79% (74)	4.68% (88)	32.16% (1,662)		0.69% (49)	5.36% (36)
	Ar. trappei-like Att1452-6, Att1456-7						0.73% (42)		
Nursery AMF	Uncultured *Acaulospora* spp.	35.16% (3,328)	18.92% (1,121)	3.76% (156)	16.12% (303)	9.44% (488)	21.02% (1,212)	8.83% (630)	15.48% (104)
	Uncultured *Archaeospora* spp.	4.07% (385)	37.15% (2,201)	0.24% (10)				0.41% (29)	
	Uncultured *Rhizophagus* spp.	0.78% (74)	0.39% (23)		0.43% (8)		0.10% (6)	0.38% (27)	6.10% (41)
Environ-mental AMF	Environmental *Rhizophagus* spp. [P]	14.85% (1,406)	0.07% (4)						
	Environmental *Glomus* spp. [P]							0.57% (41)	1.34% (9)
	AMF newly detected by 454	23.44% (2,220)	40.83% (2,419)	72.84% (3,019)	71.50% (1,344)	37.16% (1,921)	67.20% (3,874)	82.76% (5,904)	61.90% (416)
	Contamination (non-AMF)								
	Introduced AMF species	21.69% (2,053)	2.64% (156)	23.15% (959)	11.97% (225)	53.39% (2,759)	11.67% (673)	7.05% (503)	15.18% (102)
	None introduced AMF species	78.31% (7,413)	97.36% (5,768)	76.85% (3,185)	88.03% (1,655)	46.61% (2,409)	88.33% (5,091)	92.95% (6,631)	84.82% (570)
	Number of total reads	**9,466**	**5,924**	**4,144**	**1,880**	**5,168**	**5,765**	**7,134**	**672**

Table 34: AMF species detected via 454 GS FLX sequencing of *Tabebuia chrysantha* in the in the Nursery experiment No. 3 (nursery and field phase). The percentage of sequence reads per AMF species are given, the according number of reads in brackets. Trends are marked with a gray triangle in the according direction. ***P*:** environmental sequences first sequenced from *Podocarpus oleifolius* roots and nodules. **Nursery AMF:** AMF detected in nursery roots by Sanger sequencing, **introduced AMF species:** AMF applied as inoculum mix, **non-introduced AMF species:** all AMF not introduced by the inoculum mixture, **AMF newly detected by 454:** AMF sequences not identified in any Ecuadorian sample before by Sanger-sequencing.

3.2.4.5 AMF diversity

Up to 16 AMF species per root sample could be detected in the tree seedlings by 454 sequencing (Table 35). In *Cedrela montana* and *Tabebuia chrysantha* roots a lower number of AMF species was found during the nursery stage compared to the field. The contrary was true for roots of *Heliocarpus americanus* seedlings, which harbored more AMF in the nursery than in the field. Despite the high background mycorrhization present in all treatments the highest number of AMF species detected in *T. chrysantha* roots was found in almost all cases in the +AMF treatments (Table 35). In addition, *C. montana* tended to show a higher number of AMF species when inoculated. Seedlings of *H. americanus* showed increased numbers of AMF species in the control and HF treatment in the nursery. After out-planting the number of detected AMF species considerably decreased in the non-AMF treatments, whereas the numbers within the non-AMF treatments decreased slightly or were stable from nursery to field phase.

	Sample point	Treatment	*Cedrela montana*	*Heliocarpus americanus*	*Tabebuia chrysantha*
Nursery phase	1	control	n.d.	**14**	7
	1	HF	6	**15**	7
	1	-AMF + LF	5	7	8
	1	+AMF + LF	**7**	6	**13**
	1	+AMF	**9**	13	**11**
	2	control	n.d.	**14**	4
	2	HF	6	**13**	8
	2	-AMF + LF	**8**	11	6
	2	+AMF + LF	**7**	11	**9**
	2	+AMF	3	9	**11**
Field phase	3	control	11	8	10
	3	HF	**15**	**10**	10
	3	-AMF + LF	10	**12**	**12**
	3	+AMF + LF	**12**	**10**	11
	3	+AMF	**12**	8	**16**
	4	control	10	7	7
	4	HF	10	5	8
	4	-AMF + LF	10	6	8
	4	+AMF + LF	10	**10**	**14**
	4	+AMF	**15**	**9**	**14**

Table 35: Number of detected AMF species per treatment. AMF treatments marked in gray, highest number of AMF species per sampling point is marked in bold. Treatments descriptions are as follows **control:** control treatment, **HF:** High fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

The comparison of average number of AMF species detected in *C. montana* and *T. chrysantha* seedling roots revealed a lower number of AMF species in the nursery than in the field and vice versa for *H. americanus* (Table 36).

	Treatments	*Cedrela montana*	*Heliocarpus americanus*	*Tabebuia chrysantha*
Nursery phase	Non-AMF	6 (6.25)	12 (12.33)	6 (6.67)
	AMF	6 (6.5)	9	11
	In total	6 (6.375)	11 (11.3)	7 (7.3)
Field phase	Non-AMF	11	8	9 (9.17)
	AMF	12 (12.25)	9 (9.25)	13 (13.75)
	In total	11 (11.5)	8 (8.5)	11

Table 36: Average number of AMF species persistent in the tree seedling roots in the nursery and on the reforestation plots. Average was calculated taking all treatments (in total), non-AMF and AMF-treatments into account. Numbers of AMF species are shown in round figures, calculated numbers are written in brackets.

A shift in AMF composition between nursery and field phase was detected in seedlings of *C. montana and H. americanus*, but not in *T. chrysantha*. In roots of *C. montana* additional AMF appeared, which were mainly environmental AMF species formerly detected and sequenced from *Podocarpus oleifolius* roots and nodules from the natural forest (Appendix Table A9). In the roots of *H. americanus* seedlings *Cetraspora* sequences were detected, which were not found in the 454 sequences of the nursery samplings (Appendix Table A10). Interestingly, no shift in AMF species composition could be observed in *T. chrysantha*, as the detected fungal sequences covering all glomeromycotan main lineages except *Paraglomerales* appear in the nursery as well as in the field (Appendix Table A11).

3.3 Nursery experiment No. 4 and No. 4A

In these experiments individual AMF inocula were applied to tree seedlings of *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha*, to specify potential AMF-plant preferences.

3.3.1 Inoculum efficiency tested on *Plantago lanceolata* as host plant

Mycorrhization rates of the seven AMF species were estimated and are shown in Fig. 30. Mycorrhization rates differed from almost zero for *Diversispora* sp. Att1449-5 up to 43% for *Rhizophagus* sp., but with high standard errors for all AMF. Inoculation by *Rhizophagus* sp. Att1451-8 resulted in the highest mycorrhization rate (43%), followed by *Ambispora* sp. Att1449-12 with 33% and *Cl. etunicatum*-like Att1449-10 with 22% after 3 months. Inoculation of *Plantago lanceolata* seedlings by *Diversispora* sp. Att1449-5, *Acaulospora* sp. nov. Att1450-1, *De. savannicola* Att1455-2 and *Ar. trappei*-like Att1456-7 resulted in low mycorrhization rates ranging from 14 to 4%. Seedlings inoculated by *Acaulospora* sp. nov. Att1450-1 showed nearly no mycorrhization at both sampling times. As *Acaulospora* was only faintly stained by methyl blue the observed mycorrhization rate does not necessarily reflect the correct situation in the roots. A subsequent spore survey of the *Acaulospora* sp. nov. Att1450-1 and *De. savannicola* Att1455-2 'test'-cultures (A, B and C) revealed sufficient numbers of spores after 6 months, indicating that successful mycorrhiza establishment has taken place. Mycorrhization rates of six out of seven AMF-*Plantago lanceolata* test cultures stayed relatively constant over the whole sampling period, except for *Ambispora* sp. Att1449-12, where the mycorrhization rate decreased from 33 to 3% within 3 months.

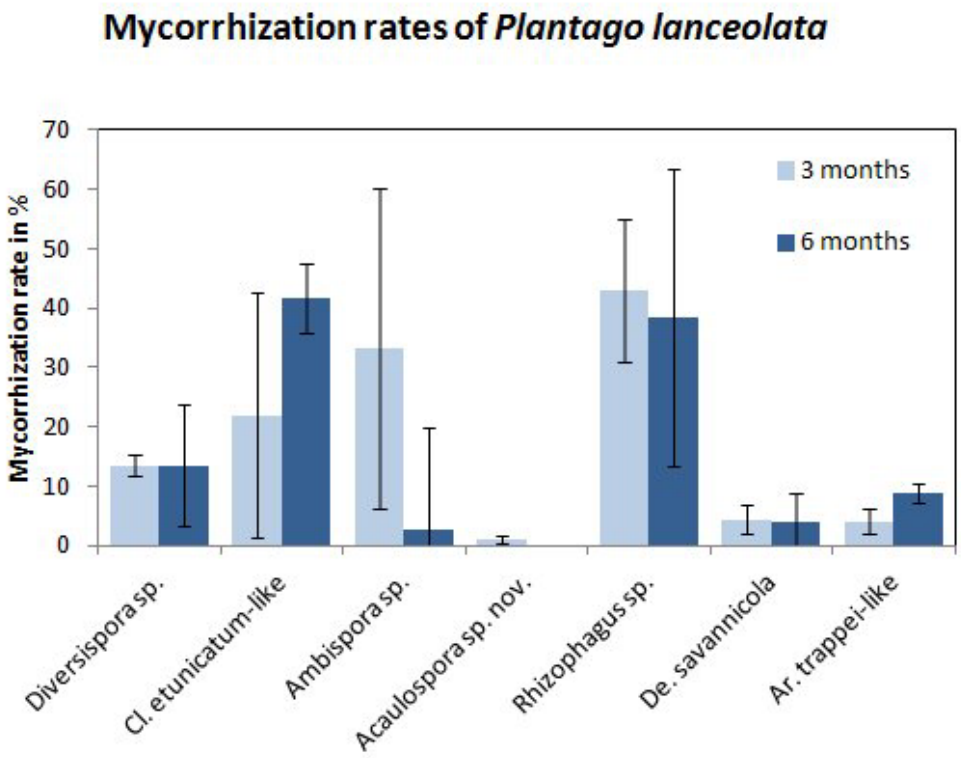


Fig. 30: Mycorrhization rates of *Plantago lanceolata* seedlings inoculated with seven individual AMF. Means ± SE are shown. Light gray bars represent the 3 months sampling, dark gray bars the 6 months sampling.

3.3.2 Inoculum efficiency in Nursery experiment No. 4

In the Nursery experiment No. 4 all three tree species, *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha*, were included.

Clear growth differences between the +AMF and –AMF treatments were visible after 5 months in the nursery (Fig. 31; Appendix Fig. A3-1 and A3-2). Seedlings inoculated with AMF performed much better than those without.

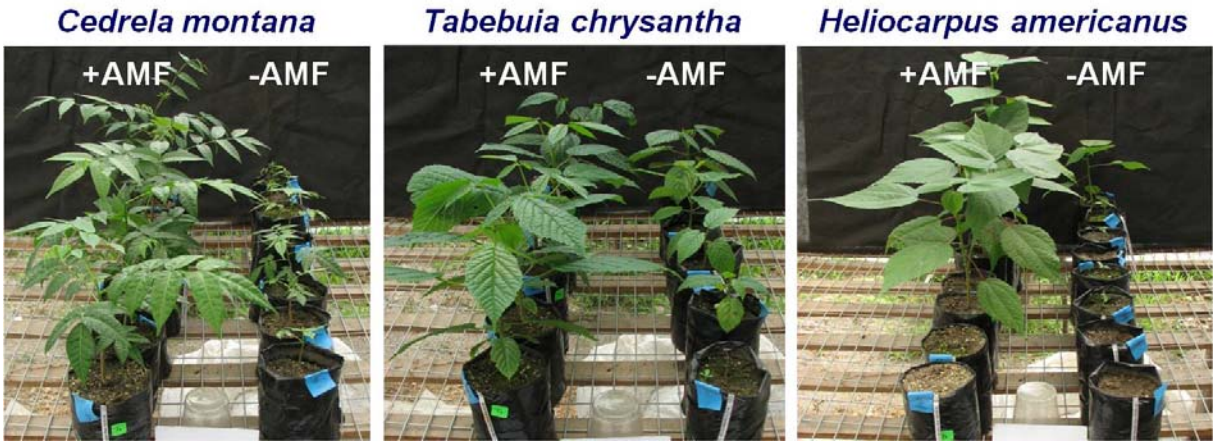


Fig. 31: Plant growth performance of tree seedling in the Nursery experiment No. 4 inoculated with *Rhizophagus* sp. Att1451-8 (after 5 months). The treatments are labeled as follows **+AMF:** living inoculum, **–AMF:** heat-killed inoculum. Both treatments received a low (¼) fertilization dose.

The *Cedrela montana* seedlings performed always better in growth when inoculated with AMF than in the -AMF treatment (Fig. 32, Table 37). This difference was more distinct after six months. AMF inoculation improved height, RCD, number of leaves, leaf area. Fresh weight and biomass of the leaves, shoots and roots were also positively affected. The mycorrhization rates were higher in +AMF compared to the -AMF treatment. Seedlings performed differently on the applied AMF species. The *Claroideoglomus etunicatum*-like Att1449-10 fungus increased biomass of leaves, shoots and roots. The seedlings reacted less to *Diversispora* sp. Att1449-5 and *Ar. trappei*-like Att1452-6. While mycorrhization rates of *Rhizophagus* sp. Att1451-8 and *Ambispora* sp. Att1449-12 were similar, seedling mortality was highest (28%) when inoculated by *Ambispora* sp. Att1449-12 also in the -AMF treatment (14%). Except for *Ambispora* sp. Att1449-12 and *Ar. trappei*-like Att1456-7, all seedlings showed lower mortality when inoculated with AMF than without inoculation.

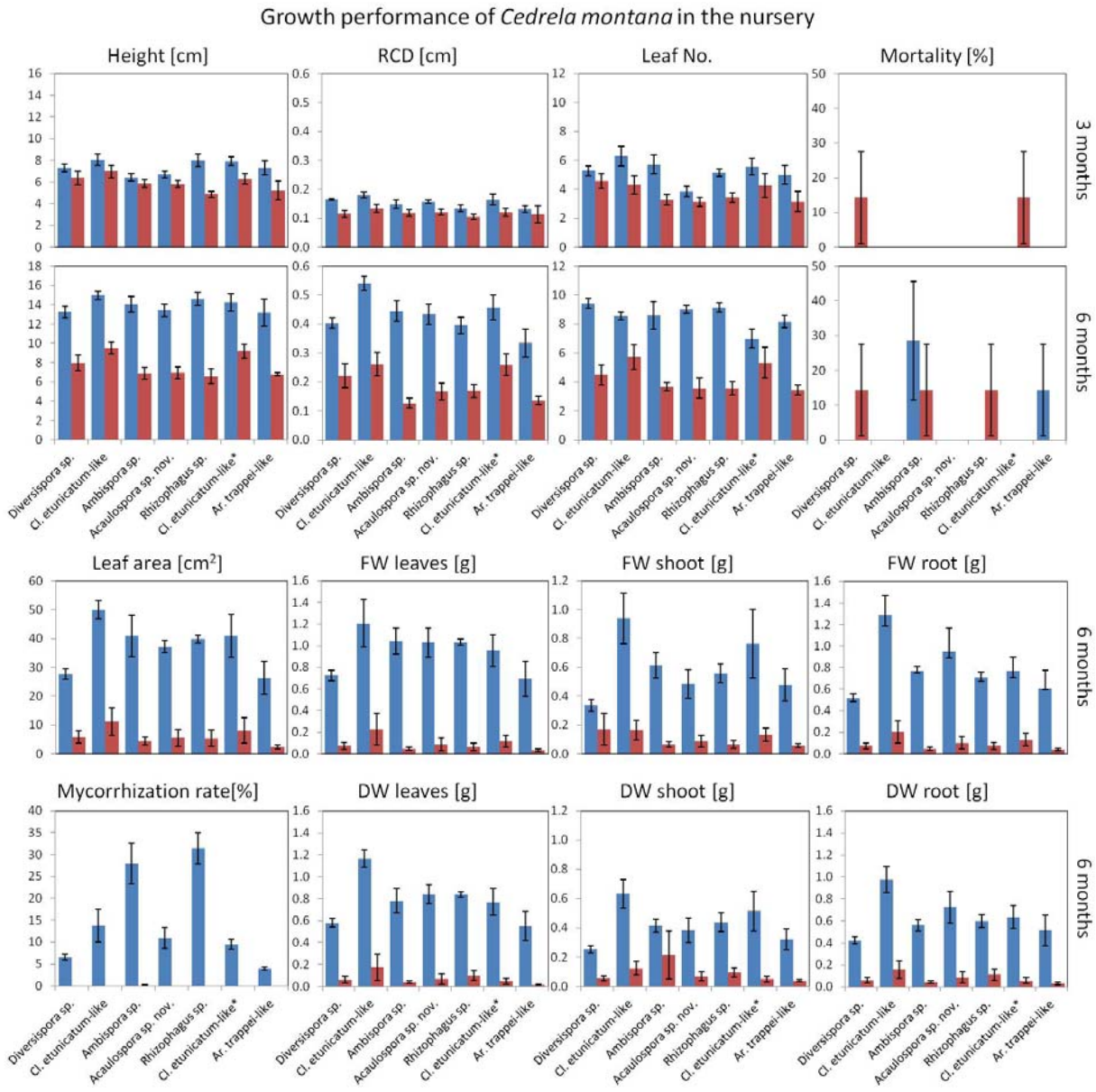


Fig. 32: Growth data of *Cedrela montana* in the Nursery experiment No. 4, after inoculation with individual AMF species. Blue bars represent +AMF (living inoculum), red bars –AMF (heat-killed inoculum) treatment. Both treatments received a low (¼) fertilization dose. Means ± SE are shown. The *Cl. etunicatum*-like multispore culture is marked with ***** because it most likely contains more than one AMF species.

Time [mo]	AMF	Height [cm]	RCD [cm]	Leaves	Mortality [%]	Leaf area [cm²]	Fresh weight [g]			Biomass [g]			Mycorrhization rate [%]
							Leaves	Shoot	Root	Leaves	Shoot	Root	
3	Att1449-5	7.3 ± 0.36	0.17 ± 0.003	5 ± 0.3	0.00 ± 0.00								
3	Att1449-10	8.0 ± 0.49	0.18 ± 0.010	6 ± 0.7	0.00 ± 0.00								
3	Att1449-12	6.4 ± 0.33	0.15 ± 0.014	6 ± 0.7	0.00 ± 0.00								
3	Att1450-1	6.7 ± 0.32	0.16 ± 0.006	4 ± 0.4	0.00 ± 0.00								
3	Att1451-8	8.0 ± 0.59	0.13 ± 0.011	5 ± 0.2	0.00 ± 0.00								
3	Att1451-18*	7.9 ± 0.40	0.16 ± 0.019	6 ± 0.6	0.00 ± 0.00								
3	Att1456-7	7.3 ± 0.68	0.13 ± 0.011	5 ± 0.7	0.00 ± 0.00								
3	Att1449-5	6.4 ± 0.64	0.12 ± 0.012	5 ± 0.5	14.29 ± 13.23								
3	Att1449-10	7.0 ± 0.56	0.13 ± 0.014	4 ± 0.6	0.00 ± 0.00								
3	Att1449-12	5.9 ± 0.37	0.12 ± 0.010	3 ± 0.3	0.00 ± 0.00								
3	Att1450-1	5.8 ± 0.31	0.12 ± 0.009	3 ± 0.3	0.00 ± 0.00								
3	Att1451-8	4.9 ± 0.27	0.10 ± 0.009	3 ± 0.3	0.00 ± 0.00								
3	Att1451-18*	6.3 ± 0.48	0.12 ± 0.014	4 ± 0.8	14.29 ± 13.23								
3	Att1456-7	5.2 ± 0.87	0.11 ± 0.029	3 ± 0.7	0.00 ± 0.00								
6	Att1449-5	13.3 ± 0.61	0.40 ± 0.018	9 ± 0.3	0.00 ± 0.00	27.61 ± 1.833	0.722 ± 0.0471	0.335 ± 0.0394	0.515 ± 0.0404	0.579 ± 0.0391	0.254 ± 0.0239	0.421 ± 0.0325	6.53 ± 0.637
6	Att1449-10	15.0 ± 0.43	0.54 ± 0.024	9 ± 0.3	0.00 ± 0.00	49.95 ± 3.105	1.206 ± 0.2176	0.940 ± 0.1760	1.286 ± 0.1805	1.165 ± 0.0797	0.632 ± 0.0986	0.977 ± 0.1195	13.73 ± 3.736
6	Att1449-12	14.0 ± 0.83	0.44 ± 0.035	9 ± 1.0	28.57 ± 17.07	40.89 ± 7.189	1.044 ± 0.1206	0.613 ± 0.0881	0.767 ± 0.0449	0.782 ± 0.1075	0.414 ± 0.0451	0.559 ± 0.0488	27.87 ± 4.674
6	Att1450-1	13.4 ± 0.63	0.43 ± 0.035	9 ± 0.3	0.00 ± 0.00	37.22 ± 2.104	1.028 ± 0.1360	0.484 ± 0.0983	0.948 ± 0.2175	0.841 ± 0.0845	0.384 ± 0.0830	0.725 ± 0.1440	10.93 ± 2.375
6	Att1451-8	14.6 ± 0.67	0.40 ± 0.028	9 ± 0.3	0.00 ± 0.00	39.77 ± 1.313	1.032 ± 0.0258	0.558 ± 0.0666	0.705 ± 0.0531	0.839 ± 0.0242	0.439 ± 0.0640	0.595 ± 0.0597	31.47 ± 3.585
6	Att1451-18*	14.2 ± 0.92	0.46 ± 0.042	7 ± 0.7	0.00 ± 0.00	40.89 ± 7.458	0.957 ± 0.1469	0.763 ± 0.2400	0.766 ± 0.1272	0.769 ± 0.1212	0.516 ± 0.1365	0.634 ± 0.1032	9.47 ± 1.122
6	Att1456-7	13.2 ± 1.41	0.34 ± 0.048	8 ± 0.4	14.29 ± 13.23	26.37 ± 5.684	0.695 ± 0.1621	0.476 ± 0.1092	0.609 ± 0.1662	0.550 ± 0.1317	0.324 ± 0.0709	0.512 ± 0.1406	4.00 ± 0.267
6	Att1449-5	8.0 ± 0.80	0.22 ± 0.041	5 ± 0.7	14.29 ± 13.23	5.82 ± 2.079	0.074 ± 0.0332	0.170 ± 0.1111	0.073 ± 0.0278	0.062 ± 0.0279	0.055 ± 0.0182	0.060 ± 0.0226	0.00 ± 0.000
6	Att1449-10	9.5 ± 0.62	0.26 ± 0.040	6 ± 0.9	0.00 ± 0.00	11.11 ± 4.735	0.226 ± 0.1466	0.164 ± 0.0661	0.204 ± 0.1022	0.177 ± 0.1182	0.123 ± 0.0481	0.158 ± 0.0801	0.00 ± 0.000
6	Att1449-12	6.9 ± 0.62	0.13 ± 0.017	4 ± 0.3	14.29 ± 13.23	4.28 ± 1.345	0.045 ± 0.0147	0.064 ± 0.0172	0.047 ± 0.0143	0.037 ± 0.0124	0.215 ± 0.1632	0.040 ± 0.0117	0.13 ± 0.119
6	Att1450-1	6.9 ± 0.62	0.17 ± 0.029	4 ± 0.7	0.00 ± 0.00	5.49 ± 2.944	0.084 ± 0.0605	0.088 ± 0.0399	0.102 ± 0.0601	0.067 ± 0.0491	0.068 ± 0.0309	0.084 ± 0.0497	0.00 ± 0.000
6	Att1451-8	6.6 ± 0.77	0.17 ± 0.022	4 ± 0.4	0.00 ± 0.00	5.35 ± 2.846	0.064 ± 0.0355	0.066 ± 0.0286	0.072 ± 0.0354	0.099 ± 0.0444	0.094 ± 0.0315	0.111 ± 0.0506	0.00 ± 0.000
6	Att1451-18*	9.2 ± 0.73	0.26 ± 0.038	5 ± 1.0	14.29 ± 13.23	8.07 ± 4.374	0.116 ± 0.0534	0.132 ± 0.0450	0.131 ± 0.0588	0.048 ± 0.0283	0.049 ± 0.0218	0.054 ± 0.0271	0.00 ± 0.000
6	Att1456-7	6.8 ± 0.18	0.14 ± 0.015	3 ± 0.3	0.00 ± 0.00	2.33 ± 0.716	0.031 ± 0.0126	0.056 ± 0.0149	0.037 ± 0.0128	0.017 ± 0.0047	0.039 ± 0.0082	0.031 ± 0.0110	0.00 ± 0.000

Table 37: Growth of *Cedrela montana* in the nursery experiment No. 4, after inoculation with individual AMF species. Means ± SE are shown. Data marked in gray represent the +AMF (living inoculum), data without gray background the –AMF (heat-killed inoculum) treatment. The *Cl. etunicatum*-like multispore culture is marked with * because it most likely contains more than one AMF species. AMF cultures are represented by their Att-number, Att1449-5: *Diversispora* sp., Att1449-10: *Cl. etunicatum*-like, Att1449-12: *Ambispora* sp., Att1450-1: *Acaulospora* sp. nov., Att1451-8: *Rhizophagus* sp., Att1451-18: *Cl. etunicatum*-like*, Att1456-7: *Ar. trappei*-like.

Seedlings of *Heliocarpus americanus* showed improved plant performance in the +AMF treatments, when compared to the -AMF treatment for almost all growth parameters, except leaf number and mortality. Leaf number seemed to be only positively affected by AMF inoculation at 3 months, independent of the individual AMF used. After 6 months a higher leaf number was found in the -AMF treatment. This effect becomes apparent especially for *Cl. etunicatum*-like Att1449-10 and *Rhizophagus* sp. Att1451-8 (Fig. 33, Table 38). Inoculation by *Cl. etunicatum*-like species (Att1449-10 and Att1451-18* (ms)) showed an increase in height, RCD and leaf number, fresh weight, root and aboveground (shoot and leaves) biomass when compared to the other AMF used. The same tendency was seen for *Ar. trappei*-like Att1456-7. Mortality of the seedlings inoculated by *Cl*. *etunicatum*-like Att1451-18* (ms), *Rhizophagus* sp. Att1451-8 and *Archaeospora* sp. Att1456-2 was lower in the 3 months sampling than in the -AMF treatment. All other +AMF treatments resulted in an increased or similar mortality rate as the -AMF treatment. After 6 months, mortality of nearly all plants in the -AMF and +AMF treatments was similar, only seedlings inoculated by *Acaulospora* sp. nov. Att1450-1, *Cl. etunicatum*-like Att1451-18* (ms) and *Ar. trappei*-like Att1456-7 showed decreased mortality rates. However inoculation by *Diversispora* sp. Att1449-5 slightly increased mortality after 6 months. Mycorrhization rates were almost zero in the –AMF treatments.

AMF inoculation seemed to slightly increase biomass of *H. americanus*, with minor variations in the growth parameters, such as a reduced leaf number in the +AMF treatment after 6 months. Height and RCD were slightly increased in all +AMF treatments, while the mortality showed no general tendency.

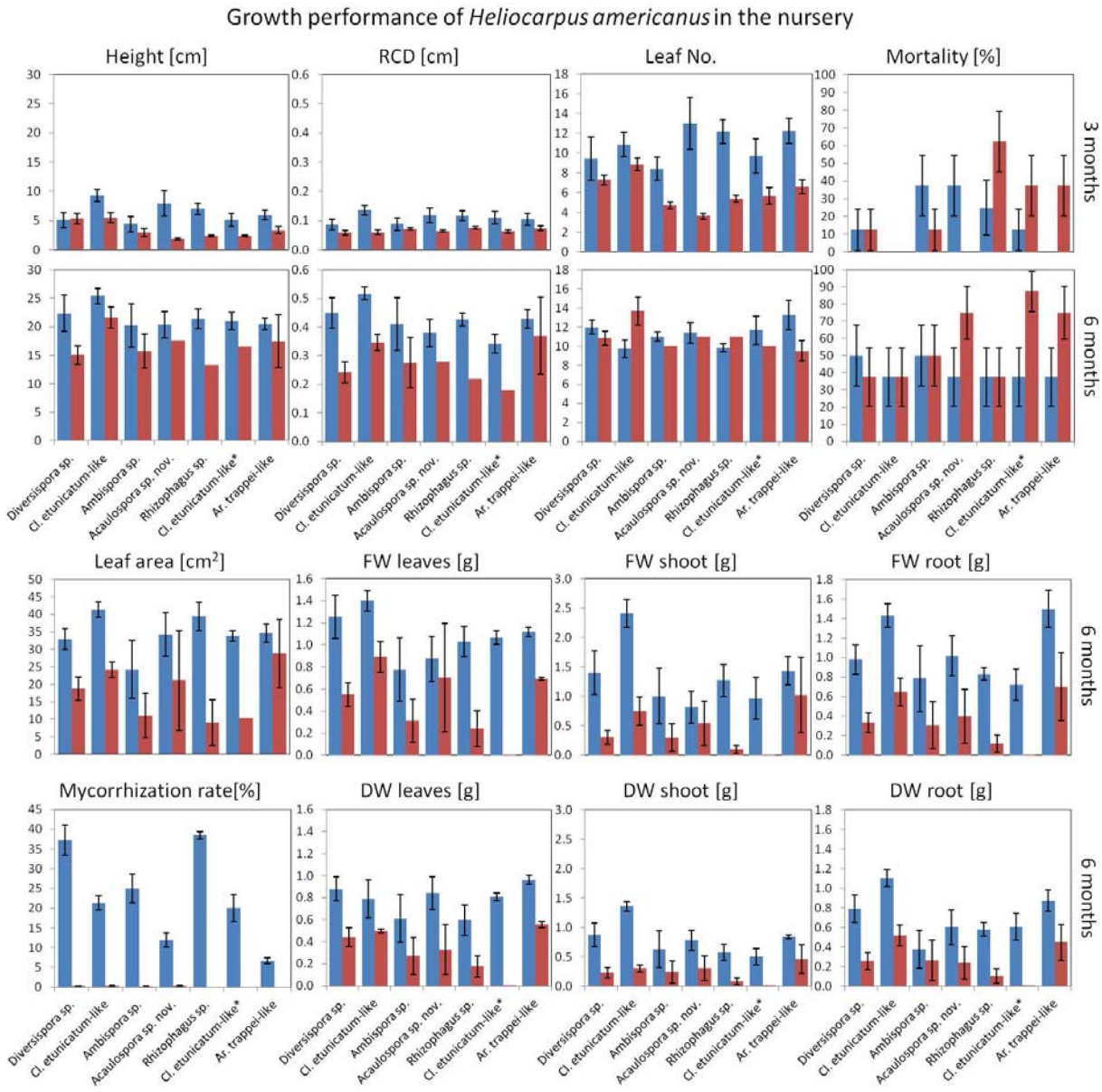


Fig. 33: Growth data of *Heliocarpus americanus* in the Nursery experiment No. 4, after inoculation with individual AMF species. Blue bars represent +AMF (living inoculum), red bars –AMF (heat-killed inoculum) treatment. Both treatments received a low (¼) fertilization dose. Means ± SE are shown. The *Cl. etunicatum*-like multispore culture is marked with ***** because it most likely contains more than one AMF species.

Time [mo]	AMF	Height [cm]	RCD [cm]	Leaves	Mortality [%]	Leaf area [cm²]	Fresh weight [g]			Biomass [g]			Mycorrhization rate [%]
							Leaves	Shoot	Root	Leaves	Shoot	Root	
3	Att1449-5	5.1 ± 1.30	0.09 ± 0.018	9 ± 2.2	12.50 ± 11.69								
3	Att1449-10	9.3 ± 1.00	0.14 ± 0.017	11 ± 1.3	0.00 ± 0.00								
3	Att1449-12	4.4 ± 1.33	0.09 ± 0.020	8 ± 1.2	37.50 ± 17.12								
3	Att1450-1	8.0 ± 2.11	0.12 ± 0.025	13 ± 2.6	37.50 ± 17.12								
3	Att1451-8	7.0 ± 0.99	0.12 ± 0.017	12 ± 1.2	25.00 ± 15.31								
3	Att1451-18*	5.1 ± 1.11	0.11 ± 0.021	10 ± 1.7	12.50 ± 11.69								
3	Att1456-7	6.0 ± 0.83	0.11 ± 0.021	12 ± 1.3	0.00 ± 0.00								
3	Att1449-5	5.4 ± 0.89	0.06 ± 0.008	7 ± 0.9	12.50 ± 11.69								
3	Att1449-10	5.5 ± 0.89	0.06 ± 0.008	9 ± 1.2	0.00 ± 0.00								
3	Att1449-12	3.0 ± 0.66	0.07 ± 0.004	5 ± 1.2	12.50 ± 11.69								
3	Att1450-1	1.9 ± 0.14	0.07 ± 0.004	4 ± 0.5	0.00 ± 0.00								
3	Att1451-8	2.4 ± 0.15	0.08 ± 0.005	5 ± 0.5	62.50 ± 17.12								
3	Att1451-18*	2.4 ± 0.08	0.06 ± 0.005	6 ± 0.7	37.50 ± 17.12								
3	Att1456-7	3.3 ± 0.61	0.07 ± 0.007	7 ± 1.3	37.50 ± 17.12								
6	Att1449-5	22.4 ± 3.17	0.45 ± 0.054	12 ± 0.7	50.00 ± 17.68	32.97 ± 2.915	1.254 ± 0.1969	1.399 ± 0.3699	0.981 ± 0.1507	0.880 ± 0.1116	0.871 ± 0.2015	0.790 ± 0.1378	37.17 ± 3.796
6	Att1449-10	25.4 ± 1.34	0.52 ± 0.022	10 ± 0.9	37.50 ± 17.12	41.32 ± 2.225	1.399 ± 0.0892	2.412 ± 0.2354	1.428 ± 0.1196	0.788 ± 0.1735	1.358 ± 0.0845	1.102 ± 0.0881	21.33 ± 1.799
6	Att1449-12	20.2 ± 3.82	0.41 ± 0.092	11 ± 0.5	50.00 ± 17.68	24.25 ± 8.302	0.776 ± 0.2873	1.002 ± 0.4762	0.785 ± 0.3365	0.614 ± 0.2166	0.632 ± 0.3108	0.379 ± 0.1932	25.00 ± 3.625
6	Att1450-1	20.3 ± 2.31	0.38 ± 0.048	11 ± 1.1	37.50 ± 17.12	34.33 ± 6.178	0.873 ± 0.2041	0.815 ± 0.2734	1.017 ± 0.2045	0.841 ± 0.1479	0.779 ± 0.1730	0.601 ± 0.1747	11.87 ± 1.803
6	Att1451-8	21.4 ± 1.75	0.43 ± 0.023	10 ± 0.4	37.50 ± 17.12	39.47 ± 4.018	1.027 ± 0.1358	1.270 ± 0.2679	0.831 ± 0.0620	0.596 ± 0.1379	0.573 ± 0.1386	0.578 ± 0.0690	38.53 ± 0.912
6	Att1451-18*	21.0 ± 1.60	0.34 ± 0.032	12 ± 1.5	37.50 ± 17.12	33.87 ± 1.572	1.065 ± 0.0627	0.965 ± 0.3574	0.720 ± 0.1600	0.807 ± 0.0355	0.501 ± 0.1365	0.605 ± 0.1359	20.00 ± 3.523
6	Att1456-7	20.5 ± 1.06	0.43 ± 0.033	13 ± 1.5	37.50 ± 17.12	34.58 ± 2.610	1.116 ± 0.0418	1.432 ± 0.2384	1.500 ± 0.1930	0.962 ± 0.0385	0.839 ± 0.0294	0.872 ± 0.1115	6.67 ± 0.777
6	Att1449-5	15.1 ± 1.63	0.24 ± 0.037	11 ± 0.7	37.50 ± 17.12	18.81 ± 3.324	0.548 ± 0.1073	0.306 ± 0.1183	0.334 ± 0.1027	0.440 ± 0.0860	0.227 ± 0.0908	0.256 ± 0.0871	0.27 ± 0.146
6	Att1449-10	21.7 ± 1.86	0.35 ± 0.028	14 ± 1.5	37.50 ± 17.12	24.21 ± 2.270	0.890 ± 0.1380	0.747 ± 0.2380	0.650 ± 0.1405	0.498 ± 0.0178	0.305 ± 0.0613	0.515 ± 0.1078	0.30 ± 0.166
6	Att1449-12	15.8 ± 3.01	0.28 ± 0.088	10 ± 0.0	50.00 ± 17.68	11.11 ± 6.293	0.313 ± 0.1960	0.298 ± 0.2320	0.307 ± 0.2411	0.271 ± 0.1709	0.239 ± 0.1862	0.264 ± 0.2088	0.17 ± 0.144
6	Att1450-1	17.6 ± 0.00	0.28 ± 0.000	11 ± 0.0	75.00 ± 15.31	21.12 ± 14.217	0.703 ± 0.4918	0.541 ± 0.3775	0.398 ± 0.2792	0.328 ± 0.2284	0.308 ± 0.2137	0.237 ± 0.1661	0.33 ± 0.236
6	Att1451-8	13.3 ± 0.00	0.22 ± 0.000	11 ± 0.0	87.50 ± 11.69	9.01 ± 6.519	0.244 ± 0.1602	0.101 ± 0.0677	0.120 ± 0.0874	0.175 ± 0.1006	0.083 ± 0.0561	0.101 ± 0.0737	0.00 ± 0.000
6	Att1451-18*	16.5 ± 0.00	0.18 ± 0.000	10 ± 0.0	37.50 ± 17.12	10.39 ± 0.000	0.007 ± 0.0000	0.006 ± 0.0000	0.004 ± 0.0000	0.005 ± 0.0000	0.005 ± 0.0000	0.003 ± 0.0000	0.00 ± 0.000
6	Att1456-7	17.5 ± 4.60	0.37 ± 0.134	10 ± 1.1	75.00 ± 15.31	28.79 ± 9.809	0.693 ± 0.0112	1.024 ± 0.6347	0.701 ± 0.3458	0.556 ± 0.0263	0.459 ± 0.2459	0.447 ± 0.1840	0.00 ± 0.000

Table 38: Growth of *Heliocarpus americanus* in the Nursery experiment No. 4, after inoculation with individual AMF species. Means ± SE are shown. Data marked in gray represent the +AMF (living inoculum), data without gray background the –AMF (heat-killed inoculum) treatment. The *Cl. etunicatum*-like multispore culture is marked with ***** because it most likely contains more than one AMF species. AMF cultures are represented by their Att-number, **Att1449-5:** *Diversispora* sp., **Att1449-10:** *Cl. etunicatum*-like, **Att1449-12:** *Ambispora* sp., **Att1450-1:** *Acaulospora* sp. nov., **Att1451-8:** *Rhizophagus* sp., **Att1451-18:** *Cl. etunicatum*-like*, **Att1456-7:** *Ar. trappei*-like.

Seedlings of *Tabebuia chrysantha* reacted quite distinct to inoculation with different AMF. AMF-plant combinations with *Diversispora* sp. Att1449-5, *Cl. etunicatum*-like Att1449-10 and *Rhizophagus* sp. Att1451-8 improved growth most effective (Fig. 34, Table 39). These AMF increased height, RCD, leaf number, leaf area and especially the fresh weight and biomass of the leaves, shoot and roots. The mortality rate of the +AMF seedlings after 3 months was zero while some non-inoculated plants died. This trend continued after 6 months with low mortality for almost all plants in the +AMF treatment. Only plants inoculated by *Acaulospora* sp. nov. Att1450-1 and *Cl. etunicatum*-like* Att1451-18 (ms) showed equal mortality rates for +AMF and -AMF. The -AMF treatment showed nearly no mycorrhization. Plants inoculated by *Rhizophagus* sp. Att1451-8 showed the highest mycorrhization (71%).

The *T. chrysantha* seedlings grew better when inoculated with AMF than the non-inoculated ones. Some specific AMF-plant combinations performed best. In these cases the seedlings showed an increase of all growth parameters measured, especially in biomass.

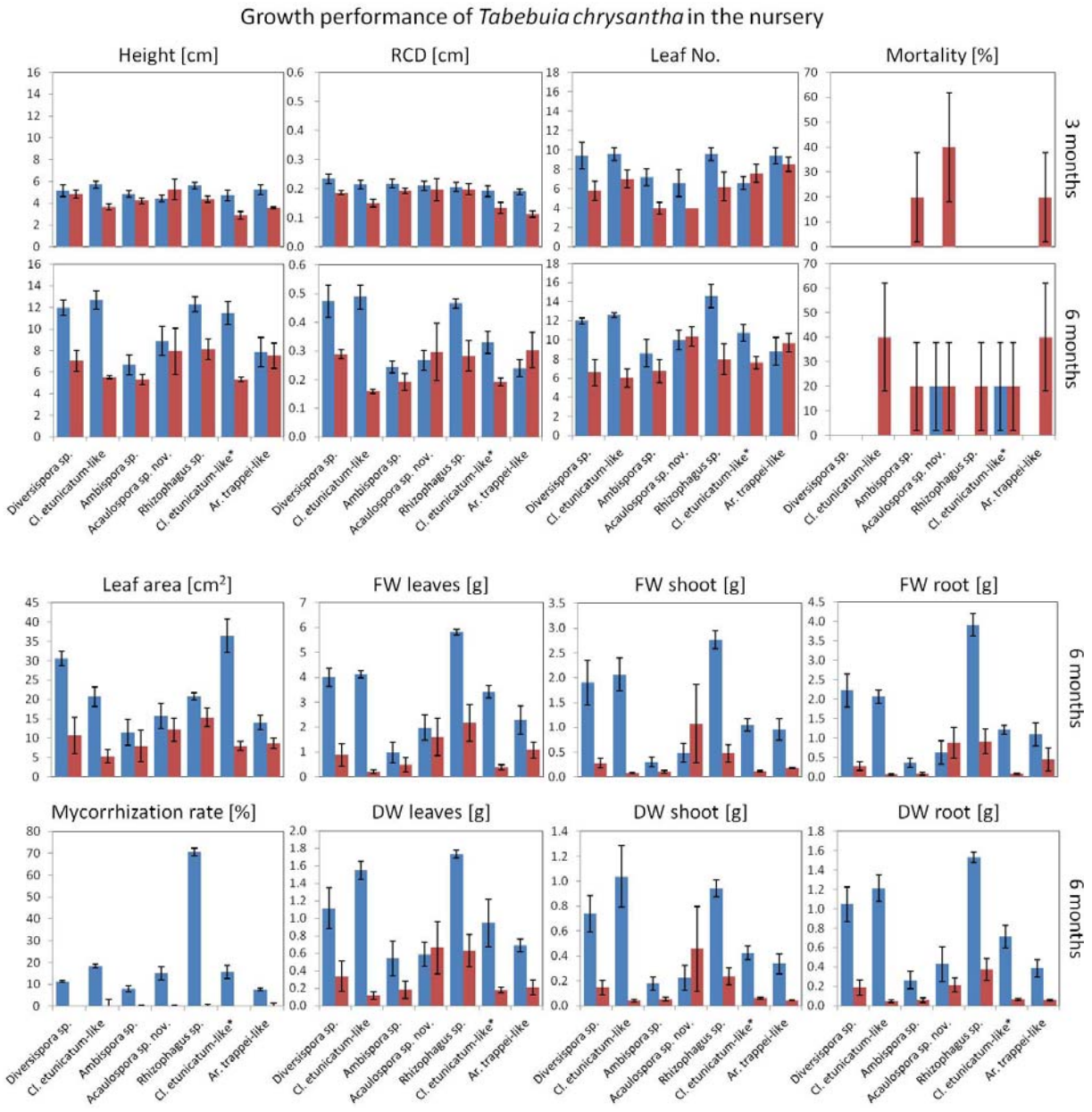


Fig. 34: Growth data of *Tabebuia chrysantha* in the Nursery experiment No. 4, after inoculation with individual AMF species. Blue bars represent +AMF (living inoculum), red bars –AMF (heat-killed inoculum) treatment. Both treatments received a low (¼) fertilization dose. Means ± SE are shown. The *Cl. etunicatum*-like multispore culture is marked with * because it most likely contains more than one AMF species.

Time [mo]	AMF	Height [cm]	RCD [cm]	Leaves	Mortality [%]	Leaf area [cm²]	Fresh weight [g]			Biomass [g]			Mycorrhization rate [%]
							Leaves	Shoot	Root	Leaves	Shoot	Root	
3	Att1449-5	5.2 ± 0.53	0.23 ± 0.016	9 ± 1.3	0.00 ± 0.00								
3	Att1449-10	5.7 ± 0.33	0.21 ± 0.015	10 ± 0.7	0.00 ± 0.00								
3	Att1449-12	4.8 ± 0.30	0.22 ± 0.015	7 ± 0.9	0.00 ± 0.00								
3	Att1450-1	4.4 ± 0.30	0.21 ± 0.017	7 ± 1.4	0.00 ± 0.00								
3	Att1451-8	5.6 ± 0.28	0.21 ± 0.016	10 ± 0.7	0.00 ± 0.00								
3	Att1451-18*	4.7 ± 0.51	0.19 ± 0.019	7 ± 0.7	0.00 ± 0.00								
3	Att1456-7	5.2 ± 0.44	0.19 ± 0.010	9 ± 0.8	0.00 ± 0.00								
3	Att1449-5	4.8 ± 0.37	0.19 ± 0.007	6 ± 1.0	0.00 ± 0.00								
3	Att1449-10	3.6 ± 0.29	0.15 ± 0.013	7 ± 0.9	0.00 ± 0.00								
3	Att1449-12	4.2 ± 0.27	0.19 ± 0.009	4 ± 0.6	20.00 ± 17.89								
3	Att1450-1	5.3 ± 0.96	0.20 ± 0.038	4 ± 0.0	40.00 ± 21.91								
3	Att1451-8	4.4 ± 0.30	0.20 ± 0.019	6 ± 1.5	0.00 ± 0.00								
3	Att1451-18*	2.9 ± 0.37	0.13 ± 0.018	8 ± 0.9	0.00 ± 0.00								
3	Att1456-7	3.6 ± 0.06	0.11 ± 0.012	9 ± 0.8	20.00 ± 17.89								
6	Att1449-5	12.0 ± 0.74	0.47 ± 0.056	12 ± 0.3	0.00 ± 0.00	30.56 ± 1.784	3.989 ± 0.3648	1.901 ± 0.4465	2.223 ± 0.4296	1.113 ± 0.2340	0.739 ± 0.1463	1.047 ± 0.1781	11.33 ± 0.327
6	Att1449-10	12.7 ± 0.86	0.49 ± 0.043	13 ± 0.2	0.00 ± 0.00	20.64 ± 2.490	4.120 ± 0.1506	2.066 ± 0.3326	2.062 ± 0.1687	1.550 ± 0.1065	1.038 ± 0.2463	1.213 ± 0.1367	18.40 ± 0.896
6	Att1449-12	6.7 ± 0.93	0.24 ± 0.021	9 ± 1.4	0.00 ± 0.00	11.45 ± 3.369	0.987 ± 0.4185	0.302 ± 0.0973	0.362 ± 0.1227	0.542 ± 0.2003	0.177 ± 0.0543	0.262 ± 0.0897	7.87 ± 1.501
6	Att1450-1	8.9 ± 1.35	0.27 ± 0.036	10 ± 1.0	20.00 ± 17.89	15.71 ± 3.256	1.981 ± 0.5098	0.487 ± 0.1923	0.632 ± 0.2981	0.590 ± 0.1386	0.224 ± 0.0996	0.429 ± 0.1813	15.00 ± 2.963
6	Att1451-8	12.3 ± 0.70	0.47 ± 0.016	15 ± 1.2	0.00 ± 0.00	20.74 ± 0.919	5.812 ± 0.1183	2.772 ± 0.1850	3.908 ± 0.2775	1.735 ± 0.0467	0.943 ± 0.0682	1.532 ± 0.0508	70.53 ± 1.637
6	Att1451-18*	11.5 ± 1.05	0.33 ± 0.038	11 ± 0.9	20.00 ± 17.89	36.48 ± 4.346	3.415 ± 0.2364	1.049 ± 0.1296	1.210 ± 0.1207	0.946 ± 0.2695	0.423 ± 0.0551	0.715 ± 0.1170	15.67 ± 2.856
6	Att1456-7	7.8 ± 1.38	0.24 ± 0.030	9 ± 1.5	0.00 ± 0.00	14.04 ± 1.783	2.278 ± 0.5737	0.958 ± 0.2186	1.099 ± 0.3038	0.689 ± 0.0772	0.336 ± 0.0820	0.386 ± 0.0889	7.73 ± 0.553
6	Att1449-5	7.0 ± 0.99	0.29 ± 0.016	7 ± 1.4	0.00 ± 0.00	10.67 ± 4.613	0.889 ± 0.4418	0.276 ± 0.1001	0.277 ± 0.1168	0.338 ± 0.1771	0.145 ± 0.0581	0.184 ± 0.0801	0.00 ± 0.000
6	Att1449-10	5.5 ± 0.14	0.16 ± 0.008	6 ± 0.9	40.00 ± 21.91	5.37 ± 1.686	0.198 ± 0.0749	0.079 ± 0.0150	0.064 ± 0.0203	0.118 ± 0.0418	0.042 ± 0.0119	0.046 ± 0.0142	0.00 ± 3.215
6	Att1449-12	5.3 ± 0.49	0.19 ± 0.029	7 ± 1.2	20.00 ± 17.89	7.94 ± 3.994	0.502 ± 0.2862	0.097 ± 0.0261	0.081 ± 0.0302	0.184 ± 0.1004	0.052 ± 0.0162	0.058 ± 0.0214	0.00 ± 0.671
6	Att1450-1	8.0 ± 2.13	0.30 ± 0.100	10 ± 1.0	40.00 ± 21.91	12.19 ± 3.026	1.611 ± 0.7542	1.074 ± 0.7924	0.880 ± 0.4065	0.665 ± 0.3019	0.456 ± 0.3402	0.213 ± 0.0752	0.00 ± 0.650
6	Att1451-8	8.2 ± 0.96	0.28 ± 0.053	8 ± 1.6	20.00 ± 17.89	15.42 ± 2.467	2.170 ± 0.7435	0.478 ± 0.1745	0.912 ± 0.3189	0.630 ± 0.1843	0.234 ± 0.0689	0.373 ± 0.1123	0.00 ± 0.732
6	Att1451-18*	5.3 ± 0.20	0.19 ± 0.014	8 ± 0.7	0.00 ± 0.00	7.98 ± 1.189	0.393 ± 0.1064	0.118 ± 0.0155	0.077 ± 0.0222	0.177 ± 0.0309	0.061 ± 0.0091	0.066 ± 0.0109	0.00 ± 0.000
6	Att1456-7	7.5 ± 1.18	0.30 ± 0.063	10 ± 1.0	40.00 ± 21.91	8.66 ± 1.403	1.078 ± 0.3282	0.177 ± 0.0155	0.449 ± 0.2950	0.208 ± 0.0850	0.044 ± 0.0035	0.058 ± 0.0106	0.00 ± 1.343

Table 39: Growth of *Tabebuia chrysantha* in the Nursery experiment No. 4, after inoculation with individual AMF species. Means ± SE are shown. Data marked in gray represent the +AMF (living inoculum), data without gray background the -AMF (heat-killed inoculum) treatment. The *Cl. etunicatum*-like multispore culture is marked with ***** because it most likely contains more than one AMF species. AMF cultures are represented by their Att-number, **Att1449-5:** *Diversispora* sp., **Att1449-10:** *Cl. etunicatum*-like, **Att1449-12:** *Ambispora* sp., **Att1450-1:** *Acaulospora* sp. nov., **Att1451-8:** *Rhizophagus* sp., **Att1451-18:** *Cl. etunicatum*-like*, **Att1456-7:** *Ar. trappei*-like.

3.3.3 Effect of inoculation on *Cedrela montana* in Nursery experiment No. 4A

The experiment was performed to investigate the effects on plant performance when applying AMF with and without fertilizer. *Cedrela montana* showed high mortality rates (Fig. 35-1 and Fig. 35-2, Table 40). However, the seedlings in both +AMF treatments performed better in height, RCD, leaf number and leaf area after 6 months in the nursery than the non-inoculated plants. The *C. montana* seedlings showed increased fresh weight and biomass, when inoculated with *Cl. etunicatum*-like Att1449-10, *Ambispora* sp. Att1449-12 (when not fertilized), *Acaulospora* sp. nov. Att1450-1 and *Ar. trappei*-like Att1456-7. These four AMF-plant associations also reduced mortality rates compared to the non-inoculated seedlings. No *C. montana* seedling died in the +AMF (T2) when inoculated by *Acaulospora* sp. nov. Att1450-1 and in the +AMF + LF treatment (T4) when inoculated by *Ar. trappei*-like Att1456-7. Mycorrhization rates were in general higher in the +AMF treatments, except for *Ambispora* sp. Att1449-12, where the -AMF + LF treatment (T3) showed higher mycorrhizal colonization than the +AMF treatment (T4). The different seedling performance was also photographically documented after 5 months in the nursery (see Appendix Fig. A4-1 and A4-2).

C. montana seedlings showed similar response as in the No. 4 experiment. Seedlings performed better when inoculated with AMF. Only fertilized seedlings showed an increase in biomass (Fig. 35-1 and Fig. 35-2, Table 40).

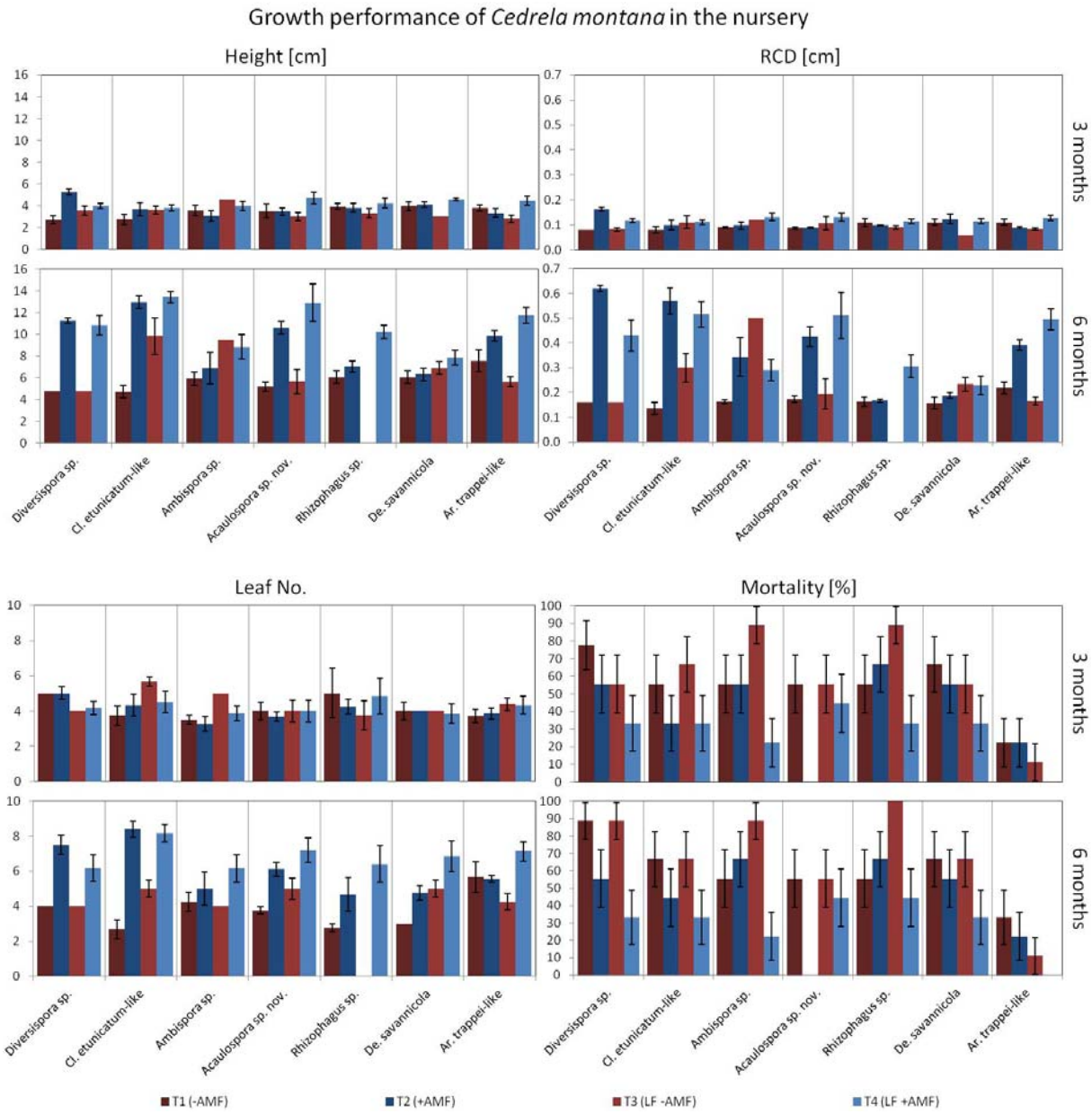


Fig. 35-1: Height, RCD, leaf numbers and mortality of *Cedrela montana* in the Nursery experiment No. 4A, after inoculation with individual AMF species. Blue bars represent the +AMF treatments, red bars the –AMF treatments (heat-killed inoculum), **LF:** indicate low fertilization. Means ± SE are shown.

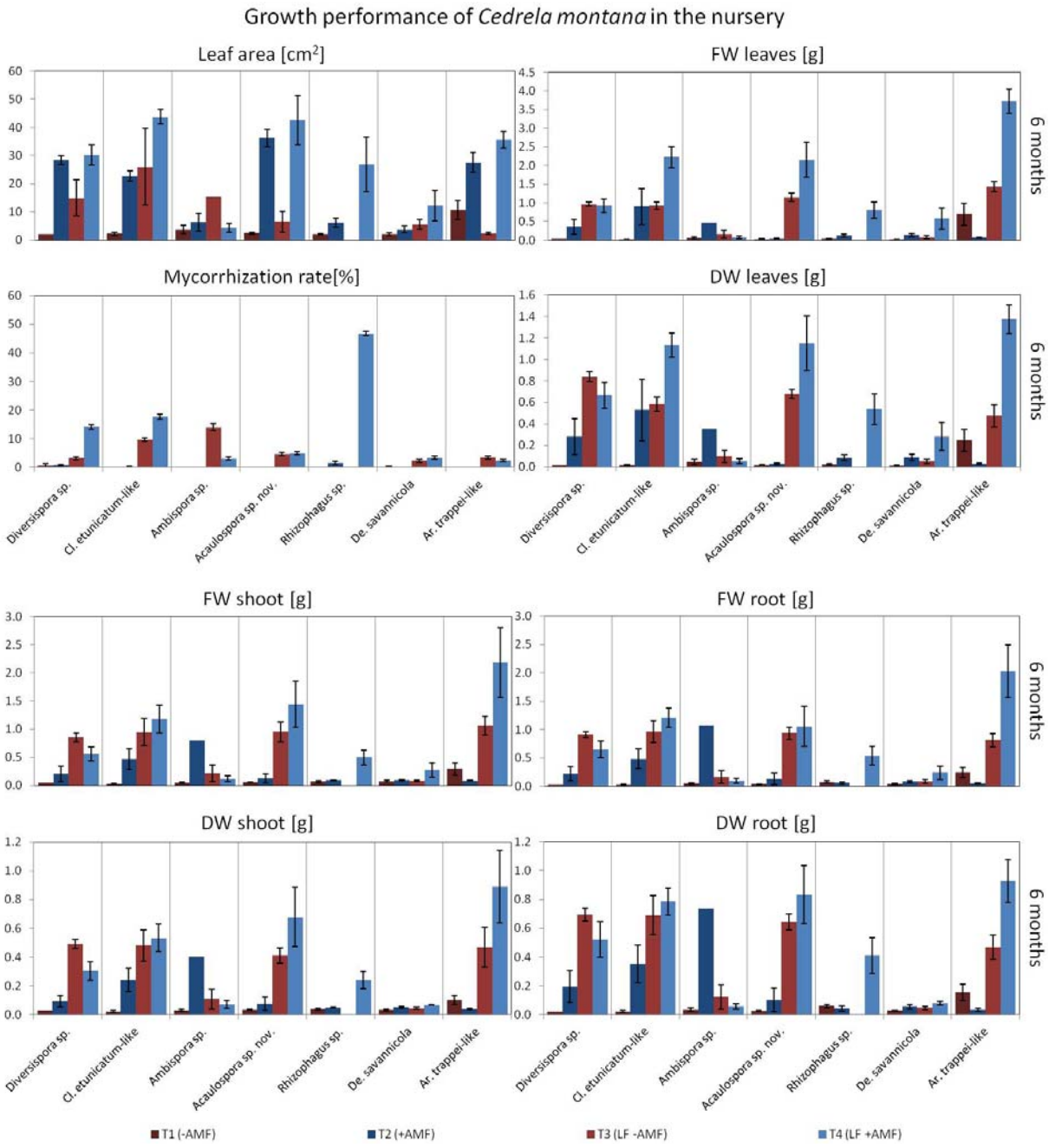


Fig. 35-2: Leaf area, mycorrhization rates, fresh weight and biomass (dry weight) of *Cedrela montana* in the Nursery experiment No. 4A, after inoculation with individual AMF species. Blue bars represent the +AMF treatments, red bars the –AMF treatments (heat-killed inoculum), **LF:** indicate low fertilization. Means ± SE are shown.

Time [mo]	Treat-ment	AMF	Height [cm]	RCD [cm]	Leaves	Mortality [%]	Leaf area [cm^2]	Fresh weight [g]			Biomass [g]			Mycorrhization rate [%]
								Leaves	Shoot	Root	Leaves	Shoot	Root	
3	T1	Att1449-5	2.7 ± 0.35	0.08 ± 0.000	5 ± 0.0	77.78 ± 13.86								
3	T1	Att1449-10	2.8 ± 0.45	0.08 ± 0.012	4 ± 0.5	55.56 ± 16.56								
3	T1	Att1449-12	3.6 ± 0.48	0.09 ± 0.004	4 ± 0.3	55.56 ± 16.56								
3	T1	Att1450-1	3.5 ± 0.63	0.09 ± 0.004	4 ± 0.5	55.56 ± 16.56								
3	T1	Att1451-8	3.9 ± 0.28	0.11 ± 0.016	5 ± 1.4	55.56 ± 16.56								
3	T1	Att1455-2	4.0 ± 0.39	0.11 ± 0.012	4 ± 0.5	66.67 ± 15.71								
3	T1	Att1456-7	3.8 ± 0.30	0.11 ± 0.011	4 ± 0.4	22.22 ± 13.86								
3	T2	Att1449-5	5.3 ± 0.29	0.16 ± 0.007	5 ± 0.4	55.56 ± 16.56								
3	T2	Att1449-10	3.7 ± 0.63	0.10 ± 0.021	4 ± 0.6	33.33 ± 15.71								
3	T2	Att1449-12	3.1 ± 0.48	0.10 ± 0.013	3 ± 0.4	55.56 ± 16.56								
3	T2	Att1450-1	3.5 ± 0.31	0.09 ± 0.003	4 ± 0.3	0.00 ± 0.00								
3	T2	Att1451-8	3.8 ± 0.40	0.10 ± 0.002	4 ± 0.4	66.67 ± 15.71								
3	T2	Att1455-2	4.1 ± 0.22	0.12 ± 0.019	4 ± 0.0	55.56 ± 16.56								
3	T2	Att1456-7	3.3 ± 0.37	0.09 ± 0.004	4 ± 0.3	22.22 ± 13.86								
3	T3	Att1449-5	3.6 ± 0.41	0.08 ± 0.006	4 ± 0.0	55.56 ± 16.56								
3	T3	Att1449-10	3.6 ± 0.37	0.11 ± 0.024	6 ± 0.3	66.67 ± 15.71								
3	T3	Att1449-12	4.6 ± 0.00	0.12 ± 0.000	5 ± 0.0	88.89 ± 10.48								
3	T3	Att1450-1	3.0 ± 0.35	0.11 ± 0.027	4 ± 0.6	55.56 ± 16.56								
3	T3	Att1451-8	3.3 ± 0.41	0.09 ± 0.006	4 ± 0.8	88.89 ± 10.48								
3	T3	Att1455-2	3.0 ± 0.00	0.06 ± 0.000	4 ± 0.0	55.56 ± 16.56								
3	T3	Att1456-7	2.8 ± 0.32	0.08 ± 0.004	4 ± 0.4	11.11 ± 10.48								
3	T4	Att1449-5	4.0 ± 0.26	0.12 ± 0.008	4 ± 0.4	33.33 ± 15.71								
3	T4	Att1449-10	3.8 ± 0.30	0.11 ± 0.009	5 ± 0.6	33.33 ± 15.71								
3	T4	Att1449-12	4.0 ± 0.41	0.13 ± 0.015	4 ± 0.4	22.22 ± 13.86								
3	T4	Att1450-1	4.7 ± 0.53	0.13 ± 0.016	4 ± 0.6	44.44 ± 16.56								
3	T4	Att1451-8	4.3 ± 0.44	0.11 ± 0.009	5 ± 1.0	33.33 ± 15.71								
3	T4	Att1455-2	4.6 ± 0.10	0.12 ± 0.011	4 ± 0.5	33.33 ± 15.71								
3	T4	Att1456-7	4.5 ± 0.42	0.13 ± 0.011	4 ± 0.5	0.00 ± 0.00								
6	T1	Att1449-5	4.8 ± 0.00	0.16 ± 0.000	4 ± 0.0	88.89 ± 10.48	2.15 ± 0.000	0.038 ± 0.0000	0.043 ± 0.0000	0.026 ± 0.0000	0.019 ± 0.0000	0.030 ± 0.0000	0.021 ± 0.0000	0.67 ± 0.644
6	T1	Att1449-10	4.7 ± 0.59	0.14 ± 0.024	3 ± 0.5	66.67 ± 15.71	2.25 ± 0.524	0.022 ± 0.0084	0.031 ± 0.0083	0.026 ± 0.0082	0.016 ± 0.0071	0.022 ± 0.0075	0.022 ± 0.0071	0.00 ± 0.000
6	T1	Att1449-12	6.0 ± 0.61	0.16 ± 0.007	4 ± 0.5	55.56 ± 16.56	3.71 ± 1.419	0.065 ± 0.0292	0.048 ± 0.0070	0.048 ± 0.0171	0.047 ± 0.0228	0.032 ± 0.0048	0.036 ± 0.0125	0.00 ± 0.000
6	T1	Att1450-1	5.2 ± 0.41	0.17 ± 0.014	4 ± 0.2	55.56 ± 16.56	2.45 ± 0.257	0.029 ± 0.0069	0.056 ± 0.0063	0.037 ± 0.0054	0.021 ± 0.0053	0.033 ± 0.0034	0.027 ± 0.0031	0.00 ± 0.000
6	T1	Att1451-8	6.1 ± 0.58	0.16 ± 0.019	3 ± 0.2	55.56 ± 16.56	2.08 ± 0.310	0.034 ± 0.0088	0.065 ± 0.0122	0.075 ± 0.0150	0.023 ± 0.0063	0.037 ± 0.0066	0.059 ± 0.0129	0.00 ± 0.000
6	T1	Att1455-2	6.1 ± 0.59	0.16 ± 0.024	3 ± 0.0	66.67 ± 15.71	2.03 ± 0.406	0.021 ± 0.0075	0.076 ± 0.0203	0.042 ± 0.0085	0.014 ± 0.0050	0.033 ± 0.0057	0.028 ± 0.0036	0.22 ± 0.220
6	T1	Att1456-7	7.6 ± 0.98	0.22 ± 0.024	6 ± 0.9	33.33 ± 15.71	10.61 ± 3.386	0.696 ± 0.3033	0.294 ± 0.1053	0.242 ± 0.0897	0.247 ± 0.1010	0.101 ± 0.0321	0.154 ± 0.0572	0.00 ± 0.000
6	T2	Att1449-5	11.2 ± 0.24	0.62 ± 0.012	8 ± 0.6	55.56 ± 16.56	28.40 ± 1.653	0.357 ± 0.1953	0.212 ± 0.1356	0.222 ± 0.1242	0.281 ± 0.1673	0.092 ± 0.0402	0.194 ± 0.1088	0.73 ± 0.350
6	T2	Att1449-10	13.0 ± 0.58	0.57 ± 0.053	8 ± 0.5	44.44 ± 16.56	22.70 ± 1.813	0.901 ± 0.4827	0.472 ± 0.1829	0.482 ± 0.1757	0.529 ± 0.2841	0.241 ± 0.0838	0.351 ± 0.1306	0.22 ± 0.220
6	T2	Att1449-12	6.9 ± 1.47	0.34 ± 0.077	5 ± 0.9	66.67 ± 15.71	6.28 ± 3.119	0.472 ± 0.0000	0.793 ± 0.0000	1.077 ± 0.0000	0.353 ± 0.0000	0.402 ± 0.0000	0.740 ± 0.0000	0.00 ± 0.000
6	T2	Att1450-1	10.6 ± 0.59	0.43 ± 0.040	6 ± 0.4	0.00 ± 0.00	36.19 ± 3.021	0.048 ± 0.0151	0.124 ± 0.0808	0.132 ± 0.1030	0.028 ± 0.0087	0.076 ± 0.0463	0.103 ± 0.0806	0.00 ± 0.000
6	T2	Att1451-8	7.1 ± 0.52	0.17 ± 0.007	5 ± 1.0	66.67 ± 15.71	6.16 ± 1.493	0.127 ± 0.0361	0.089 ± 0.0075	0.057 ± 0.0201	0.086 ± 0.0255	0.049 ± 0.0009	0.045 ± 0.0168	1.56 ± 0.540

Time [mo]	Treat-ment	AMF	Height [cm]	RCD [cm]	Leaves	Mortality [%]	Leaf area [cm^2]	FW leaves	FW shoot	FW root	DW leaves	DW shoot	DW root	Mycorrhization rate [%]
6	T2	Att1455-2	6.3 ± 0.56	0.19 ± 0.013	5 ± 0.4	55.56 ± 16.56	3.75 ± 1.213	0.137 ± 0.0459	0.096 ± 0.0144	0.079 ± 0.0188	0.089 ± 0.0288	0.050 ± 0.0084	0.056 ± 0.0150	0.00 ± 0.000
6	T2	Att1456-7	9.9 ± 0.49	0.39 ± 0.021	6 ± 0.2	22.22 ± 13.86	27.56 ± 3.541	0.068 ± 0.0150	0.087 ± 0.0095	0.052 ± 0.0095	0.030 ± 0.0063	0.040 ± 0.0041	0.035 ± 0.0073	0.00 ± 0.000
6	T3	Att1449-5	4.8 ± 0.00	0.16 ± 0.000	4 ± 0.0	88.89 ± 10.48	15.00 ± 6.334	0.964 ± 0.0579	0.854 ± 0.0779	0.911 ± 0.0524	0.837 ± 0.0484	0.492 ± 0.0322	0.695 ± 0.0443	3.17 ± 0.601
6	T3	Att1449-10	9.8 ± 1.66	0.30 ± 0.057	5 ± 0.5	66.67 ± 15.71	26.02 ± 13.673	0.923 ± 0.0972	0.949 ± 0.2407	0.961 ± 0.1934	0.584 ± 0.0647	0.481 ± 0.1078	0.692 ± 0.1361	9.73 ± 0.652
6	T3	Att1449-12	9.5 ± 0.00	0.50 ± 0.000	4 ± 0.0	88.89 ± 10.48	15.30 ± 0.000	0.161 ± 0.0961	0.215 ± 0.1473	0.165 ± 0.1107	0.101 ± 0.0560	0.108 ± 0.0685	0.123 ± 0.0834	14.00 ± 1.273
6	T3	Att1450-1	5.7 ± 1.12	0.20 ± 0.061	5 ± 0.6	55.56 ± 16.56	6.49 ± 3.758	1.153 ± 0.1162	0.954 ± 0.1754	0.934 ± 0.1043	0.679 ± 0.0408	0.411 ± 0.0538	0.644 ± 0.0558	4.67 ± 0.606
6	T3	Att1451-8	n.d.	n.d.	n.d.	100.00 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	T3	Att1455-2	6.9 ± 0.57	0.23 ± 0.027	5 ± 0.5	66.67 ± 15.71	5.52 ± 1.741	0.083 ± 0.0353	0.081 ± 0.0105	0.087 ± 0.0330	0.052 ± 0.0229	0.045 ± 0.0074	0.047 ± 0.0115	2.33 ± 0.546
6	T3	Att1456-7	5.6 ± 0.46	0.17 ± 0.015	4 ± 0.5	11.11 ± 10.48	2.42 ± 0.235	1.436 ± 0.1310	1.065 ± 0.1671	0.811 ± 0.1202	0.476 ± 0.1012	0.470 ± 0.1370	0.468 ± 0.0825	3.47 ± 0.550
6	T4	Att1449-5	10.8 ± 0.91	0.43 ± 0.062	6 ± 0.8	33.33 ± 15.71	30.27 ± 3.574	0.918 ± 0.1867	0.562 ± 0.1229	0.647 ± 0.1513	0.665 ± 0.1200	0.303 ± 0.0623	0.520 ± 0.1240	14.13 ± 0.845
6	T4	Att1449-10	13.4 ± 0.52	0.52 ± 0.051	8 ± 0.5	33.33 ± 15.71	43.77 ± 2.637	2.228 ± 0.2834	1.185 ± 0.2481	1.209 ± 0.1653	1.133 ± 0.1132	0.532 ± 0.0951	0.785 ± 0.0938	17.73 ± 0.855
6	T4	Att1449-12	8.9 ± 1.11	0.29 ± 0.044	6 ± 0.8	22.22 ± 13.86	4.29 ± 1.511	0.075 ± 0.0300	0.119 ± 0.0504	0.100 ± 0.0443	0.056 ± 0.0229	0.070 ± 0.0275	0.057 ± 0.0180	3.07 ± 0.565
6	T4	Att1450-1	12.9 ± 1.73	0.51 ± 0.093	7 ± 0.7	44.44 ± 16.56	42.54 ± 8.780	2.155 ± 0.4634	1.443 ± 0.4123	1.055 ± 0.3511	1.151 ± 0.2515	0.678 ± 0.2057	0.833 ± 0.2006	4.93 ± 0.577
6	T4	Att1451-8	10.2 ± 0.58	0.31 ± 0.046	6 ± 1.0	44.44 ± 16.56	26.85 ± 9.639	0.802 ± 0.2239	0.500 ± 0.1322	0.538 ± 0.1626	0.538 ± 0.1413	0.240 ± 0.0600	0.412 ± 0.1227	46.80 ± 0.825
6	T4	Att1455-2	7.9 ± 0.66	0.23 ± 0.037	7 ± 0.9	33.33 ± 15.71	12.13 ± 5.376	0.580 ± 0.2804	0.275 ± 0.1265	0.239 ± 0.1193	0.283 ± 0.1315	0.067 ± 0.0038	0.080 ± 0.0140	3.33 ± 0.576
6	T4	Att1456-7	11.7 ± 0.72	0.50 ± 0.043	7 ± 0.6	0.00 ± 0.00	35.57 ± 2.932	3.729 ± 0.3185	2.186 ± 0.6192	2.030 ± 0.4569	1.372 ± 0.1313	0.889 ± 0.2521	0.928 ± 0.1485	2.40 ± 0.493

Table 40: Growth parameters of *Cedrela montana* in the nursery experiment No4. A, after inoculation with individual AMF species. Means ± SE are shown. Data marked in gray represent the +AMF (living inoculum), data without gray background the –AMF (heat-killed inoculum) treatments. The treatments as follows are used in the table **T1:** -AMF, **T2:** +AMF, **T3:** +AMF + LF, **T4:** -AMF + LF. **n.d.:** no data available. **LF:** low fertilization. AMF cultures are represented by their Att-number, **Att1449-5:** *Diversispora* sp., **Att1449-10:** *Cl. etunicatum*-like, **Att1449-12:** *Ambispora* sp., **Att1450-1:** *Acaulospora* sp. nov., **Att1451-8:** *Rhizophagus* sp., **Att1455-2:** *De. savannicola*, **Att1456-7:** *Ar. trappei*-like. **FW:** fresh weight, **DW:** biomass.

4 Discussion

Arbuscular mycorrhiza (AM) is a widespread symbiosis and members of the AM-forming *Glomeromycota* are found in all kinds of habitats (Börstler *et al.* 2010). Some of these AM fungi (AMF) are generalists and can form symbiosis with a wide range of host plants especially in nutrient poor tropical soils (Janos 1987). As more and more tropical forests are destroyed and Ecuador has the highest deforestation rate in South America (FAO 2006), reforestation is an important option to preserve biodiversity. The aim of this study was to identify native AMF and their potential role in reforestation of abandoned pastures in Ecuador Andes. The studies were carried out in collaboration with the forestry research group of TU Munich project C2.1 Günter, Mosandl, Stimm, Weber in the frame of the DFG RU816 "Biodiversity and Sustainable Management of a Megadiverse Mountain Ecosystem in South Ecuador" (www.tropicalmountainforest.org).

Former reforestation on abandoned pastures in the research area Reserva Biológica San Francisco (RBSF) in South Ecuador was investigated by Aguirre Mendoza (2007). These reforestation attempts were carried out by use of seedlings of native Ecuadorian trees, e.g. *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha*, and foreign tree species, such as *Eucalyptus* spp. and *Pinus* spp. A nursery experiment (No. 1) was performed by the research group of Kottke and Oberwinkler (project A6 in DFG RU402; Functionality in a Tropical Mountain Rainforest: Diversity, Dynamic Processes and Utilization Potentials under Ecosystem Perspectives, www.bergregenwald.de) to investigate influence of a natural inoculum (forest soil and tree roots from pristine forest) on maintenance of tree seedlings in the nursery. It was shown that inoculation with soil from natural stands improved tree seedling performance in the nursery (Urgiles *et al.* 2009). Additional reforestation attempts on the pastures suffered from high mortality rates for some species (Aguirre Mendoza 2007). It was hypothesized that survival of native tree seedlings could potentially be improved by appropriate mycorrhization in the nursery (Aguirre Mendoza 2007). Thus, we aimed to find out which Ecuadorian AMF were best suited to improve seedling performance for reforestation.

4.1 Ecuadorian AMF

We expected a large diversity of AMF in the research area (RBSF) as Ecuador is a hotspot of plant biodiversity (Jorgensen & León-Yánez 1999) and plant diversity is discussed to correspond with AMF biodiversity (van der Heijden *et al.* 1998b). *Plantago lanceolata* was used to isolate and cultivate AMF from Ecuador, because of its known high mycorrhiza

dependency (Šmilauer 2001, van der Heijden *et al.* 2002). Although our pot-culturing was successful it is unlikely that we trapped all AMF from the soil or root samples. The incomplete trapping of AMF by culturing became clear after applying 454 techniques, which showed additionally non-introduced AMF colonizing roots of the nursery seedlings. We isolated five AMF species from the trap culturing approach using mycorrhizas from the previous Nursery experiment No. 1 (Urgiles *et al.* 2009) and two AMF species from rhizosphere soil of *Cedrela montana* on a reforestation plot in the RBSF. Characterization of the seven cultured AMF was achieved by combining morphological and molecular methods according to e.g. Walker *et al.* (2007), Gamper *et al*. (2009), Błaszkowski *et al.* (2012) and Goto *et al.* (2010), as solely morphological characterization of AMF is often misleading. The latter was shown for *Ambispora brasiliensis* (Goto *et al.* 2008) characterized on spore morphology only, revised to *Acaulospora brasiliensis* based on molecular methods (Krüger *et al*. 2011). Thus, the combination of morphological and molecular characterization can prevent incorrect assignment of AMF. Similar problems would have occurred for solely morphological characterization of the herein described AMF from Ecuador, as the described AMF cultures of *Claroideoglomus etunicatum*-like (Att1449-10, Att1451-6 and Att1456-11) morphologically differ in the Melzer's reaction and their spores vary in size and color. However on the molecular level the cultures belong to the same species. *Scutellospora savannicola* (now *Dentiscutata savannicola*) was previously described from Cuba by Ferrer & Herrera (1981). All other six pot-cultured AMF species were found to be so far undescribed species.

Former studies in the RBSF area by Beck *et al.* (2007) and Haug *et al.* (2010) showed that there is a large richness of native tropical trees associated with multiple AMF. Phylogenetic analyses showed a high diversity of isolated AMF in the present thesis as the cultured Ecuadorian AMF covered nearly all lineages of the *Glomeromycota*, except *Paraglomus*. Haug *et al.* (2010) found mainly *Glomus* and only few sequences of *Acaulospora*, *Archaeospora* and *Paraglomus.* The restriction to mainly *Glomeraceae* might be due to the used primer set which amplifies only a part of the lineages of *Glomeromycota* (*Claroideoglomeraceae* (former *Glomus* group B), *Acaulosporaceae, Archaeosporales* and *Paraglomerales*). We enlarged the molecular characterization to all glomeromycotan lineages also taking *Gigasporaceae*, *Entrophosporaceae*, *Pacisporaceae* and *Diversisporaceae* into account, using an AMF specific primer set for all AMF (Krüger *et al.* 2009). Molecular characterization is also hampered by using the nuclear SSU rRNA gene as a marker, which was recently shown to resolve AMF only above species level (Krüger *et al.* 2012). Molecular characterization for AMF cultures is now best proceeded by a ca. 1.5 kb rDNA fragment covering the SSU_{part}-ITS-LSU_{part} as described in Krüger *et al.* (2009) or a ca. 3 kb rDNA fragment covering the SSU_{full}-ITS-LSU_{part} (Stockinger *et al.* 2009, 2010, Krüger *et al.* 2012). These methods were herein used for characterization of the Ecuadorian AMF

cultures and provided species-level resolution. A comparison with SSU rDNA sequences published in Kottke *et al.* 2008 and Haug *et al.* 2010 from AMF out of the RBSF revealed no closely related glomeromycotan sequences described in this PhD thesis (data not shown). Nevertheless, the SSU rDNA only resolves AMF from genus to sub-genus level and the used set of primers amplifying different regions within the SSU rDNA only overlapping in approximately 400 bp, thus comparison may be error prone.

On the basis of morphological and molecular characterization preliminary names for the isolated and cultured AMF were given. The culturing of AMF in pot cultures for inoculum production is a convenient method as it is easy and cheap, but it also carries some disadvantages as contaminations cannot be excluded (Ijdo *et al.* 2010), even when produced in a closed system with sunbags protecting against e.g. nematodes, springtails, root pathogens etc. Millner & Kitt (1992) argued that pot culturing hampers production of pure inoculum without residua of substrate. Our inoculum consisted of a mixture of seven different AMF species and contained the culturing substrate (autoclaved Oil dry-sand mixture) as carrier. Jansa *et al.* (2008) showed that inoculation with a mixture of several AMF can improve P uptake more than the host plant could achieve with only one single AMF. We detected an improved P-content of seedling roots and leaves when compared to the control, especially for *T. chrysantha*. Van der Heijden *et al.* (1998a) found that the mycorrhiza dependency of plants is quite variable to AMF species and/or communities. Nevertheless, we expected positive growth response of the tree seedlings to the isolated AMF, as they originated from roots of *C*. *montana* or *H*. *americanus* and were pre-adapted to these tree species and nursery conditions. Furthermore, by covering nearly all main lineages of the *Glomeromycota* and their functional differences a positive plant response of the tree seedlings was likely, although natural AMF occurrence at field sites may be larger (Allen *et al.* 1995, 2001).

Quality control and tracing of introduced AMF by 454 amplicon sequencing

Identification of AMF by use of the LSU rDNA D2 domain is hampered by length polymorphism and limited fragment length which makes robust phylogeny challenging. Therefore the 3 kb long rDNA fragment (SSU_{full}-ITS-LSU_{part} rDNA) was used as suitable reference alignment and phylogenetic backbone, as the 454-sequenced 400 bp reads of the LSU D2 domain alone comprises insufficient phylogenetic resolution (Stockinger *et al.* 2010).

We could show a broad diversity of AMF found via 454 sequencing. Sequences from all orders of the *Glomeromycota* with a maximum of 16 AMF species per root system were found. The predominance of *Glomeraceae* by Haug *et al.* (2010) in *Cedrela montana*

seedlings on the reforestation plots could not be confirmed, as AMF belonging to *Claroideoglomus*, *Archaeospora* and *Acaulospora* were also present in high percentages. Sequences belonging to the latter genus were also found by Haug *et al.* (2010).

However, we could confirm the findings of Haug *et al.* (2010) in reference to nursery raised seedlings. Beside *Glomeraceae* also sequences belonging to the family of *Acaulosporaceae, Claroideoglomeraceae* and *Archaeosporaceae* were detected. Nevertheless, we could extend these findings by detecting also AMF species of *Gigasporaceae*. This discrepancy is due to the different primers used in the studies. Primers used by Haug *et al.* (2010) discriminate against several groups among the *Glomeromycota,* amplifying the less variable and short SSU rDNA sequences, whereas the primers used in this study amplify all glomeromycotan fungi (Krüger *et al.* 2009). Tracing of AMF on species level was possible by a suitable reference alignment used as phylogenetic backbone including 'barcoded' AMF (SSU_{full}-ITS-LSU_{part} rDNA) as proposed in Stockinger *et al.* (2010).

The 454 analysis of the seedling roots showed that the AMF applied with the inoculum mix could be traced over the whole nursery and field phase. The fast growing and generalist AMF *Rhizophagus* sp. Att1451-8, Att1456-1 was predominant in almost all roots of the tree seedlings independent on fertilization and sampling point (nursery or field phase). Due to the ubiquity of *Rhizophagus* spp. and their hyphal network regeneration ability this was somehow expected. In some cases *Acaulospora* spp. were present in similar or higher percentages than *Rhizophagus* spp. in the seedling roots, also abundant sequence reads of *Claroideoglomus* spp. and *Archaeospora* spp. could be detected. *Acaulospora* sp. nov. Att1450-1 and *Cl. etunicatum*-like Att1449-10, Att1456-1, Att1456-11 were also persistent, especially in the roots of *T. chrysantha*. *Diversispora* sp. Att1449-5 was only found in low amounts in *C. montana* and *H. americanus*, whereas the AMF was frequently found in the roots of *T. chrysantha*. Since the nursery substrate consisted of sand from an agricultural field and there is evidence that the steam sterilization of the standard nursery substrate was incomplete, it is likely that some of the isolated AMF species originated from this site. *Acaulosporaceae, Claroideoglomus etunicatum* (Becker & Gerdemann 1977) and *Diversispora celata* (Gamper *et al.* 2009) were previously found in anthropogenic influenced agricultural fields. Only *Ar. trappei*-like Att1452-6, Att1456-7 was less present or even absent in the roots of the tree seedlings. This may due to the fact that *Archaeospora trappei* was first found in lily fields of Oregon and coastal areas of California (Ames & Lindeman 1976), quite different habitats from the reforestation site. Beside *Diversispora* sp. Att1449-5, all isolated AMF clearly separate phylogenetically from other published glomeromycotan sequences and are so far undescribed. Therefore, conclusions concerning habitats and plant-preferences remain speculative.

AMF-preferences were visible by *C. montana* and *H. americanus*, whereas the seedlings of *T. chrysantha* harbored all introduced AMF except *Ar. trappei*-like. High abundance of AMF originating from the Nursery experiment No. 1 (Urgiles *et al.* 2009) was detected in the tree seedlings roots. For example the 'nursery' *Rhizophagus* spp. and *Acaulospora* spp. were frequent. Interestingly the 'nursery' *Archaeospora* spp. was more abundant than the introduced *Ar. trappei*-like Att1452-6, Att1456-7 isolates especially in the +AMF + LF treatment.

The mycorrhization of seedlings, not inoculation with vital AMF, was high in all treatments in the nursery phase and increased further after out-planting on the reforestation plots. Most probably a natural re-colonization of the seedlings roots with local AMF took place, as discussed in White *et al.* (2008). Urgiles *et al.* (2009) indicated colonization by *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora* species by microscopic observations of the observed seedlings roots. Sequences belonging to *Glomeraceae* and *Claroideoglomeraceae* in the roots of *C. montana* seedlings, sequences of *Glomeraceae* in *H. americanus* and additional sequences of *Archaeosporales* in both tree species were confirmed by molecular research of Haug *et al.* (2010). Our results so far approve these findings, but enlarged them in case of AMF species diversity and plant-AMF effects on the tree seedlings. We could further show that 5 cm of tree seedling roots can inhabit up to 16 AMF species, dependent on tree species and analyzed part of the root system. This is consistent with the studies of Scheublin *et al.* (2004) and Alguacil *et al.* (2011), which showed that the composition of the AMF community varies between plant species.

454 sequencing is an effective choice to identify persisting AMF in seedling roots compared to traditional identification techniques, as large amount of samples can be time and cost efficiently processed (Fierer *et al.* 2008; Jumpponen *et al.* 2010). However, processing the enormous amount of bioinformatical data (herein: 497,374 sequences >300 bp, excluding singletons and doubletons) is time-consuming, as up to now no suitable all-in-one program is available and therefore several programs have to be used for quality check, barcode identification, read clustering, aligning and calculation of phylogenetic trees (see 2.4.3). Furthermore, analyses of the short sequence reads without an appropriate sequence database may lead to misinterpretations (Tedersoo *et al.* 2010). Due to the different amount of reads per sample we cannot exclude that in some cases only predominant AMF sequences were detected and the sample preparation (used primers, PCR bias, etc.) might have an impact on the results. Therefore analysis of the 454 data has to be taken with care, as the real situation in the nursery and field may be different. Nevertheless, high-throughput sequencing makes analysis of AMF communities more precise, as sequence types are achieved which probably never got amplified via the classical way of cloning and sequencing (Sogin *et al.* 2006; Öpik *et al.* 2009) improving our knowledge of AMF.

Efficiency of inoculum in Nursery experiment No. 3

In this study AMF originating from the research area in Ecuador were used for inoculation of tree seedlings to prevent spreading of foreign species into ecosystems (Pringle *et al.* 2009). However, invasive species may have had already repercussions on native local communities and impact on rare and endangered species (Wilcove & Master 2005, Gurevitch & Padilla 2004). Changes in composition of AMF communities dependent on the host species analyzed (Wubet *et al.* 2009) could be confirmed as analysis of the 454 sequencing revealed different AMF colonizing the seedlings roots. Additional inoculation with single AMF inocula, as performed in the Nursery experiment No. 4 and 4A confirmed that individual AMF-plant associations performed better in growth than others, as also observed by van der Heijden *et al.* (1998a). Furthermore Klironomos (2003) stated that plant response to locally adapted AMF was larger than associations with exotic fungal species.

Effects of AMF inoculation in the nursery phase

Our main criterion for an improved reforestation was to increase the survival of the native tree seedlings. We could show that mortality rates of the inoculated tree seedlings partly decreased in the AMF-treatments in a 6 months nursery phase, as described in Guadarrama *et al.* (2004). Turjaman *et al.* (2006) further investigated the effect of AMF on two plant species that produce so-called nontimber forest products (e.g. latex, seeds, flowers, fruits etc.) to promote forest conservation. They found after a 6 months nursery phase, beside increased N and P content, also increased survival rates of the seedlings when inoculated with AMF compared to the control. In our study a significantly reduced mortality of *Cedrela montana* and *Tabebuia chrysantha* was observed. In contrast to the study of Guadarrama *et al.* (2004), the fast growing pioneer *Heliocarpus americanus* did not profit from AMF inoculation alone. The influence of AMF inoculation on the measured growth parameter differed between the three tree species due to different AM-dependency.

Cedrela montana reacted positively to AMF inoculation by increased height, RCD and leaf numbers compared to the control plants, as also illustrated in Urgiles *et al.* (2009). The effects on fertilization of *C. montana* in the herein described Nursery experiment No. 3 were more apparent as the seedlings reacted with increases of height, RCD, number of leaves, leaf and shoot biomass to both fertilization strengths. Therefore we can state that *C. montana* reacts positively in growth to AMF inoculation and/or fertilizer. The latter reaction was unexpectedly strong probably due to low amounts of minerals in the standard nursery substrate.

Heliocarpus americanus reacted positively to fertilization, which was expected as *H. americanus* is a pioneer plant (fast growing) and thought to be less AM-dependent. However, the measured growth parameters of the +AMF + LF treatment reached the same or even

significantly better values than the ones in the high fertilization treatment. Urgiles *et al.* (2009) also reported that high fertilization strength as well as the mycorrhizal treatments with low fertilization improved growth parameters of the seedlings. Therefore nutrient uptake of *H. americanus* seedlings seems to be improved by AMF inoculation.

Tabebuia chrysantha reacted best to high fertilization after 3 months in the nursery. After 6 months this effect changed and the +AMF + LF treatment showed similar results as high fertilization. Mortality rate was significantly reduced by inoculation with AMF, independent of fertilization. Haug *et al.* 2010 found that *T. chrysantha* seedlings, raised in the nursery and sampled later on the reforestation plots, were colonized by a variety of AMF species. Aguirre Mendoza (2007) argued that the low mortality rates of *T. chrysantha* planted on the pastures might dependent on the mycorrhization of the roots, as abundant AMF were found in the roots of the seedlings. Thus high AMF-dependence can be assumed for *T. chrysantha*.

Inoculation of AMF can have beneficial effects on nutrient transport to the plant under greenhouse conditions especially for P, Zn and N (e.g. Khade & Rodrigues 2009, Espinoza-Victoria *et al.* 1993, Smith & Read 2008). In the nursery phase of experiment No. 3 the inoculated tree seedlings of *T. chrysantha* showed higher amounts of P in leaves and roots compared to the control. *Cedrela montana* showed no clear nutrient improvement when inoculated, whereas seedlings of *Heliocarpus americanus* showed increased P values in roots and leaves in the AMF treatments (LF + AMF and +AMF). The P content of the inoculated seedling of *T. chrysantha* and *C. montana* (leaves and roots) increased in the nursery phase (3 to 6 months), while for *H. americanus* leaves and roots is decreased. Therefore we can state that inoculation with AMF can improve phosphorus uptake in roots and leaves, which imply an active phosphor transport between the native tree seedlings and AMF colonizing the roots. Raju *et al.* (1988) e.g. reported increases in S, K, Cu and decreases in Mn, Fe and Zn of inoculated plants in acidic soils, when compared to the control. We can only partial confirm this observation, as *C. montana* showed these effects in the +AMF treatment, whereas *H. americanus* and *T. chrysantha* showed these tendencies in the +AMF and the +AMF+LF treatments. P amount increased in almost all tree seedlings, when inoculated with the AMF mixture as shown widely in literature (Khade & Rodrigues 2009, Espinoza-Victoria *et al.* 1993, Smith & Read 2008). However we could not confirm a constant increase of K as stated in Khade & Rodrigues (2009).

In general, an improved plant performance was observed after AMF inoculation, although up to 20% mycorrhization of a ‘background’ AMF community was visible in the non-AMF treatments and therefore effects may be less significant. The steam sterilization in the nursery was obviously not fully efficient, the open greenhouse system (disposure of soil, dust etc.) and a rotation of treatment setup by placing bags in different positions throughout the

running experiment might have caused cross-contaminations. Also the mite attack biased growth performance and leaf number values especially of *C. montana*. *S*eedlings were also affected through drought stress due to the changes in the watering regime. Due to this complex influences an uneven plant performance in the greenhouse was visible. Variations were, however, compensated by the high number of seedlings in the Nursery experiment No. 3 resulting in significant differences although standard errors were high.

Effects of AMF inoculation in the reforestation phase

No significant differences in growth performance of the sampled tree seedlings on the reforestation plots were observed. As only a total of six plants per treatment of each tree species was sampled, and due to the high variation in soil quality, altitude, light conditions, etc. on the reforestation plots, low significance was expected. These findings were confirmed during the two year-sampling on the pastures by Palomeque (2012).

Increases in S, K, Cu and decreases in Al, Mn, Fe and Zn of inoculated plants in acidic soils (Raju *et al.* 1988) could be mainly confirmed for *H. americanus*, but only partial for *C. montana* and *T. chrysantha*. Increased nutrients were detected in *C. montana* seedlings solely in the +AMF treatment, but in both +AMF treatments (LF + AMF and +AMF) for *T. chrysantha* and *H. americanus*. The P amount increased in all leaves of the tree seedlings when inoculated with the AMF mixture (Khade & Rodrigues 2009, Espinoza-Victoria *et al.* 1993, Smith & Read 2008).

Sampling of all out-planted tree seedlings revealed reduced mortality rates of the AMF-inoculated *T. chrysantha* seedlings. Whereas inoculation appeared to have no significant effect on the seedlings of *C. montana* and *H. americanus.* High variability of the measured data can have several reasons. First, AMF from the field colonized all tree seedlings after out-planting as shown from the 454 data. The fast mycorrhization on the plots may well be explained by the high presence and diversity of AMF on the abandoned pastures (Haug *et al.* 2010). The “field inoculum” may be the reason that all *T. chrysantha* seedlings reached similar growth values over time. Second, the conditions on the plots varied strongly, e.g. in quality of the soil, water regime, light conditions, altitude of the plot (1,800 - 2,100 m a.s.l.) and even weather types, depending on wind direction (Emck 2007).

In summary, the tree seedlings showed positive growth when inoculated with AMF in the nursery phase, as previously reported by Urgiles *et al.* (2009). The positive growth effects disappeared after planting of the seedlings on the reforestation plots (abandoned pasture). Palomeque (2012) confirmed our findings as no significant growth differences between the surviving tree seedlings in the field after 2 years were observed. Bashan *et al.* (2012) also reported fewer positive growth effects of leguminous trees, when inoculated with native AMF after 2.5 years on a restoration site in the southern Sonoran desert. Inoculation by native

AMF seems to be important in the nursery phase in the early stage of seedling establishment, and later to overcome the planting shock on the pastures, where AMF from the field colonize the roots of the seedlings over time. Several studies reported that AMF can have positive effects on restoration sites, e.g. a higher native vegetation coverage (Noyd *et al.* 1995, 1996, Smith *et al.* 1998) or an improved plant performance on ground with low phosphorus level (Johnson 1998). However in some cases inoculation with AMF is inappropriate, dependent on the field conditions (e.g. high phosphorus level) and a natural re-colonization can take place over time (White *et al.* 2008).

The main improvement was a significantly reduced mortality rate of the tree seedlings in the AMF treatments of *Tabebuia chrysantha* in the field. Palomeque (2012) also found a significantly increased survival in the *T. chrysantha* seedlings (94%) when inoculated with AMF compared to the remaining treatments (survival rates of >70%), on the reforestation plots 2 years after out-planting. These results indicate a positive correlation of decreased mortality with AMF inoculation of the *T. chrysantha* seedlings on the reforestation plots as a higher P level in the inoculated tree seedlings was found. 454 analyses showed that non-introduced AMF re-colonized the roots in the field. Similar observations are reported by White *et al.* (2008). One may argue that due to high background mycorrhization in the nursery (under nursery standard practice) and mycorrhizal re-colonization in the field inoculation by AMF did not result in relevant changes. However the control and +AMF treatment showed different significances in seedling growth especially in the nursery. Comparison between the –AMF + LF and the +AMF + LF treatment in some cases showed large differences of the measured parameters. Therefore we can state that AMF inoculation on its own has nearly no fertilization effect and addition of local AMF (under nursery standard practice) did make a difference.

Native AMF inoculum performance

The isolation and characterization of local AMF revealed a high percentage of new fungal species never described before. Our AMF inoculum mix was applied according to Schmidt *et al.* (2005) who suggested using young fungal inoculum and mixing the inoculum with soil as preferable to application in layers or point inoculation. Due to several changes in the experimental design between Nursery experiment No. 3 (application of substrate-inoculum-mixture) and No. 4/4A (point inoculation), such as changed sterilization procedure, inoculation with individual AMF instead of a mixture and usage of a different seedling batch, it cannot be stated if point inoculation was superior to the application of the substrate-inoculum-mixture. Age, storage, strength and amount of the applied inoculum can influence inoculation success. The efficiency of inocula was tested after suggestions of Dalpé (1991) by setting up test cultures. Our test cultures with the host plant *P. lanceolata* showed a

sufficient efficiency of the mixed inoculum, even when applied in low amounts. The efficiency of our AMF inoculum mix should not have suffered through transport and storage at 4°C. Dalpé & Monreal (2004) stated that semi-dry inocula can be stored over long terms (1-2 years) at 5°C, or even at room temperatures (20 to 25°C) without losing viability and efficiency.

The question arises if inoculation by worldwide spread AMF is also suitable or superior to local AMF as Haug *et al.* (2010) stated the finding of worldwide appearing AMF on the pastures at RBSF. This is questionable as the used primers in Haug *et al.* (2010) only resolve AMF on the genus-level. Our results indicate that mostly local AMF were present in the seedling roots on the pasture, or species such as *Glomus macrocarpum* which are hardly cultivable and not available in commercial inocula. Nevertheless, effects on growth performance of native tree seedlings by a widespread ubiquist AMF (*Rhizophagus irregularis* DAOM197198) was tested in the Nursery experiment No. 5 (Urgiles *et al.* in preparation) and will show further results in near future.

4.1.1 Effects of individual AMF strains

Although it is known that host plants usually contain more than a single AMF (e.g. Scheublin *et al.* 2004, Šmilauer 2001, Haug *et al.* 2010), little is known about AMF-plant specificities. Therefore we also applied the characterized AMF as individual inocula and conducted an additional nursery experiment (No. 4), to find out if there might be AMF most suitable for the individual tree species' growth performance in the nursery.

Effect of inoculation by individual AMF on native tree seedlings

The seedlings of *Cedrela montana, Heliocarpus americanus* and *Tabebuia chrysantha* all reacted with increased height, RCD, leaf numbers, leaf area and biomass production when inoculated by individual AMF. Differences between inoculated and non-inoculated seedlings in some cases were quite strong showing increase of up to three times in growth compared to the non-AMF treatment. *Cedrela montana* and *H. americanus* did not show as clear AMF preference as *T. chrysantha*, because most of the used AMF yielded similar increases of the growth parameters. *Claroideoglomus etunicatum*-like Att1449-10 performed best in case of growth of *C. montana*, whereas a specific AMF preference of *H. americanus* could not be detected. The *T. chrysantha* seedlings showed clear AMF preferences for *Diversispora* sp. Att1449-5, *Cl. etunicatum*-like Att1449-10 and *Rhizophagus* sp. Att1451-8.

***Effect of inoculation and fertilization of individual AMF on* Cedrela montana**

Improved growth performance in height, RCD, leaf number and leaf area after 6 months of the inoculated *Cedrela montana* seedlings was detected. Low fertilization solely resulted in

increased biomass production. Due to the changed sterilization procedure and less root colonization of 'background-AMF' in the controls a clear AMF preference of *C. montana* was observed in this nursery experiment (No. 4A).

Inoculation with individual AMF resulted in different growth response dependent on AMF and according tree species, as illustrated in Nursery experiment No. 4 and 4A. Results are similar to several studies, which showed different growth effects of AMF species on plants (e.g. Klironomos 2003, van der Heijden *et al.* 1998a) and changes in AMF communities in different hosts (e.g. crops, herbs and trees) indicating an AMF-plant preference (e.g. Torrecillas *et al.* 2012, Bashan *et al.* 2012, Alguacil *et al.* 2011, Scheublin *et al.* 2004). Effects concerning individual AMF were more pronounced than in the above described Nursery experiment No. 3 most probably due to more effective sterilization procedure of the standard nursery substrate and therefore lacking background mycorrhization of the seedling roots. Although almost all tree seedlings reacted positively in growth when inoculated by individual AMF, effects between the individual inocula varied highly in the measured growth parameters and in other studies some AMF-plant associations were found to perform better than others (van der Heijden *et al.* 1998a; Klironomos 2003), indicating AMF-plant preferences (Croll *et al.* 2008). Positive growth reactions of *C. montana* to low fertilization were observed in both nursery experiments. Jansa *et al.* (2008) also investigated the effects of single species versus mixed AMF inoculum and revealed a more beneficial effect (e.g. phosphorus uptake) when a mixed inoculum was applied. Our results show that the growth effects of the individual AMF species did not exceed the ones achieved by applying the inoculum mix in terms of height and RCD, but in fresh weight and biomass, confirming partially the findings of Jansa *et al.* (2008).

Our result showed a different performance of the used tree species in specific AMF-plant associations, also dependent on the applied fertilization level. Low fertilization in combination with AMF inoculation is suggested for *H. americanus* and *T. chrysantha*, whereas *C. montana* reacted prior to AMF inoculation and irregularly to fertilization. *Acaulospora* sp. nov. Att1450-1, *Claroideoglomus etunicatum*-like Att1449-10/Att1451-6/Att1456-11, *Rhizophagus* sp. Att1451-8/Att1456-1 and *Diversispora* sp. Att1449-5 were persistent in the seedlings roots and could successfully traced by 454 sequencing. However, they performed different in the three tree species. *Cedrela montana* reacted with improved growth predominantly to *Cl. etunicatum*-like Att1449-10 and Att1451-18 (multispore culture), *Acaulospora* sp. nov. Att1450-1, *Rhizophagus* sp. Att1451-8 and interestingly to *Ambispora* sp. Att1449-12. *Heliocarpus americanus* showed improved growth when inoculated with *Diversispora* sp. Att1449-5, *Cl*. *etunicatum*-like Att1449-10 and *Ar*. *trappei*-like Att1456-7. Effects in seedling growth of the individual AMF inocula varied in *C. montana* and *H. americanus* depending on measured growth parameter. The clearest AMF-preference

was visible in *T. chrysantha* seedlings, which reacted with improved growth to *Diversispora* sp. Att1449-5, *Cl. etunicatum*-like Att1449-10 (also Att1451-18) and *Rhizophagus* sp. Att1451-8.

We can now specify the best performing AMF according to the used tropical tree species, which will improve further inocula and reforestation in Ecuador. Finally we can state that *Acaulospora* sp. nov. Att1450-1, *Cl*. *etunicatum*-like Att1449-10, *Rhizophagus* sp. Att1451-8 and *Diversispora* sp. Att1449-5 in combination with reduced fertilization showed improved seedling growth in all native tree species. *Dentiscutata savannicola* Att1455-2 was herein tested solely with *C. montana* seedlings, which showed improved growth. Therefore this AMF may be another major candidate for future inoculum, but this question will be answered in a subsequent experiment (No. 5) in the Ecuadorian nursery. The dominant AMF *Gl*. *macrocarpum* could also be added in future inoculum as it was found in all seedling roots, especially in *T. chrysantha* in high percentages (up to 75%). Other aspects as application of inoculum, point-inoculation vs. substrat-inoculum-mix and amount of AMF in the inoculum (individual AMF vs. mixed-AMF inoculum) cannot be answered sufficiently, as the growth effects between the different nursery experiment were influenced by a variety of factors (e.g. watering regime, mite attack, cross-contaminations, different sterilization procedure etc.). Nevertheless, the application of individual AMF resulted in improved growth of *C. montana* and *T. chrysantha*, when compared to the AMF-mixture, whereas *H. americanus* reacted vice versa. Altough the main aspect of the study could be solved some questions still remain, which may be answered in Nursery experiment No. 5.

5 Conclusion

The main aim in this study was to decrease mortality of native tree seedlings with inoculation of AMF, as former reforestation attempts in the research area (RBSF) were hampered by high mortality rates. In the herein described Nursery experiments we could show that distinct AMF-plant associations performed differently in growth and improved seedling performance. We could further confirm plant-AMF-preferences by the native tree species. Via 454 sequencing we could monitor the introduced AMF species in the roots of the seedlings and their persistence over time. In the field phase we could show that inoculation with AMF significantly reduced mortality rates of *Tabebuia chrysantha*, but not of *Cedrela montana* and *Heliocarpus americanus*. We assume that the applied inoculum mixture was most suitable for *T. chrysantha*, as the introduced AMF species were more abundant in the roots of this tree species than in the others.

All AMF applied in the nursery experiment were characterized on the morphological and molecular level. However, the question if the culture Att1449-12 contains an *Ambispora* sp. could not be finally clarified, as the 'inhabitant' could not be adequately characterized and the isolate (strain) performed unusually in the nursery experiments. It may be that something else (e.g. fungi or bacteria) in this culture partially facilitate seedling growth as the plants reacted positively to this single spore inoculum and mycorrhizal colonization rates were appropriate. This question may be answered by the results in the ongoing Nursery experiment No. 5 (not described herein).

The mixed AMF inoculum was produced in closed pot cultures in the greenhouse of the Genetics, Department Biology I, LMU Munich, as this is a simple and cost-effective way to produce inoculum. The effectiveness of the semi-dry AMF inoculum was appropriate, as a fast transport to Ecuador took place and further storage was done at 4°C. Also application of an inoculum mixture carrying diverse AMF is more natural-like, since more than one AMF species is present in one root system. Additionally, test cultures with *Plantago lanceolata* as host showed sufficient mycorrhizal root colonization of the inoculum.

Future inoculum should also take the detected background AMF into account, predominantly *Gl. macrocarpum*, *Rh. irregularis* and *Ac. brasiliensis*-like, as these AMF were found in high percentages (454 sequence reads) in the seedling roots in the nursery and the field and may be useful for an efficient reforestation. The number of AMF species used in the inoculum may be adapted in future, as five AMF species in an inoculum may be inappropriate. We detected up to 15 AMF species in one root system of the tropical tree species in the nursery and up to 16 in the field. Nevertheless, our results indicate that application of up to three different AMF,

well adapted to a tree species, may sufficient. Except for mortality rates of the *T. chrysantha* seedlings, no significantly improved growth parameters were observed in the field phase which could also be hampered by different abiotic aspects (e.g. soil, altitude etc.). It also may be that the applied inoculum was underdosed. Therefore, future inocula should consist of our main candidates *Acaulospora* sp. nov. Att1450-1, *Cl. etunicatum*-like Att1449-10, *Rhizophagus* sp. Att1451-8 and *Diversispora* sp. Att1449-5 in combination with reduced fertilization, with adaptation to *C. montana* (plus *Ambispora* sp. Att1449-12) and *H. americanus* (*Archaeospora* sp. Att1456-7). In addition nursery and planting conditions have to be improved, as mycorrhization cannot do the whole job.

5.1 Outlook

Further studies and nursery experiments have to be carried out to improve our knowledge about AMF and their benefits on tropical tree seedlings. An additional nursery experiment (No. 5) was established in Ecuador testing some of these aspects, as different amounts of fertilizer, applied in layers, an improved sterilization procedure, and point inoculation of single species inocula of native AMF and a foreign AMF (*Rh. intraradices* DAOM197198) on *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha*, to corroborate the findings of the herein described nursery experiments.

Future inoculum may be improved dependent on application, either by addition of further AMF species or reducing the inoculum to the dominating AMF species, as re-colonization of the surviving plants takes place in the field (White *et al.* 2008).

High-throughput sequencing will advance in future, as the third-generation sequencing is now on the market, which makes longer sequence reads possible, e.g. the GS FLX+ system from Roche (up to 800 bp read lengths for amplicons, second quarter 2013) and the Pac Bio R2 sequencer (Pacific Bioscience). The latter will probably revolutionize the community analyses of AMF in combination with other high-throughput technologies or by using circular consensus sequence (CCS) reads. The new C3 chemistry of PacBio allows amplifications to an average of ca. 8.5 kb DNA fragments, which decrease the high error rates with increasing CCS reads, e.g. of a 1.5 kb fragment to ≥99% (5 pass). This will be sufficient for adequate and robust phylogenetic analyses and may improve our knowledge about AMF diversity.

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8 Appendix

8.1 Figures and tables

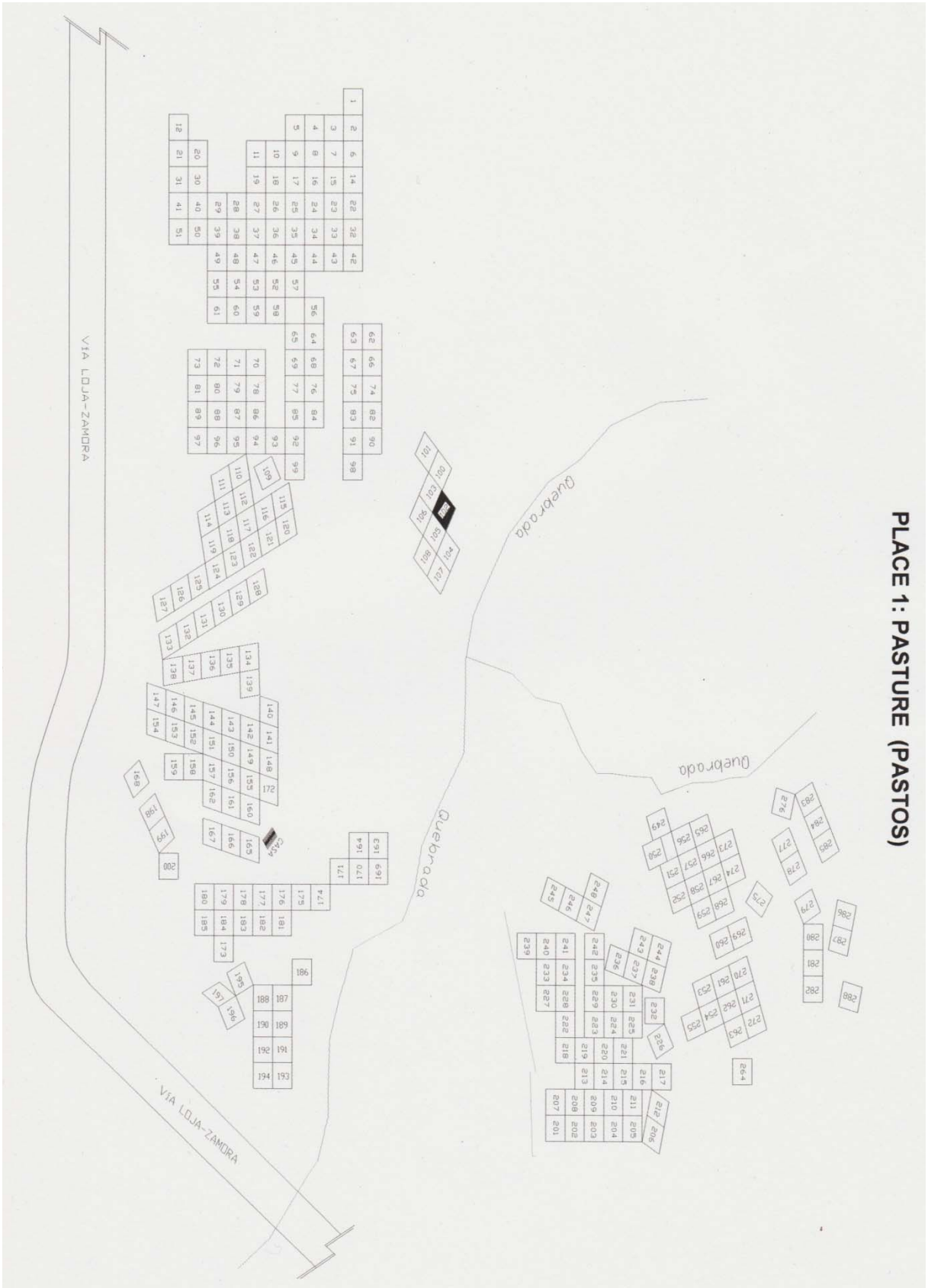


Fig. A1: Map of the reforestation plots near the ECSF in Ecuador. Overview of the location of all reforestation plots on the upper and lower pastures.

		Primer sequence (5'->3'direction)				
	Primer	**GS FLX Titanium Primer A (forward) or B (reversed)**	**Key**	**MID**	**AMF primer**	**Length**
forward	**454_LSUD2Bf-MID1**	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACGAGTGCGT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID2	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACGCTCGACA	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID3	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	AGACGCACTC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID7	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CGTGTCTCTA	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID9	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TAGTATCAGC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID11	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TGATACGTCT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID12	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TACTGAGCTA	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID14	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CGAGAGATAC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID15	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ATACGACGTA	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID16	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TCACGTACTA	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID17	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CGTCTAGTAC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID18	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TCTACGTAGC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID19	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TGTACTACTC	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID26	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACATACGCGT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID27	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACGCGAGTAT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID28	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACTACTATGT	GTGAAATTGTTRAWARGGAAACG	63nt

	Primer	GS FLX Titanium Primer A or B	Key	MID	AMF primer	Length
	454_LSUD2Bf-MID29	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ACTGTACAGT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID30	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	AGACTATACT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID31	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	AGCGTCGTCT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID32	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	AGTACGCTAT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID33	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	ATAGAGTACT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID34	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CACGCTACGT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID35	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CAGTAGACGT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID36	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	CGACGTGACT	GTGAAATTGTTRAWARGGAAACG	63nt
	454_LSUD2Bf-MID37	CCATCTCATCCCTGCGTGTCTCCGAC	TCAG	TACACACACT	GTGAAATTGTTRAWARGGAAACG	63nt
reverse	**454_LSUmBr1**	CCTATCCCCTGTGTGCCTTGGCAGTC	TCAG		DAACACTCGCATATATGTTAGA	52nt
	454_LSUmBr2	CCTATCCCCTGTGTGCCTTGGCAGTC	TCAG		AACACTCGCACACATGTTAGA	51nt
	454_LSUmBr3	CCTATCCCCTGTGTGCCTTGGCAGTC	TCAG		AACACTCGCATACATGTTAGA	51nt
	454_LSUmBr4	CCTATCCCCTGTGTGCCTTGGCAGTC	TCAG		AAACACTCGCACATATGTTAGA	52nt
	454_LSUmBr5	CCTATCCCCTGTGTGCCTTGGCAGTC	TCAG		AACACTCGCATATATGCTAGA	51nt

Table A1: Primer sequences used for the GS FLX Titanium amplicon sequencing (Roche) in detail. Complete sequences of the used 454 primers for amplicon sequencing are shown. One primer consists of the GS FLX Titanium primer A (forward) or B (reversed), the key sequences (used for calibration of the GS FLX sequencer), the according MID sequence (only on the forward primer, as we used unidirectional sequencing) and the designed primer to amplify the specific product. **nt:** nucleotides.

Att-No.	Name	Voucher	Size (length × breadth) in µm	Shape	Appearance	Color	Melzers reaction	Muronym
1449-5	*Diversispora* sp.	W5349				10YR 7/1 - 8/2 - 8/4 (white to very pale brown) [MSC]	no reaction	A(UL)
		W5661	95.7 (25 to 135) × 96 (27 to 140)	globose to subglobose to broad ellipsoidalal	single	colorless to white	no reaction	
1449-10	*Claroideoglomus etunicatum*-like	W5333	116 (80 to 188) × 113 (88 to 165)	globose to subglobose	single or in loose clusters	7.5R 8/6-7/8-6/8-5/8 (reddish yellow to strong brown) [MSC]	very slow pink reaction of outermost wall component	A(EL)
		W5668	114 (67 to 167) × 114 (67 to 203)	globose to ovoid	single or in loose clusters	colorless to yellow to yellow-brown rarely grayish olive	no reaction	
1450-1	*Acaulospora* sp. nov.	W5350				7YR 5/6 (strong brown) [MSC]	two reactive wall components	
		W5666	194 (78 to 232) × 195 (87 to 255)	globose to subglobose to broad ellipsoidal	single	peach to orange yellow to orange		

Att-No.	Name	Voucher	Size (length × breadth) in µm	Shape	Appearance	Color	Melzers reaction	Muronym
1450-1	*Acaulospora* sp. nov.	W5693	191 (158 to 225) × 196 (155 to 233)	globose to obvoid	single		two reactive wall components	
		W5741	186 (158 to 228) × 191 (163 to 228)	globose to ovoid	single	10YR 6/8 (brownish yellow) to 7.5YR 6/8 (strong brown) [MSC]	Component 2 and part of group 3 reacting purple, but only in some specimens	A(UL)B(F) C(FFbFp)
1451-6	*Claroideoglomus etunicatum*-like	W5335	99 (56 to 142) × 99 (56 to 141)	globose to broad ellipsoidal, also pyriform	single or in loose clusters	hyaline to 2.5Y 8/6 - 8/8 (yellow) [MPT]	no reaction	A(EL)
	Funneliformis cf. *vesiculiferus*	W5472	50 (35 to 67) × 47 (34 to 66)	globose to subglobose to broad ellipsoidal	in tight clusters	colorless to pale yellowish cream (colorless - 3) [RBG]	rapid blood red	
	glomoid unknown	W5553	184 (150 to 203) × 184 (155 to 200)	globose	single	white (1) [RBG]	evanescent component pink	A(EL)
	Claroideoglomus etunicatum-like	W5554				pale pinkish cream to ochre to sienna (4-9-11) [RBG]	no reaction	A(EL)

Att-No.	Name	Voucher	Size (length × breadth) in µm	Shape	Appearance	Color	Melzers reaction	Muronym
1451-6	*Glomus* sp.	W5555				colorless or pale yellow small spores with yellow brown larger spores in a cluster.		A(EL)
	Claroideoglomus etunicatum-like	W5595	117 (74 to 139) × 117 (70 to 139)	globose to subglobose to broad ellipsoidal		olivaceous		
	Glomoid unknown	W5596	192 (183 to 200) × 190 (180 to 208)	globose to subglobose		whitish		
	Claroideoglomus etunicatum-like	W5667	101 (40 to 160) × 99 (39 to 144)	globose to subglobose to broad ellipsoidal	single or in loose clusters	white to yellow to yellow brown	no reaction	A(EL) maybe A(ELU)
1451-8	*Rhizophagus* sp.	W5338				hyaline to pale yellow	no reaction	
		W5662	86 (37 to 160) × 58 (29 to 114)	subglobose to ellipsoidal to ovoid	spores in roots tightly packed together (juxtaposed)	colorless to pale yellow	no reaction	A(UL)?

Att-No.	Name	Voucher	Size (length × breadth) in µm	Shape	Appearance	Color	Melzers reaction	Muronym
1452-6	*Archaeospora trappei*-like	W5340	63 (51 to max. 78) × 62 (51 to max. 77)	globose to subglobose to broad ellipsoidal to ovoid to obovoid	single	hyaline	no reaction	
		W5670				hyaline	no reaction	
1456-1	*Rhizophagus* sp.	W5336						
		W5664	59 (38 to 109) × 48 (31 to 104)	globose to subglobose to broad ellipsoidal to ovoid, also irregular	spores in roots	very pale yellow	no reaction	
1456-7	*Archaeospora trappei*-like	W5337						
		W5663	60 (43 to max. 69) × 60 (43 to max. 83)	globose to subglobose to broad ellipsoidal	single	colorless, hyaline	no reaction	A(F)B(L)?
1456-11	*Claroideoglomus etunicatum*-like	W5348				10YR 6/8 (brownish yellow) [MSC]	no reaction	A(UL)?

Att-No.	Name	Voucher	Size (length × breadth) in µm	Shape	Appearance	Color	Melzers reaction	Muronym
1456-11	*Claroideoglomus etunicatum*-like	W5669	124 (76 to 173) × 122 (80 to 181)	globose to broad ellipsoidal, also clavate	single or in loose clusters	white, light gray to brownish-yellow	no reaction	
1455-2	*Dentiscutata savannicola*	W5538	321 (215 to 585) × 367 (235 to 510)	globose to broad ellipsoidal to ovoid, also pyriform and obovoid	single	10YR 8/1 becoming 8/4 to 7/8 (- 3/6 when moribund)White becoming yellow to brownish yellow to dark yellowish brown when dead) [MSC]	rapid outer blood red. Inner slowly purple.	A(EL)B(F) C(***)
		W5893				colorless to ivory to pale yellowish cream to yellowish cream to pale ochraceous (colourless-1-2-3-5-6) [RBG]	rapid reaction - inner and outer walls darkening to blood red	

Table A2: Voucher information of the Ecuadorian cultures in detail. Different color charts were used; **[MSC]**: Munsell Soil Chart, **[MPT]**: Munsell plant tissue Chart and **[RBG]**: RBG chart.

Clone No.	Sample description	DNA region	DNA source	Date	Culture species	Primer	Clones	Closest BLAST hits
MK051	N1-1 60°C	SSU-ITS-LSU	roots	12.10.2007	unknown	SSUmAf/ LR4+2bp	5	uncultured *Acaulospora* sp., *Ac. paulinae*
MK052	N3-1 60°C	SSU-ITS-LSU	roots	12.10.2007	unknown	SSUmAf/ LR4+2bp	7	uncultured *Archaeospora* sp., uncultured *Glomeromycete*, *Ac. paulinae*
MK053	1-1 62°C	SSU-ITS-LSU	roots	12.10.2007	unknown	SSUmAf/ LR4+2bp	4	uncultured *Archaeospora* sp., uncultured *Glomeromycete*, *Acaulospora* sp, uncultured *Acaulospora* sp.
MK054	1-1 62°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	*Ac. laevis*, *Acaulospora* sp., uncultured *Glomus* clone, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
MK055	3-1 62°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	uncultured *Glomus* clone, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
MK056	1-1 64°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	*Acaulospora* sp., *Glomus* sp. MUCL43206
MK057	5-1 64°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	uncultured *Glomus* clone, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
MK058	1-1 66°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	*Acaulospora* sp., uncultured *Acaulospora* clone, uncultured *Glomus* clone
MK059	3-1 66°C AML	SSU	roots	12.10.2007	unknown	AML1/AML2	8	uncultured *Glomus* clone, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
CK006	*P. oleifolius*. – TOPO blunt	SSU-ITS-LSU	nodules	11.01.2008	unknown	SSUmC/LSUmB	2	*Gl. coronatum*, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
CK007	*P. oleifolius* - pJET1.2_2µl ligation	SSU-ITS-LSU	nodules	11.01.2008	unknown	SSUmC/LSUmB	6	uncultured *Glomus* sp., *Glomus* sp. MUCL43206, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*, *Gl. diaphanum*
CK008	*P. oleifolius* - pJET1.2_4µl ligation	SSU-ITS-LSU	nodules	11.01.2008	unknown	SSUmC/LSUmB	8	*Glomus* sp. MUCL43206, uncultured *Glomus* sp., *Glomus* mycorrhizal symbiont of *Marchantia foliacea*, *Gl. diaphanum*
CK009	N3 – ABC (pooled)	SSU-ITS-LSU	roots	17.04.2008	unknown	SSUmAf/LSUmA	3	*Glomus* mycorrhizal symbiont of *Marchantia foliacea*, *Glomus* sp. MUCL43206, *Gl. diaphanum*
CK010	N8 – ABC (pooled)	SSU-ITS-LSU	roots	17.04.2008	unknown	SSUmAf/LSUmA	2	*De. heterogama*, *De. reticulata*, *Gigaspora* sp.
CK011	N1 – A	SSU-ITS-LSU	roots	17.04.2008	unknown	SSUmC/LSUmB	7	uncultured *Archaeospora* sp.
CK012	N2 – C	SSU-ITS-LSU	roots	17.04.2008	unknown	SSUmC/LSUmB	4	*Ar. trappei*, *Rh. intraradices*

Clone No.	Sample description	DNA region	DNA source	Date	Culture species	Primer	Clones	Closest BLAST hits
CK014	W5198 - A	SSU-ITS-LSU	spore	17.04.2008	*De. savannicola*	SSUmAf/LSUmA	2	*De. heterogama*, *Gi. rosea*, *Scutellospora* sp.
CK016	N3 JenaKit +BSA	SSU-ITS-LSU	roots	07.05.2008	unknown	SSUmAf/LSUmA	3	*Rh. intraradices*, uncultures *Glomus* sp.
CK017	N3 JenaKit +BSA	SSU-ITS-LSU	roots	07.05.2008	unknown	SSUmC/LSUmB	1	*Glomus* mycorrhizal symbiont of *Marchantia foliacea*
CK018	*P. oleifolius* JenaKit	SSU-ITS-LSU	roots w/o nodules	07.05.2008	unknown	SSUmAf/LSUmA	1	uncultured *Acaulospora* sp., *De. heterogama*
CK019	*P. oleifolius* JenaKit +BSA	SSU-ITS-LSU	roots w/o nodules	07.05.2008	unknown	SSUmAf/LSUmA	1	*Glomus* sp. MUCL43206
CK020	*P. oleifolius* JenaKit +BSA	SSU-ITS-LSU	roots with nodules	07.05.2008	unknown	SSUmAf/LSUmA	13	*Acaulospora* sp., *Scutellospora* sp.
CK021	N3 JenaKit +BSA	SSU-ITS-LSU	roots	07.05.2008	unknown	SSUmAf/LSUmA	5	*Glomus* sp. MUCL43206, *Rh. intraradices*
CK022	Att1449-5	SSU-ITS-LSU	spore	26.08.2008	*Diversispora* sp.	SSUmC/LSUmB	3	*Gl. versiforme* BEG47, *Gl. aurantium*, *Gl. eburneum*; *Gl.* cf. *etunicatum* W2423
CK023	Att1450-1	SSU-ITS-LSU	spore	26.08.2008	*Acaulospora* sp.nov.	SSUmC/LSUmB	4	uncultured *Acaulospora* sp., *Ac. mellea*, *Ac. collosica*
CK024	Att1451-8	SSU-ITS-LSU	roots	26.08.2008	*Rhizophagus* sp.	SSUmAf/LSUmA	4	*Glomus* sp. MUCL43206, *Rh. intraradices* AFTOL 845, uncultured *Glomus* sp. (Kottke)
CK025	Att1456-1	SSU-ITS-LSU	roots	26.08.2008	*Rhizophagus* sp.	SSUmC/LSUmB	4	uncultured *Glomus* clone S1R2, *Gl. diaphanum*, *Glomus* mycorrhizal symbiont of *Marchantia foliacea*
CK026	Att1456-7	SSU-ITS-LSU	spore	26.08.2008	*Ar. trappei*-like	SSUmC/LSUmB	6	*Ar. trappei*
CK027	Att1456-11	SSU-ITS-LSU	spore	26.08.2008	*Cl. etunicatum*-like	SSUmC/LSUmB	3	*Cl. claroideum*, *Cl. etunicatum* BEG92
CK028	Att1449-10	SSU-ITS-LSU	spore	03.09.2008	*Cl. etunicatum*-like	SSUmC/LSUmB	12	*Cl. etunicatum* BEG92, C*l. claroideum*
CK029	Att1451-6	SSU-ITS-LSU	spore	03.09.2008	*Cl. etunicatum*-like	SSUGap/LSUmA	1	*Cl. etunicatum* BEG92
CK030	Att1451-6	SSU-ITS-LSU	spore	03.09.2008	*Cl. etunicatum*-like	SSUmC/LSUmB	6	*Cl. etunicatum*, uncultured *Glomus* clone Pa127
CK031	Att1451-6 - D	SSU-ITS-LSU	spore	03.09.2008	*Cl. etunicatum*-like	SSUmC/LSUmB	2	*Gi. rosea*, *Gi. margarita*
CK034	Att1452-6 - B_{NEW}	SSU-ITS-LSU	spore	02.12.2008	*Ar. trappei*-like	SSUmC/LSUmB	5	uncultured *Archaeospora* sp., *Ar. trappei*
CK035	Att1449-10 - D	SSU	spore	02.12.2008	*Cl. etunicatum*-like	SSU128/Geo11b	10	*Cl. etunicatum* UFPE06, *Glomus* sp., *Cl. lamellosum*
CK036	Att1449-10 - C	SSU	spore	02.12.2008	*Cl. etunicatum*-like	GeoA2/ITS1Frc	9	*Cl. etunicatum* UFPE06, *Glomus* sp., *Cl. lamellosum*
CK037	Att1451-6 - D	SSU	spore	02.12.2008	*Cl. etunicatum*-like	SSU128/Geo11b	7	*Cl. etunicatum* UFPE06, *Glomus* sp., *Cl. lamellosum*

Clone No.	Sample description	DNA region	DNA source	Date	Culture species	Primer	Clones	Closest BLAST hits
CK038	Att1451-6 - C	SSU	spore	02.12.2008	*Cl. etunicatum*-like	GeoA2/ITS1Frc	5	*Cl. etunicatum* UFPE06, *Glomus* sp., *Cl. lamellosum*
CK039	Att1449-5 - A	SSU	spore	17.12.2008	*Diversispora* sp.	GeoA1/ITS1Frc	13	*Gl. versiforme* BEG47
CK040	Att1449-5 - C	SSU	spore	17.12.2008	*Diversispora* sp.	GeoA1/ITS1Frc	8	*Gl. versiforme* BEG47, *Gl*. cf. *etunicatum* W2423
CK041	Att1450-1 - B	SSU	spore	17.12.2008	*Acaulospora* sp.nov.	GeoA1/ITS1Frc	12	*Ac. spinosa*, *Ac. longula*, *Ac. rugosa*
CK042	Att1456-7 - C	SSU	spore	17.12.2008	*Ar. trappei*-like	GeoA1/ITS1Frc	8	*Ar. trappei* Att186-1
CK043	Att1456-11 - A	SSU	spore	17.12.2008	*Cl. etunicatum*-like	GeoA1/ITS1Frc	5	*Glomus* sp., *Gl. etunicatum* UFPE06, *Gl. lamellosum*
CK044	Att1456-11 - B	SSU	spore	17.12.2008	*Cl. etunicatum*-like	GeoA1/ITS1Frc	6	*Cl. etunicatum* UFPE06, *Cl. lamellosum*, *Glomus* sp.
CK045	Att1452-6 - D	SSU	spore	17.12.2008	*Ar. trappei*-like	GeoA2/Geo11b	11	*Ar. trappei* Att186-1, *Am. fennica*
CK048	Att1451-8 - A	SSU	roots	28.01.2009	*Rhizophagus* sp.	GeoA1/ITS1Frc	9	*Rh. intraradices* AFTOL-ID 845
CK049	Att1456-1 - C	SSU	roots	28.01.2009	*Rhizophagus* sp.	GeoA2/Geo11b	4	*Rh. intraradices* AFTOL-ID 845
MK107	att1449-12 - B	SSU-ITS-LSU	roots	25.02.2009	*Rhizophagus* sp.	SSUmC/LSUmB	15	uncultured *Archaeospora* sp., *Am. leptoticha*
CK051	Att1456-13 - A	SSU-ITS-LSU	spore	26.03.2009	*Cl. etunicatum*-like	SSUmA/LSUmA	1	*Cl. etunicatum* BEG92, *Cl. claroideum*
CK052	Att1456-13 - C	SSU-ITS-LSU	spore	26.03.2009	*Cl. etunicatum*-like	SSUmA/LSUmA	5	*Cl. etunicatum* BEG92, *Cl. claroideum*
CK054	Att1455-2 - A	SSU-ITS-LSU	spore	26.03.2009	*De. savannicola*	SSUmC/LSUmB	12	*De. heterogama* AFTOL-ID 138, *Scutellospora* sp.
CK055	Att1455-2 - B	SSU-ITS-LSU	spore	26.03.2009	*De. savannicola*	SSUmC/LSUmB	8	*De. heterogama* AFTOL-ID 138, *Scutellospora* sp., *De. heterogama* BEG35
CK056	Att1455-2 - E	SSU-ITS-LSU	spore	26.03.2009	*De. savannicola*	SSUmC/LSUmB	16	*De. heterogama* AFTOL-ID 138, *Scutellospora* sp., *De. heterogama* BEG35
CK057	N2 – B	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	15	*Ar. trappei*, *Glomus* sp. MUCL43203
CK058	N3 – B	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	1	*Ac. scrobiculata*
CK059	N5 – B	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	2	*Ac. scrobiculata*
CK060	N6 - B	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	2	uncultured *Archaeospora* sp., *Ar. trappei*
CK061	N8 - B	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	5	*Glomus* sp. MUCL43206
CK062	*P. oleifolius* (1:10 diluted)	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	3	*Glomus* sp. MUCL43206
CK063	N3 (1:10 diluted)	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	4	uncultured *Glomus* sp., *Cl. etunicatum*

Clone No.	Sample description	DNA region	DNA source	Date	Culture species	Primer	Clones	Closest BLAST hits
CK064	N4 (1:10 diluted)	SSU-ITS-LSU	roots	10.05.2009		SSUmA/LSUmA	3	*Ac. scrobiculata*
CK065	Att1451-6 – ABC (pooled)	SSU-ITS-LSU	3 spores	17.07.2009	*Cl. etunicatum*-like	SSUmC/LSUmB	17	*Cl. etunicatum* BEG92, *De. heterogama*
CK069	Att1455-2 - E	SSU	spore	15.01.2010	*De. savannicola*	GeoA1/ITS1Frc	3	*De. heterogama*
CK073	Att1451-6 - A	SSU	spore	18.01.2010	*Cl. etunicatum*-like	GeoA2/Geo11b	17	*Glomus* sp. PM1.2, *Cl. etunicatum* UFPE06
CK074	Att1455-2 - A	SSU	spore	18.01.2010	*De. savannicola*	GeoA2/Geo11b	11	*De. heterogama* AFTOL-ID 138
CK075	Att1455-2 - C	SSU	spore	18.01.2010	*De. savannicola*	GeoA2/Geo11b	6	*De. heterogama* AFTOL-ID 138
CK090	Att1450-1	SSU-ITS-LSU	spore	12.11.2010	*Acaulospora* sp.nov.	SSUmA/LSUmA	12	*Ac. leavis*, *Ac. koskei*
CK091	Att1449-5	SSU-ITS-LSU	spore	12.11.2010	*Diversispora* sp.	SSUmA/LSUmA	10	*Gl. versiforme* BEG47

Table A3: Plasmid sequences either from the Ecuadorian AMF cultures, the nursery or environmental samples. Detailed overview of the different sequences achieved from Ecuadorian samples with the according BLAST hits at the listed dates.

Cedrela montana		Nursery - 3 months							Nursery - 6 months				
		Treatment							Treatment				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	c	b	bc	bc	Height	Fisher-LSD	a	c	c	b	b
(n=210)	Tukey's HSD	a	b	b	b	b	(n=189)	Tukey's HSD	a	b	b	b	b
RCD	Fisher-LSD	a	c	bc	ab	bc	RCD	Fisher-LSD	a	bc	c	b	bc
(n=210)	Tukey's HSD	a	b	b	ab	b	(n=189)	Tukey's HSD	a	b	b	ab	b
Leave No.	Fisher-LSD	a	ab	c	b	bc	Leave No.	Fisher-LSD	a	a	b	a	a
(n=210)	Tukey's HSD	a	ab	c	bc	bc	(n=189)	Tukey's HSD	a	ab	b	a	a
Leaf area	Fisher-LSD	ab	b	a	ab	ab	Leaf area	Fisher-LSD	a	b	b	ab	ab
(n=11)	Tukey's HSD	a	a	a	a	a	(n=42)	Tukey's HSD	a	a	a	a	a
Fresh root	Fisher-LSD	No data					Fresh root	Fisher-LSD	a	ab	b	ab	ab
	Tukey's HSD						(n=18)	Tukey's HSD	a	a	a	a	a
Fresh shoot	Fisher-LSD	No data					Fresh shoot	Fisher-LSD	a	b	b	ab	ab
	Tukey's HSD						(n=18)	Tukey's HSD	a	a	a	a	a
Fresh leaves	Fisher-LSD	No data					Fresh leaves	Fisher-LSD	a	a	a	a	a
	Tukey's HSD						(n=18)	Tukey's HSD	a	a	a	a	a
Dry root	Fisher-LSD	a	b	ab	ab	ab	Dry root	Fisher-LSD	a	ab	b	b	ab
(n=10)	Tukey's HSD	a	a	a	a	a	(n=39)	Tukey's HSD	a	a	a	a	a

Parameter	Test	Control					HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control
Dry shoot	Fisher-LSD	No data					Dry shoot	Fisher-LSD	a	b	b	ab	ab
	Tukey's HSD						(n=39)	Tukey's HSD	a	ab	b	ab	ab
Dry leaves	Fisher-LSD	a	b	ab	ab	ab	Dry leaves	Fisher-LSD	a	ab	b	a	a
(n=10)	Tukey's HSD	a	a	a	a	a	(n=39)	Tukey's HSD	a	ab	b	ab	ab
Mortality	Fisher-LSD	a	a	a	a	a	Mortality	Fisher-LSD	b	b	a	a	ab
(n=210)	Tukey's HSD	a	a	a	a	a	(n=189)	Tukey's HSD	ab	b	ab	a	ab
Myc rate	Fisher-LSD	a	a	a	a	b	Myc rate	Fisher-LSD	a	b	ab	c	d
(n=10)	Tukey's HSD	a	a	a	ab	b	(n=18)	Tukey's HSD	a	a	a	b	c

		Reforestation - June 2009							Reforestation - November 2009				
		Treatment							Treatment				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	a	a	a	a	Height	Fisher-LSD	a	ab	b	ab	ab
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
RCD	Fisher-LSD	a	ab	b	ab	ab	RCD	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Leave No.	Fisher-LSD	a	a	a	a	a	Leave No.	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	Parameter	Test	Control	HF
Leaf area	Fisher-LSD	a	a	a	a	a	Leaf area	Fisher-LSD	a	ab	b	ab	ab
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh root	Fisher-LSD	ab	b	ab	b	b	Fresh root	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh shoot	Fisher-LSD	a	a	a	a	a	Fresh shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh leaves	Fisher-LSD	a	a	a	a	a	Fresh leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry root	Fisher-LSD	No data					Dry root	Fisher-LSD	No data				
	Tukey's HSD							Tukey's HSD					
Dry shoot	Fisher-LSD	a	a	a	a	a	Dry shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry leaves	Fisher-LSD	a	a	a	a	a	Dry leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Mortality	Fisher-LSD	All values are equal.					Mortality	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD						(n=6)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	a	a	a	a	a	Myc rate	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Heliocarpus americanus		Nursery - 3 months							Nursery - 6 months				
		Treatment							Treatment				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	c	c	d	b	Height	Fisher-LSD	a	c	b	c	a
(n=210)	Tukey's HSD	a	bc	b	c	a	(n=49-52)	Tukey's HSD	a	bc	b	c	a
RCD	Fisher-LSD	a	c	bc	d	b	RCD	Fisher-LSD	a	ab	ab	b	ab
(n=210)	Tukey's HSD	a	bc	b	c	b	(n=49-52)	Tukey's HSD	a	ab	ab	b	ab
Leave No.	Fisher-LSD	a	a	a	a	a	Leave No.	Fisher-LSD	b	b	ab	ab	a
(n=210)	Tukey's HSD	a	a	a	a	a	(n=49-52)	Tukey's HSD	ab	b	b	b	a
Leaf area	Fisher-LSD	a	b	b	b	ab	Leaf area	Fisher-LSD	a	b	b	b	a
(n=21)	Tukey's HSD	a	ab	b	ab	ab	(n=42)	Tukey's HSD	a	b	b	b	a
Fresh root	Fisher-LSD	a	a	a	a	a	Fresh root	Fisher-LSD	a	b	ab	b	a
(n=9)	Tukey's HSD	a	a	a	a	a	(n=42)	Tukey's HSD	a	ab	ab	b	a
Fresh shoot	Fisher-LSD	a	a	a	a	a	Fresh shoot	Fisher-LSD	a	cb	b	c	a
(n=9)	Tukey's HSD	a	a	a	a	a	(n=42)	Tukey's HSD	ab	c	bc	c	a
Fresh leaves	Fisher-LSD	a	b	ab	ab	a	Fresh leaves	Fisher-LSD	a	c	b	c	a
(n=9)	Tukey's HSD	a	b	ab	ab	ab	(n=42)	Tukey's HSD	a	b	b	b	a
Dry root	Fisher-LSD	a	ab	b	b	ab	Dry root	Fisher-LSD	a	bc	ab	c	a
(n=21)	Tukey's HSD	a	a	a	a	a	(n=42)	Tukey's HSD	ab	bc	abc	c	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	Parameter	Test	Control	HF
Dry shoot	Fisher-LSD	a	c	abc	bc	ab	Dry shoot	Fisher-LSD	a	bc	b	c	a
(n=21)	Tukey's HSD	a	b	ab	ab	ab	(n=42)	Tukey's HSD	ab	c	bc	c	a
Dry leaves	Fisher-LSD	a	b	b	b	a	Dry leaves	Fisher-LSD	a	c	b	c	a
(n=21)	Tukey's HSD	a	b	b	b	a	(n=42)	Tukey's HSD	a	b	b	b	a
Mortality	Fisher-LSD	a	a	a	a	a	Mortality	Fisher-LSD	b	a	a	a	a
(n=210)	Tukey's HSD	a	a	a	a	a	(n=210)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	ab	a	b	c	d	Myc rate	Fisher-LSD	a	ab	b	d	c
(n=12)	Tukey's HSD	a	a	ab	bc	c	(n=18)	Tukey's HSD	a	a	a	b	b
		Reforestation - June 2009							**Reforestation - November 2009**				
		Treatment							**Treatment**				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	b	ab	ab	ab	Height	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
RCD	Fisher-LSD	a	a	a	a	a	RCD	Fisher-LSD	a	b	ab	ab	ab
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Leave No.	Fisher-LSD	a	a	a	a	a	Leave No.	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	Parameter	Test	Control	HF
Leaf area	Fisher-LSD	a	ab	ab	ab	b	Leaf area	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh root	Fisher-LSD	a	b	ab	ab	a	Fresh root	Fisher-LSD	a	b	ab	ab	ab
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh shoot	Fisher-LSD	a	b	ab	ab	a	Fresh shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	b	ab	ab	ab	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh leaves	Fisher-LSD	a	a	a	a	a	Fresh leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry root	Fisher-LSD Tukey's HSD	No data					Dry root	Fisher-LSD Tukey's HSD	No data				
Dry shoot	Fisher-LSD	a	b	ab	ab	ab	Dry shoot	Fisher-LSD	a	b	ab	ab	ab
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry leaves	Fisher-LSD	a	a	a	a	a	Dry leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Mortality	Fisher-LSD	Data values are all equal.					Mortality	Fisher-LSD	a	a	a	a	b
(n=6)	Tukey's HSD						(n=6)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	a	a	a	a	b	Myc rate	Fisher-LSD	a	a	a	ab	b
(n=6)	Tukey's HSD	a	a	a	a	b	(n=6)	Tukey's HSD	ab	ab	a	ab	b

Tabebuia chrysantha		Nursery - 3 months							Nursery - 6 months				
		Treatment							Treatment				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	b	c	b	bc	a	Height	Fisher-LSD	a	b	a	b	a
(n=210)	Tukey's HSD	ab	b	ab	b	a	(n=49-52)	Tukey's HSD	a	b	a	b	a
RCD	Fisher-LSD	b	c	bc	bc	a	RCD	Fisher-LSD	a	bc	ab	c	a
(n=210)	Tukey's HSD	ab	c	bc	bc	a	(n=49-52)	Tukey's HSD	ab	b	ab	b	a
Leave No.	Fisher-LSD	a	b	b	b	a	Leave No.	Fisher-LSD	a	b	b	c	b
(n=210)	Tukey's HSD	a	c	bc	bc	ab	(n=49-52)	Tukey's HSD	a	b	b	c	b
Leaf area	Fisher-LSD	a	d	bc	cd	ab	Leaf area	Fisher-LSD	a	c	b	c	b
(n=21)	Tukey's HSD	a	c	ab	bc	ab	(n=42)	Tukey's HSD	a	bc	b	c	ab
Fresh root	Fisher-LSD	a	b	b	b	b	Fresh root	Fisher-LSD	a	c	bc	b	ab
(n=9)	Tukey's HSD	a	ab	ab	b	ab	(n=42)	Tukey's HSD	a	b	ab	b	a
Fresh shoot	Fisher-LSD	a	c	ab	bc	ab	Fresh shoot	Fisher-LSD	a	bc	ab	c	a
(n=9)	Tukey's HSD	a	b	ab	b	ab	(n=42)	Tukey's HSD	a	b	ab	b	a
Fresh leaves	Fisher-LSD	a	b	b	b	ab	Fresh leaves	Fisher-LSD	a	cd	bc	d	ab
(n=9)	Tukey's HSD	a	ab	ab	b	ab	(n=42)	Tukey's HSD	a	cd	bc	d	ab
Dry root	Fisher-LSD	a	b	b	b	ab	Dry root	Fisher-LSD	a	b	ab	b	a
(n=21)	Tukey's HSD	a	b	ab	ab	ab	(n=42)	Tukey's HSD	ab	bc	abc	c	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	Parameter	Test	Control	HF
Dry shoot	Fisher-LSD	a	c	abc	bc	ab	Dry shoot	Fisher-LSD	a	b	a	b	a
(n=21)	Tukey's HSD	a	b	ab	ab	a	(n=42)	Tukey's HSD	a	b	ab	b	a
Dry leaves	Fisher-LSD	a	b	b	b	a	Dry leaves	Fisher-LSD	a	c	b	c	ab
(n=21)	Tukey's HSD	a	b	ab	b	a	(n=42)	Tukey's HSD	a	bc	ab	c	a
Mortality	Fisher-LSD	ab	b	ab	ab	ab	Mortality	Fisher-LSD	ab	b	a	ab	a
(n=210)	Tukey's HSD	ab	b	ab	a	a	(n=210)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	No data					Myc rate	Fisher-LSD	a	a	a	b	c
(n=12)	Tukey's HSD						(n=18)	Tukey's HSD	a	a	a	b	c
		Reforestation - June 2009 SHADED							**Reforestation - November 2009 SHADED**				
		Treatment							**Treatment**				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	a	a	a	a	Height	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
RCD	Fisher-LSD	a	a	a	a	a	RCD	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Leave No.	Fisher-LSD	a	a	a	a	a	Leave No.	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	Parameter	Test	Control	HF
Leaf area	Fisher-LSD	a	a	a	a	a	Leaf area	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh root	Fisher-LSD	a	a	a	a	a	Fresh root	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh shoot	Fisher-LSD	a	a	a	a	a	Fresh shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh leaves	Fisher-LSD	a	a	a	a	a	Fresh leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry root	Fisher-LSD Tukey's HSD	No data					Dry root	Fisher-LSD Tukey's HSD	No data				
Dry shoot	Fisher-LSD	a	a	a	a	a	Dry shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry leaves	Fisher-LSD	a	a	a	a	a	Dry leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Mortality	Fisher-LSD	a	a	a	a	a	Mortality	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	a	a	a	a	a	Myc rate	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

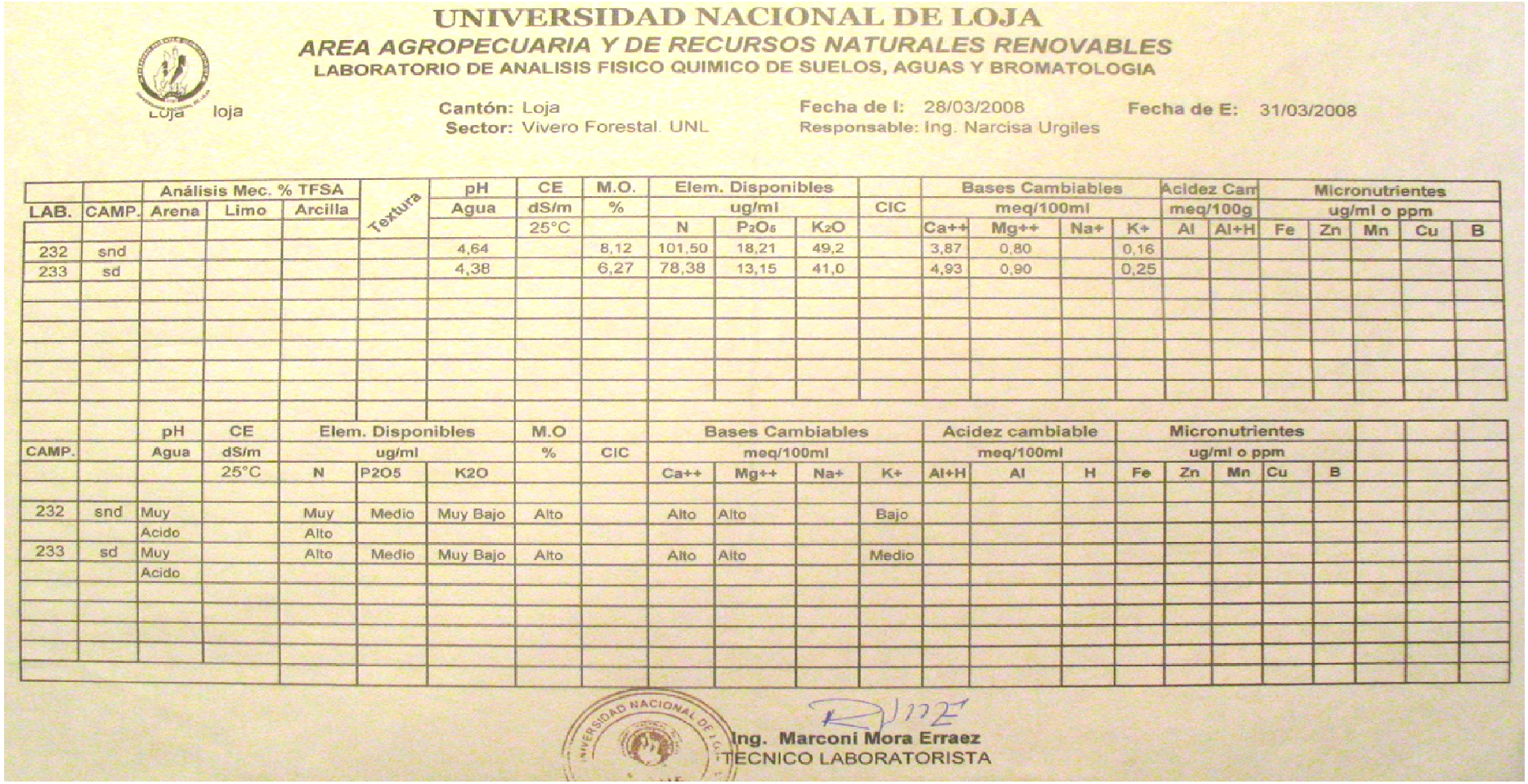
		Reforestation - June 2009 UNSHADED							Reforestation - November 2009 UNSHADED				
		Treatment							Treatment				
Parameter	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Parameter**	**Test**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Height	Fisher-LSD	a	a	a	a	a	Height	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
RCD	Fisher-LSD	a	b	ab	b	ab	RCD	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Leave No.	Fisher-LSD	a	ab	ab	ab	b	Leave No.	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Leaf area	Fisher-LSD	a	b	ab	ab	ab	Leaf area	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh root	Fisher-LSD	a	b	ab	b	ab	Fresh root	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh shoot	Fisher-LSD	a	b	ab	b	ab	Fresh shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Fresh leaves	Fisher-LSD	a	ab	b	ab	ab	Fresh leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF	Parameter	Test	Control	HF	-AMF + LF	+AMF + LF	+AMF
Dry root	Fisher-LSD Tukey's HSD	No data					Dry root	Fisher-LSD Tukey's HSD	No data				
Dry shoot	Fisher-LSD	a	b	ab	ab	a	Dry shoot	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Dry leaves	Fisher-LSD	a	b	b	ab	ab	Dry leaves	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Mortality	Fisher-LSD	a	a	a	a	a	Mortality	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a
Myc rate	Fisher-LSD	a	a	a	a	a	Myc rate	Fisher-LSD	a	a	a	a	a
(n=6)	Tukey's HSD	a	a	a	a	a	(n=6)	Tukey's HSD	a	a	a	a	a

Table A4: Statistical results of the growth data of all tree seedlings in the Nursery experiment No. 3. The different growth parameters were tested for statistical significances via the Fisher-LSD and the Tukey's HSD test. Numbers of given parameters used for analysis are given in brackets. The treatments are marked as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.

		October 2009 (n=48)					November 2009 (n=42)				
Mortality	**Treatment**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**	**Control**	**HF**	**-AMF + LF**	**+AMF + LF**	**+AMF**
Cedrela montana	Fisher-LSD	a	a	a	a	a	a	a	a	a	a
	Tukey's HSD	a	a	a	a	a	a	a	a	a	a
Heliocarpus americanus	Fisher-LSD	b	a	ab	ab	ab	b	a	ab	ab	ab
	Tukey's HSD	a	a	a	a	a	a	a	a	a	a
Tabebuia chrysantha	Fisher-LSD	b	a	ab	a	a	b	ab	ab	a	a
SHADED	Tukey's HSD	b	ab	ab	a	a	b	ab	ab	ab	a
Tabebuia chrysantha	Fisher-LSD	c	ab	bc	a	a	b	a	ab	a	a
UNSHADED	Tukey's HSD	b	ab	ab	a	ab	b	ab	ab	a	a

Table A5: Statistical results of the mortality rates of all tree seedlings on the reforestation plots in the Nursery experiment No. 3. The mortality rate was tested for statistical significances via the Fisher-LSD and the Tukey's HSD test. Numbers of given parameters used for analysis are given in brackets. The treatments are marked as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only.



UNIVERSIDAD NACIONAL DE LOJA

AREA AGROPECUARIA Y DE RECURSOS NATURALES RENOVABLES

LABORATORIO DE ANALISIS FISICO QUIMICO DE SUELOS, AGUAS Y BROMATOLOGIA

Loja loja

Cantón: Loja **Fecha de I:** 28/03/2008 **Fecha de E:** 31/03/2008

Sector: Vivero Forestal. UNL **Responsable:** Ing. Narcisa Urgiles

		Análisis Mec. % TFSA			Textura	pH	CE	M.O.	Elem. Disponibles			CIC	Bases Cambiables				Acidez Cam		Micronutrientes				
LAB.	CAMP.	Arena	Limo	Arcilla		Agua	dS/m	%	ug/ml				meq/100ml				meq/100g		ug/ml o ppm				
							25°C		N	P_2O_5	K_2O		Ca++	Mg++	Na+	K+	Al	Al+H	Fe	Zn	Mn	Cu	B
232	snd					4,64		8,12	101,50	18,21	49,2		3,87	0,80		0,16							
233	sd					4,38		6,27	78,38	13,15	41,0		4,93	0,90		0,25							

		pH	CE	Elem. Disponibles			M.O		Bases Cambiables				Acidez cambiable			Micronutrientes						
CAMP.		Agua	dS/m	ug/ml			%	CIC	meq/100ml				meq/100ml			ug/ml o ppm						
			25°C	N	P2O5	K2O			Ca++	Mg++	Na+	K+	Al+H	Al	H	Fe	Zn	Mn	Cu	B		
232	snd	Muy Acido		Muy Alto	Medio	Muy Bajo	Alto		Alto	Alto		Bajo										
233	sd	Muy Acido		Alto	Medio	Muy Bajo	Alto		Alto	Alto		Medio										

Ing. Marconi Mora Erraez
TECNICO LABORATORISTA

Fig. A2: Soil analysis of the standard nursery substrate used in the Nursery experiment No. 3. Analysis of pH, organic matter (M.O.), available elements and exchangeable bases of the standard nursery substrate before (sample No. 232) and after steam sterilizing (233) are shown in the upper part. The lower part includes the interpretations of the laboratory for the nutrient levels – high (alto), middle (medio) or low (bajo).

Part	Time point	Treatment	K	Ca	Mg	Al	Cu	Fe	Mn	Na	P	Zn	B	S	N [%]	S [%]	H [%]	C [%]
Leaves	1	control	15570	5847	2223	1146	9.15	390.25	111.17	277.16	1347	48.93	22.93	1685	1.90	0.03	7.43	43.85
Leaves	1	HF	17315	7459	2822	610	9.52	285.35	225.95	247.93	2322	56.59	23.30	2586	3.35	0.08	7.57	43.34
Leaves	1	-AMF + LF	19194	5089	2836	850	6.73	316.40	162.33	188.08	1734	54.67	22.92	1941	2.69	0.06	7.78	43.20
Leaves	1	+AMF + LF	16723	7624	3294	829	7.58	331.32	198.42	226.32	2191	68.67	27.83	2220	3.03	0.06	7.74	44.97
Leaves	1	+AMF	15395	7207	2735	571	6.59	237.15	132.13	186.60	1816	43.77	24.55	1849	2.32	0.04	7.52	44.15
Leaves	2	control	21657	8746	3133	629	11.46	295.84	167.07	130.50	2179	61.89	28.98	2766	2.35	0.09	7.45	45.38
Leaves	2	HF	24897	8462	3738	769	9.48	358.64	253.27	132.31	3035	66.33	33.72	3050	3.35	0.11	7.49	44.84
Leaves	2	-AMF + LF	16612	9607	3231	757	8.49	441.25	240.68	141.06	2275	47.74	29.25	2405	2.06	0.18	6.68	40.87
Leaves	2	+AMF + LF	17757	9425	3102	789	5.72	336.30	168.83	143.43	1877	46.87	29.00	2027	2.14	0.07	7.54	45.10
Leaves	2	+AMF	15977	8004	2745	388	9.16	195.72	99.47	106.50	2452	46.98	24.92	2089	1.83	0.05	7.48	45.25
Leaves	3	control	17410	8018	2225	793	9.29	471.60	95.43	70.98	2929	308.90	19.12	3385	2.88			44.63
Leaves	3	HF	19690	7617	1791	733	6.48	427.20	106.30	57.50	2227	162.70	29.25	1974	2.31			44.87
Leaves	3	-AMF + LF	19980	5682	1646	792	7.74	472.80	109.40	69.15	2453	81.56	30.14	2297	2.13	n.d.	n.d.	44.72
Leaves	3	+AMF + LF	17640	5589	1991	746	7.73	576.40	111.80	51.27	2125	91.21	22.79	1655	2.22			45.35
Leaves	3	+AMF	18480	6619	2543	693	8.71	431.10	95.47	194.60	3375	135.20	23.15	2753	2.55			44.29
Leaves	4	control	20510	9371	3829	1142	13.13	513.50	81.62		2555	213.80	37.80	2229	2.20			45.15
Leaves	4	HF	25080	5920	1983	1038	8.93	516.80	71.17		2548	108.60	49.39	1539	1.82			45.52
Leaves	4	-AMF + LF	23420	7977	4926	532	12.65	381.60	64.31	n.d.	4092	157.60	32.40	2918	2.79	n.d.	n.d.	46.27
Leaves	4	+AMF + LF	18570	8497	3677	1184	9.63	723.10	88.49		2239	135.90	33.45	2782	2.19			44.92
Leaves	4	+AMF	21250	8100	4095	662	11.26	419.70	93.90		3069	259.00	34.86	2385	2.50			45.92
Roots	1	control	17016	3619	1825	20608	13.64	6179.02	1124.04	393.74	1141	150.48	12.98	1324	1.58	0.02	5.60	33.84
Roots	1	HF	17425	3917	2438	24219	12.81	7738.28	1519.62	303.63	1672	155.65	13.52	1858	1.90	0.05	5.08	30.09
Roots	1	-AMF + LF	19660	3491	2038	20934	13.41	6252.49	1751.42	287.62	1284	143.12	13.45	1867	1.95	0.04	5.65	33.91
Roots	1	+AMF + LF	18527	3313	1987	19557	9.44	6072.47	1531.48	268.70	1501	205.82	12.52	1908	1.83	0.05	5.73	34.53
Roots	1	+AMF	20729	3265	1784	16368	10.30	4820.75	1401.16	319.59	1595	150.56	11.95	2001	1.82	0.06	6.02	36.26
Roots	2	control	22132	3812	2749	15250	26.79	4048.78	2667.42	415.95	1551	377.81	33.66	3134	1.63	0.13	6.66	41.14
Roots	2	HF	21725	3419	3142	12820	30.27	3359.16	4065.55	449.13	2455	316.59	35.38	4091	2.40	0.20	6.87	41.58
Roots	2	-AMF + LF	20827	3785	3269	15482	22.38	4044.84	3990.37	410.85	2204	395.48	31.59	3478	2.48	0.09	7.58	45.54
Roots	2	+AMF + LF	21046	3602	3534	14124	26.04	4127.96	3137.02	324.96	1935	354.31	27.75	3005	1.71	0.13	6.61	40.87
Roots	2	+AMF	18758	4033	3338	19043	19.01	5180.03	2366.02	422.61	2091	344.75	36.06	3183	1.72	0.15	6.39	39.09

Table A6: Detailed nutrient analysis of *Cedrela montana* in the Nursery experiment No. 3. Amounts of nutrients are given in µg/g, if not stated otherwise. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. **n.d.:** no data. Values marked in dark gray increased from sampling point 1 to 2 (nursery phase) and 3 to 4 (reforestation phase), values in light gray stayed at equal or at a similar level.

Part	Time point	Treatment	K	Ca	Mg	Al	Cu	Fe	Mn	Na	P	Zn	B	S	N [%]	S [%]	H [%]	C [%]
Leaves	1	control	23211	11941	5934	642	12.14	318.51	1629.50	48.16	1843	154.19	25.94	2000	3.07	0.07	7.41	43.33
Leaves	1	HF	22801	15007	7063	521	5.94	301.58	1800.29	34.22	2092	98.87	29.43	2244	4.03	0.07	7.41	43.27
Leaves	1	-AMF + LF	20830	3626	5854	10736	20.39	4916.77	233.65	1544.65	1662	63.47	10.01	3645	3.56	0.10	7.54	43.67
Leaves	1	+AMF + LF	19196	11053	7135	494	18.34	246.51	1006.24	44.64	2408	117.68	18.06	1634	2.11	0.05	7.63	42.60
Leaves	1	+AMF	21580	12895	7629	487	15.54	270.73	920.26	43.86	2506	79.45	20.31	2363	3.40	0.09	7.57	43.08
Leaves	2	control	18270	11140	5551	404	8.51	222.80	1693.00	69.39	1866	144.50	63.37	1378	2.29			40.81
Leaves	2	HF	13850	11430	5042	399	4.38	269.50	1577.00	189.10	1503	77.90	45.58	1748	3.13			41.63
Leaves	2	-AMF + LF	13900	9913	4906	312	4.84	195.40	1351.00	48.78	1509	64.15	42.35	1588	2.88	n.d.	n.d.	42.03
Leaves	2	+AMF + LF	15610	10330	5551	413	7.02	252.80	1193.00	69.55	1845	67.61	41.69	1554	2.58			41.30
Leaves	2	+AMF	13320	13360	8017	563	8.03	302.80	1799.00	76.60	1669	117.50	53.09	1072	1.89			39.85
Leaves	3	control	14660	10020	3680	879	7.26	519.80	897.40	570.40	1294	451.50	74.45	1010	1.69			41.64
Leaves	3	HF	18810	6010	3069	595	12.00	422.00	496.80	42.37	1728	385.50	30.15	1371	2.33			44.15
Leaves	3	-AMF + LF	17790	8291	3375	663	8.02	407.80	829.50	50.34	1645	258.10	59.86	1120	1.99	n.d.	n.d.	44.00
Leaves	3	+AMF + LF	25890	7454	3741	526	14.45	335.30	303.70	64.87	2282	311.70	31.68	1490	2.40			43.29
Leaves	3	+AMF	16600	5523	3668	806	10.77	484.20	581.20	454.30	1949	311.60	27.19	1241	2.27			42.93
Leaves	4	control	20040	13720	7166	945	18.11	741.80	896.40		1892	263.70	89.77	1451	1.86			45.59
Leaves	4	HF	22820	11240	6730	352	22.58	264.90	288.30		3706	315.20	48.95	1875	2.17			46.64
Leaves	4	-AMF + LF	21030	14260	6206	942	19.10	565.80	1583.00	n.d.	2948	249.30	70.41	1755	1.94	n.d.	n.d.	45.03
Leaves	4	+AMF + LF	24140	11870	8672	341	22.47	249.60	309.40		3316	220.20	74.57	1822	2.12			46.47
Leaves	4	+AMF	25570	12240	8258	753	25.59	582.90	1671.00		3696	144.70	115.60	2554	3.25			46.48
Roots	1	control	19530	3588	4806	15633	16.77	5685.52	254.56	2702.96	1669	75.26	10.51	2995	1.95	0.12	6.57	38.63
Roots	1	HF	28216	4134	7017	15095	12.84	7304.50	286.01	1411.93	1449	72.11	11.47	3443	2.44	0.16	6.16	36.35
Roots	1	-AMF + LF	23470	13669	6421	497	11.71	295.51	1486.92	254.78	2331	99.18	26.36	2445	1.91	0.18	6.91	40.26
Roots	1	+AMF + LF	15470	3940	6921	13857	20.56	6964.69	252.82	3097.91	1896	77.04	10.20	4336	1.89	0.23	6.75	39.27
Roots	1	+AMF	19266	3644	7319	13393	42.35	7121.15	251.15	3243.95	2092	71.40	10.18	3822	1.60	0.20	6.74	39.22
Roots	2	control	11430	3106	3904	10740	17.54	5146.00	191.30	1298.00	1266	44.32	45.12	2268	1.40			35.45
Roots	2	HF	10140	3301	4557	10210	10.98	6597.00	196.70	1557.00	1277	35.37	46.19	2733	1.60			35.14
Roots	2	-AMF + LF	10330	2771	3853	6422	10.04	3496.00	162.90	1370.00	1158	29.55	44.96	2704	1.54	n.d.	n.d.	38.42
Roots	2	+AMF + LF	11060	3462	6049	10040	15.70	6227.00	184.80	1937.00	1671	45.95	51.89	3276	1.64			35.84
Roots	2	+AMF	12990	3349	5483	8397	32.34	5100.00	211.00	1568.00	1675	38.01	60.03	2555	1.29			37.95

Table A7: **Detailed nutrient analysis of *Heliocarpus americanus* in the Nursery experiment No. 3.** Amounts of nutrients are given in µg/g, if not stated otherwise. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. **n.d.:** no data. Values marked in dark gray increased from sampling point 1 to 2 (nursery phase) and 3 to 4 (reforestation phase), values in light gray stayed at equal or at a similar level.

Plot	Part	Time point	Treatment	K	Ca	Mg	Al	Cu	Fe	Mn	Na	P	Zn	B	S	N [%]	S [%]	H [%]	C [%]
	Leaves	1	control	12996	9670	3908	1176	8.99	517.86	1152.83	63.71	1437	59.42	19.33	1929	2.61	0.43	6.98	46.38
	Leaves	1	HF	16290	11206	4638	728	7.20	366.24	913.60	81.87	1566	44.03	29.05	2219	2.94	0.18	7.47	45.44
	Leaves	1	-AMF + LF	14801	9596	4616	814	9.43	384.47	721.35	78.70	1539	43.91	19.03	1968	2.73	0.12	7.64	45.63
	Leaves	1	+AMF + LF	13540	9869	4527	749	11.32	401.01	808.88	55.15	1550	46.98	19.37	2029	2.58	0.11	7.69	45.49
	Leaves	1	+AMF	12889	8334	3400	1335	6.99	554.99	957.77	87.90	1461	66.14	17.59	1602	2.32	0.07	7.78	45.76
	Leaves	2	control	6287	11210	3891	807	4.36	508.30	1426.00	45.52	947	26.50	65.91	1351	1.91			44.49
	Leaves	2	HF	11120	8975	3402	294	4.55	192.50	910.30	25.61	1430	17.29	63.05	1610	2.27			44.77
	Leaves	2	-AMF + LF	10040	10500	3520	547	3.91	294.00	938.70	30.62	1493	22.27	73.54	1570	1.98	n.d.	n.d.	44.07
	Leaves	2	+AMF + LF	7241	10120	3519	983	5.64	640.90	918.30	39.52	1596	18.22	60.61	1549	2.12			44.40
	Leaves	2	+AMF	7856	8340	3766	607	6.78	371.80	632.30	53.33	1542	27.34	65.82	1367	2.02			44.31
Shaded	Leaves	3	control	10840	7823	2940	1675	5.64	923.20	624.40	73.67	1676	220.60	17.20	1761	1.66			44.26
Shaded	Leaves	3	HF	10450	9365	2920	1858	6.71	1002.00	1213.00	62.16	1643	582.50	21.82	1329	1.60			43.91
Shaded	Leaves	3	-AMF + LF	9433	8393	2729	1301	5.59	725.60	862.30	51.49	1632	411.90	19.01	1420	1.85	n.d.	n.d.	44.35
Shaded	Leaves	3	+AMF + LF	8498	12680	4106	1750	4.99	940.80	1210.00	51.20	1419	257.30	22.76	1427	1.57			43.41
Shaded	Leaves	3	+AMF	15980	10260	5554	418	7.11	248.90	1198.00	68.16	1881	67.99	41.67	1569	1.61			44.30
Shaded	Leaves	4	control	21440	13240	4340	1748	19.37	1030.00	753.10		1627	356.50	44.52	2202	1.96			45.86
Shaded	Leaves	4	HF	18550	9478	3191	864	9.65	539.50	1328.00		1679	225.30	42.89	1770	1.62			45.17
Shaded	Leaves	4	-AMF + LF	20330	11460	3612	1042	13.03	577.00	1137.00	n.d.	1637	514.00	28.61	2473	2.04	n.d.	n.d.	47.86
Shaded	Leaves	4	+AMF + LF	17910	7874	4167	947	9.67	578.20	1196.00		1555	440.30	37.78	1763	1.99			46.52
Shaded	Leaves	4	+AMF	16980	8411	3376	632	11.93	379.10	665.80		1744	246.00	40.12	2190	1.65			47.15
Unshaded	Leaves	3	control	6767	11870	3544	996	4.64	516.90	981.10	52.48	1164	285.60	38.00	1322	1.35			44.39
Unshaded	Leaves	3	HF	9298	10000	2711	918	5.30	484.40	1245.00	44.23	1386	249.40	41.90	1582	1.49			44.10
Unshaded	Leaves	3	-AMF + LF	8389	10150	3163	1149	5.57	589.70	951.00	58.46	1533	316.20	27.87	1669	1.54	n.d.	n.d.	43.86
Unshaded	Leaves	3	+AMF + LF	8787	9616	2817	1757	4.83	1009.00	982.90	65.96	1445	369.70	35.29	1479	1.32			43.53
Unshaded	Leaves	3	+AMF	8118	11490	4218	1200	6.48	645.80	931.30	73.74	1133	469.80	38.85	1148	1.43			43.63
Unshaded	Leaves	4	control	14900	14280	3599	1064	10.10	621.10	2098.00		1768	465.00	71.85	2915	1.43			47.12
Unshaded	Leaves	4	HF	18450	15720	3067	1149	11.29	701.60	1559.00	n.d.	1814	531.30	81.59	2090	1.54	n.d.	n.d.	46.45
Unshaded	Leaves	4	-AMF + LF	14090	13400	4169	1127	13.30	629.30	1434.00		1912	313.10	77.18	2476	1.69			46.49
Unshaded	Leaves	4	+AMF + LF	15320	7681	3754	791	9.11	507.50	1448.00		1708	429.90	38.86	1859	2.19			47.02

Plot	Part	Time point	Treatment	K	Ca	Mg	Al	Cu	Fe	Mn	Na	P	Zn	B	S	N [%]	S [%]	H [%]	C [%]
Unshaded	Leaves	4	+AMF	16710	9412	3687	418	12.70	278.70	1023.00		1820	302.60	65.51	2899	1.95			48.08
	Roots	1	control	14723	4825	4530	22028	17.58	9038.21	358.72	251.48	1006	156.19	15.41	1228	1.55	0.04	5.96	34.64
	Roots	1	HF	18252	4956	5371	22500	16.10	11717.87	407.09	343.40	1223	69.76	16.68	1831	1.99	0.06	5.60	33.33
	Roots	1	-AMF + LF	14940	5528	6330	22481	25.70	12486.34	373.47	270.32	1339	101.47	16.17	1590	1.83	0.05	5.59	33.05
	Roots	1	+AMF + LF	14949	4575	6024	21009	37.47	11148.01	378.22	263.72	1247	80.42	14.89	1827	1.85	0.05	5.83	34.52
	Roots	1	+AMF	13662	3816	4661	21558	16.41	10722.57	309.23	336.25	887	121.14	14.92	1073	1.56	0.01	5.87	34.26
	Roots	2	control	9138	4227	4744	15090	26.71	9236.00	273.80	254.30	758	88.46	49.60	1424	1.17			34.57
	Roots	2	HF	11130	2834	4188	11020	35.95	6967.00	326.10	247.50	1122	54.31	44.79	1850	1.60			35.62
	Roots	2	-AMF + LF	11280	2637	3660	10930	28.33	6258.00	235.70	567.20	1287	56.03	42.90	1849	1.47	n.d.	n.d.	37.06
	Roots	2	+AMF + LF	10500	3415	5380	16870	51.58	12330.00	298.10	413.20	1503	87.66	55.68	2123	1.51			30.21
	Roots	2	+AMF	8693	2813	4046	13400	46.63	8059.00	223.80	304.20	1330	80.86	77.73	1789	1.48			34.79

Table A8: Detailed nutrient analysis of *Tabebuia chrysantha* in the Nursery experiment No. 3. Amounts of nutrients are given in µg/g, if not stated otherwise. Treatment descriptions are as follows, **control:** control treatment, **HF:** high fertilization, **-AMF + LF:** heat-killed AMF inoculum + low fertilization, **+AMF + LF:** AMF inoculum + low fertilization, **+AMF:** AMF inoculum only. **n.d.:** no data. The reforestation plots of *T. chrysantha* are either shaded or unshaded. Values marked in dark gray increased from sampling point 1 to 2 (nursery phase) and 3 to 4 (reforestation phase), values in light gray stayed at equal or at a similar level.

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
1	3 mo.	T1	4/5/9/16/21	*Acaulospora* sp. uncultured (Ha-N4)	FRK6F-5	8126	58.30%	13938
1	3 mo.	T1	4	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	FTBZE-4	102	0.70%	13938
1	3 mo.	T1	4/9/16/21	*Claroideoglomus* sp.	GDAQJ-9	1766	12.67%	13938
1	3 mo.	T1	9/16/21	*Diversisporales* sp.	F56C1-16	4	0.03%	13938
1	3 mo.	T1	4/9/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	GCC3J-4	2461	17.66%	13938
1	3 mo.	T1	16	*Rhizophagus* sp. 1 uncultured (Cm-N3)	F4UVV-16	1479	10.61%	13938
1	3 mo.	T2	4/5/9/16/21	*Acaulospora* sp. uncultured (Ha-N4)	F0AU3-21	13865	94.92%	14607
1	3 mo.	T2	5/9/21	*Archaeospora* sp. uncultured (Ha-N6)	F90C1-5	55	0.38%	14607
1	3 mo.	T2	5	*Claroideoglomus* sp. Ecuador - sister clade	F0V45-5	486	3.33%	14607
1	3 mo.	T2	9	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	FXLL0-9	9	0.06%	14607
1	3 mo.	T2	5	*Rhizophagus* sp. 1 uncultured (Cm-N3)	F0KOE-5	192	1.31%	14607
1	3 mo.	T3	4/5/9/16/21	*Acaulospora* sp. uncultured (Ha-N4)	F01QE-4	7262	60.16%	12071
1	3 mo.	T3	4/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	F05AP-4	568	4.71%	12071
1	3 mo.	T3	9	*Archaeospora* sp.	GDRZK-9	3	0.02%	12071
1	3 mo.	T3	4/21	*Archaeospora* sp. uncultured (Ha-N2)	FV1PR-4	18	0.15%	12071
1	3 mo.	T3	9/16/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	F00CF-9	1708	14.15%	12071
1	3 mo.	T3	16/21	*Rhizophagus* sp. 1 uncultured (Cm-N3)	F1EHL-21	2507	20.77%	12071
1	3 mo.	T3	16	*Sc. spinossisima*-like	GE425-16	5	0.04%	12071
1	3 mo.	T4	4/5/9	*Acaulospora* sp. uncultured (Cm N1/N3/N5)	F16VR-9	1575	11.98%	13143
1	3 mo.	T4	4/5/9/16/21	*Acaulospora* sp. uncultured (Ha-N4)	F00DT-21	3664	27.88%	13143
1	3 mo.	T4	4/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	F0JY1-21	726	5.52%	13143
1	3 mo.	T4	4	*Archaeospora* sp. uncultured (Ha-N6)	GEZ2F-4	13	0.10%	13143
1	3 mo.	T4	5/9/21	*Archaeosporales* sp.	F1ZCG-9	682	5.19%	13143
1	3 mo.	T4	16	*Diversispora* sp. W5349/Att1449-5	GFPK1-16	33	0.25%	13143
1	3 mo.	T4	4/16	*Gl. macrocarpum*-like	F16GX-16	143	1.09%	13143
1	3 mo.	T4	4/5/9	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	F0D06-4	5521	42.01%	13143
1	3 mo.	T4	5/21	*Rhizophagus* sp. 1 uncultured (Cm-N3)	F05MF-21	786	5.98%	13143
2	6 mo.	T1	4/16	*Acaulospora* sp. uncultured (Ha-N4)	G09YI-4	3362	39.46%	8519
2	6 mo.	T1	5	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	G07JH-5	267	3.13%	8519
2	6 mo.	T1	4	*Gigasporaceae* sp.	G7FVJ-4	4	0.05%	8519
2	6 mo.	T1	4/5/9	*Glomeraceae* sp.	G0PV5-5	3380	39.68%	8519

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
2	6 mo.	T1	4/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HAPMA-21	7	0.08%	8519
2	6 mo.	T1	21	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G1JS3-21	1499	17.60%	8519
2	6 mo.	T2	4/9/16	*Acaulospora* sp. uncultured (Ha-N4)	G0WVE-16	916	9.02%	10150
2	6 mo.	T2	4	*Archaeospora* sp. uncultured (Cm-N3)	G46AC-4	12	0.12%	10150
2	6 mo.	T2	5	*Archaeospora* sp. uncultured (Ha-N6)	G5CFN-5	249	2.45%	10150
2	6 mo.	T2	5	*Claroideoglomeraceae* sp.	G0BW3-5	2072	20.41%	10150
2	6 mo.	T2	5	*Diversispora* sp. W5349/Att1449-5	HDQTB-5	8	0.08%	10150
2	6 mo.	T2	5/21	*Glomeromycota* sp.	HBW2S-21	17	0.17%	10150
2	6 mo.	T2	9	*Glomus* sp.	G0A46-9	846	8.33%	10150
2	6 mo.	T2	4/5	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G2QQ5-4	6030	59.41%	10150
2	6 mo.	T3	4/5/9/16	*Acaulospora* sp. uncultured (N4-Ha)	G5TDB-9	2437	25.12%	9700
2	6 mo.	T3	4	*Ac. tuberculata/scrobiculata*-like	HJM3P-4	11	0.11%	9700
2	6 mo.	T3	5/16/21	*Archaeospora* sp. uncultured (Cm-N3)	HIU1Y-5	7072	72.91%	9700
2	6 mo.	T3	4	*Archaeospora* sp. uncultured (Ha-N6)	G58EQ-4	34	0.35%	9700
2	6 mo.	T3	16	*Claroideoglomus* sp.	HM8K8-16	21	0.22%	9700
2	6 mo.	T3	4	**CONTAMINATION-*Cryptococcus neoformans*-like**	HHXCD-4	13	0.13%	9700
2	6 mo.	T3	21	*Gl. macrocarpum*	HKMO8-21	7	0.07%	9700
2	6 mo.	T3	16	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	G9LWV-16	105	1.08%	9700
2	6 mo.	T4	5/16	*Acaulospora* sp. nov. W5350/Att1450-1-LIKE	EVEUD-5	1775	41.39%	4288
2	6 mo.	T4	4	*Acaulospora* sp. uncultured (Ha-N4)	HNS8U-4	1442	33.63%	4288
2	6 mo.	T4	9/21	*Rhizophagus* sp. 1 uncultured (Cm-N3)	D6GG9-9	1071	24.98%	4288
3	15 mo.	T0	149	*Ac. spinosa* (ex-type)	G53GB-149	554	3.94%	14050
3	15 mo.	T0	149	*Acaulospora* sp. environmental (Po)	GVFFQ-149	21	0.15%	14050
3	15 mo.	T0	6/126/142/235	*Acaulospora* sp. uncultured (Ha-N4)	G0B8I-142	555	3.95%	14050
3	15 mo.	T0	6/142/235	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	GU31T-142	18	0.13%	14050
3	15 mo.	T0	6/126/142/149/235	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	G7NXV-142	695	4.95%	14050
3	15 mo.	T0	6/126	*Archaeospora* sp. uncultured (Cm-N3)	G0EGX-6	55	0.39%	14050
3	15 mo.	T0	142	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	GXNCL-142	3	0.02%	14050
3	15 mo.	T0	126/235	*Claroideoglomus* sp. - sister to etunicatum clade	G4GTB-126	3	0.02%	14050
3	15 mo.	T0	6/149	*Gl. macrocarpum*-like	G1RKY-149	12	0.09%	14050
3	15 mo.	T0	126/142/235	*Glomeraceae* sp.	G0KAK-126	389	2.77%	14050
3	15 mo.	T0	6/126/142/235	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G6RDY-6	11745	83.59%	14050

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
3	15 mo.	T1	39/49	*A. brasiliensis*-like	G4MBG-49	3	0.02%	13617
3	15 mo.	T1	39/49	*A. lacunosa*-like	G1AZV-39	5449	40.02%	13617
3	15 mo.	T1	39/49/60/158/212	*Acaulospora* sp. uncultured (Ha-N4)	G6HIL-158	1344	9.87%	13617
3	15 mo.	T1	39/49/158	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	G1IC6-158	1273	9.35%	13617
3	15 mo.	T1	49/60/212	*Claroideoglomus* sp.	G0IN4-49	695	5.10%	13617
3	15 mo.	T1	49	*Diversispora* sp. W5349/Att1449-5	HBTN9-49	16	0.12%	13617
3	15 mo.	T1	49	*Di. epigaea*-like	HM6XV-49	3	0.02%	13617
3	15 mo.	T1	49/60/158	*Glomeromycota* sp.	G0IKW-49	55	0.40%	13617
3	15 mo.	T1	49	*Glomus* sp.	GZW53-49	5	0.04%	13617
3	15 mo.	T1	158	*Rh. irregularis*	HET0Z-158	477	3.50%	13617
3	15 mo.	T1	49/60/158/212	*Rhizophagus* sp.	G0CQF-49	2073	15.22%	13617
3	15 mo.	T1	49/60/158	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G5TUQ-60	1975	14.50%	13617
3	15 mo.	T1	158	*Rhizophagus* sp. uncultured (Ha-N2)	HBHA9-158	243	1.78%	13617
3	15 mo.	T1	49	*Ce. gilmorei*-like	G0J6W-49	3	0.02%	13617
3	15 mo.	T1	49	*Sc. spinosissima*-like	GZ4HH-49	3	0.02%	13617
3	15 mo.	T2	54/173/233/261	*Ac. brasiliensis/alpina*-like	G5AKW-54	463	2.08%	22270
3	15 mo.	T2	85/173/261	*Ac. lacunosa*-like	GVX6L-173	9	0.04%	22270
3	15 mo.	T2	233/261	*Acaulospora* sp.	G0HA0-233	582	2.61%	22270
3	15 mo.	T2	85/173/233/261	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	HGSIH-261	6949	31.20%	22270
3	15 mo.	T2	233	*Acaulospora* sp. uncultured (Cm N1/N3/N5)	GWK4T-233	3	0.01%	22270
3	15 mo.	T2	54/85/173/233/261	*Acaulospora* sp. uncultured (Ha-N4)	GU9IB-85	5834	26.20%	22270
3	15 mo.	T2	54/261	*Archaeospora* sp. uncultured (Ha-N6)	G2YOQ-261	182	0.82%	22270
3	15 mo.	T2	85	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	HAVP1-85	22	0.10%	22270
3	15 mo.	T2	85/173/261	*Claroideoglomus* sp.	GV9O6-173	3404	15.29%	22270
3	15 mo.	T2	54/85/173/233/261	*Rhizophagus* sp. 1 uncultured (Cm-N3)	HECY1-54	4822	21.65%	22270
3	15 mo.	T3	171	*Ac. brasiliensis*-like	HGUGD-171	13	0.09%	13780
3	15 mo.	T3	159/204/238	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	GZUM4-204	1143	8.29%	13780
3	15 mo.	T3	159/238	*Acaulospora* sp. uncultured (Ha-N4)	G064V-238	2959	21.47%	13780
3	15 mo.	T3	115	*Archaeospora* sp. uncultured (Cm-N3)	G15HI-115	9	0.07%	13780
3	15 mo.	T3	238	*Archaeospora* sp. uncultured Ecuador-like	G29GU-238	213	1.55%	13780
3	15 mo.	T3	171/204	*Claroideoglomus* sp.	G022G-204	1894	13.74%	13780
3	15 mo.	T3	171	*Gigasporaceae* sp.	HFN4L-171	6	0.04%	13780
3	15 mo.	T3	171/238	*Glomeraceae* sp.	GTQGY-238	108	0.78%	13780

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
3	15 mo.	T3	115/238	*Glomeromycota* sp.	G3254-238	81	0.59%	13780
3	15 mo.	T3	159	*Gl. macrocarpum*	HFEPS-159	8	0.06%	13780
3	15 mo.	T3	159	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	G43NT-159	1694	12.29%	13780
3	15 mo.	T3	115/159/171/238	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G00KE-171	5652	41.02%	13780
3	15 mo.	T4	22/64/67/84	*Acaulospora* sp.	GWYZN-22	623	5.42%	11485
3	15 mo.	T4	22/64	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	HNA5Z-64	82	0.71%	11485
3	15 mo.	T4	67/84	*Acaulospora* sp. uncultured (Cm N1/N3/N5)	G7TBY-67	1775	15.45%	11485
3	15 mo.	T4	22/64/84/231	*Acaulospora* sp. uncultured (Ha-N4)	G4EZA-22	317	2.76%	11485
3	15 mo.	T4	64/67	*Ar. trappei*-like W5340/Att1452-6 (Ha-N6); W5337/Att1456-7 (Ha-N2)	G1KXA-64	6	0.05%	11485
3	15 mo.	T4	22/64/67/84/231	*Archaeospora* sp. uncultured (Cm-N3)	G9MQ6-67	61	0.53%	11485
3	15 mo.	T4	22/64/67/84	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	HCJCU-67	3296	28.70%	11485
3	15 mo.	T4	84	*Diversispora* sp. W5349/Att1449-5	GYFUH-84	241	2.10%	11485
3	15 mo.	T4	22/64/67/84	*Glomeraceae* sp.	HGPCU-22	815	7.10%	11485
3	15 mo.	T4	64/84	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HACRZ-64	2731	23.78%	11485
3	15 mo.	T4	64/84	*Rhizophagus* sp.	HBWNN-84	7	0.06%	11485
3	15 mo.	T4	84/231	*Rhizophagus* sp. 1 uncultured (Cm-N3)	G0DEF-231	1531	13.33%	11485
4	20 mo.	T0	126/142/280	*Ac. brasiliensis*-like	IBU36-280	747	6.66%	11223
4	20 mo.	T0	142	*Ac. spinosa* ex-type	H3X7X-142	97	0.86%	11223
4	20 mo.	T0	6/126/235	*Acaulospora* sp. uncultured (Ha-N4)	H056O-235	3397	30.27%	11223
4	20 mo.	T0	280	*Ar. trappei*-like W5337/Att1456-7 (Ha-N2)	IESKE-280	35	0.31%	11223
4	20 mo.	T0	6/235	*Archaeospora* sp. uncultured (Cm-N3)	H1JEC-235	505	4.50%	11223
4	20 mo.	T0	142	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IO31Y-142	32	0.29%	11223
4	20 mo.	T0	280	*Claroideoglomus* sp.	H03N6-280	674	6.01%	11223
4	20 mo.	T0	235/280	*Gigasporaceae* sp.	H9DPN-235	125	1.11%	11223
4	20 mo.	T0	126/142	*Rh. irregularis*	IPG00-142	267	2.38%	11223
4	20 mo.	T0	6/126/142/235/280	*Rhizophagus* sp. 1 uncultured (Cm-N3)	H037Z-280	5344	47.62%	11223
4	20 mo.	T1	49	*Ac. brasiliensis*-like	H610I-49	35	0.47%	7409
4	20 mo.	T1	49	*Acaulospora* lacunosa-like	H07M7-49	760	10.26%	7409
4	20 mo.	T1	212	*Acaulospora* sp. uncultured (N4-Ha)	H98NX-212	456	6.15%	7409
4	20 mo.	T1	60/158	*Archaeospora* sp. uncultured (Ha-N6)	H4YEG-158	32	0.43%	7409
4	20 mo.	T1	212	*Claroideoglomus* luteum-like	H0IGL-212	254	3.43%	7409
4	20 mo.	T1	212/222	*Claroideoglomus* sp.	ILRPK-212	30	0.40%	7409

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
4	20 mo.	T1	49	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	ILW9Z-49	561	7.57%	7409
4	20 mo.	T1	158/212	*Rhizophagus* sp.	H16PQ-212	2394	32.31%	7409
4	20 mo.	T1	158/212	*Rhizophagus* sp. uncultured (Ha-N2)	IC7YG-212	326	4.40%	7409
4	20 mo.	T1	60/158/212/222	*Rhizophagus* sp. 1 uncultured (Cm-N3)	H0LJ5-60	2561	34.57%	7409
4	20 mo.	T2	85/173	*Ac. brasiliensis*	H8JGO-85	109	0.83%	13066
4	20 mo.	T2	85	*Acaulospora* sp.	H2L7Q-85	58	0.44%	13066
4	20 mo.	T2	233	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	IFTRN-233	4	0.03%	13066
4	20 mo.	T2	54/173	*Acaulospora* sp. uncultured (N4-Ha)	H34T4-54	1857	14.21%	13066
4	20 mo.	T2	54/85/173/233	*Archaeospora* sp. uncultured (Cm-N3)	H07I9-54	2339	17.90%	13066
4	20 mo.	T2	54/85/182/233	*Claroideoglomus* sp.	IBAUE-85	134	1.03%	13066
4	20 mo.	T2	233	*Gl. macrocarpum*	H7MG2-233	23	0.18%	13066
4	20 mo.	T2	182	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HZ32H-182	526	4.03%	13066
4	20 mo.	T2	182/233	*Rhizophagus* sp. environmental-like (Po)	HZ56J-233	107	0.82%	13066
4	20 mo.	T2	54/85/173/182/233	*Rhizophagus* sp. 1 uncultured (Cm-N3)	H0901-233	7909	60.53%	13066
4	20 mo.	T3	115/238	*Ac. longula*-like	H9ZFO-115	32	0.16%	20182
4	20 mo.	T3	115/159/204/238	*Acaulospora* sp.	H3U3T-115	1158	5.74%	20182
4	20 mo.	T3	115/159	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	H11WS-159	1398	6.93%	20182
4	20 mo.	T3	204	*Acaulospora* sp. uncultured (N4-Ha)	IRII9-204	1052	5.21%	20182
4	20 mo.	T3	159	*Archaeospora* sp.	II61G-159	12	0.06%	20182
4	20 mo.	T3	115/159	*Archaeospora* sp. uncultured (Cm-N3)	IN7RP-159	19	0.09%	20182
4	20 mo.	T3	115	*Glomus* sp. environmental (Po)	IQC6E-115	7	0.03%	20182
4	20 mo.	T3	204/238	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HZWCK-204	184	0.91%	20182
4	20 mo.	T3	115/159/204/238	*Rhizophagus* sp. 1 uncultured (Cm-N3)	IMCKR-238	15701	77.80%	20182
4	20 mo.	T3	204	*Rhizophagus* sp. uncultured (Ha-N2)	H5IHX-204	619	3.07%	20182
4	20 mo.	T4	67	*Ac. brasiliensis/alpina*-like	IA2AV-67	76	0.54%	14091
4	20 mo.	T4	67/84/231	*Ac. colliculosa*-like	IMEO5-67	251	1.78%	14091
4	20 mo.	T4	22/64/84/231	*Acaulospora* sp.	IBHO9-64	3997	28.37%	14091
4	20 mo.	T4	64/67/231	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	H8Z1H-231	1007	7.15%	14091
4	20 mo.	T4	64/84/231	*Acaulospora* sp. uncultured (N4-Ha)	H2S5Q-84	702	4.98%	14091
4	20 mo.	T4	84/231	*Archaeospora* sp. uncultured (Cm-N3)	IHSTO-84	22	0.16%	14091
4	20 mo.	T4	67/84	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IEF4W-67	254	1.80%	14091
4	20 mo.	T4	67/84	*Claroideoglomus* sp.	IHAO0-84	64	0.45%	14091

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq.	Percent age	Total reads*
4	20 mo.	T4	22/64/67/84/231	*Diversisporales* sp.	IG5JC-22	139	0.99%	14091
4	20 mo.	T4	64	*Funneliformis* sp.	IFVZ0-64	494	3.51%	14091
4	20 mo.	T4	22/64/67/84/231	*Glomeraceae* sp.	H49JN-22	466	3.31%	14091
4	20 mo.	T4	22/64/67/84/231	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	H7D09-231	1815	12.88%	14091
4	20 mo.	T4	84	*Rh. irregularis*-like	IDASW-84	39	0.28%	14091
4	20 mo.	T4	231	*Rhizophagus* sp. environmental (Po)	IF26V-231	8	0.06%	14091
4	20 mo.	T4	64/67/84/231	*Rhizophagus* sp. 1 uncultured (Cm-N3)	ID48A-67	4757	33.76%	14091

Table A9: 454 sequence reads of *Cedrela montana* in the nursery and field phase. *: Sequence reads occurring only once (singletons) or twice (doubletons) were excluded from the analysis. The 454 sequences are listed after the related AMF species, with the according sample point, time, treatment, number of replicate or plot, number of sequence reads, percentage and total reads. The Ecuadorian AMF cultures used for inoculum are marked in dark gray, the uncultured Ecuadorian sequences achieved from the Nursery experiment No. 1 (Urgiles *et al.* 2009) in light gray, environmental sequences from *Podocarpus oleifolius* in medium gray and contaminations were written in bold. 454 reads alike to former sequences from Ecuadorian material were marked with the according tree species and/or sample code, Cm: *Cedrela montana*, Ha: *Heliocarpus americanus*, Tc: *Tabebuia chrysantha*, Po: *Podocarpus oleifolius*. The treatments are as follows **T0:** control, **T1:** high fertilization, **T2:** heat-killed AMF inoculum + low fertilization, **T3:** AMF inoculum + low fertilization and **T4:** AMF inoculum only.

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
1	3 mo.	T0	4/19/21	*Ac. brasiliensis/alpina*-like	IFFN3-4	6	0.14%	4266
1	3 mo.	T0	4/19	*Acaulospora* sp.	IM2UK-4	5	0.12%	4266
1	3 mo.	T0	4/19/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	IRLC4-21	37	0.87%	4266
1	3 mo.	T0	4/19	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	H3QAB-19	4	0.09%	4266
1	3 mo.	T0	4/9/10/19/21	*Acaulospora* sp. uncultured (Ha-N4)	IHEZC-9	2133	50.00%	4266
1	3 mo.	T0	9/21	*Archaeospora* sp.	H99C2-21	8	0.19%	4266
1	3 mo.	T0	21	*Archaeospora* sp. uncultured (N6-Ha)	IHFNC-21	5	0.12%	4266
1	3 mo.	T0	4/19/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IEIMQ-19	42	0.98%	4266
1	3 mo.	T0	4/21	*Gl. macrocarpum*	H0EUV-21	4	0.09%	4266
1	3 mo.	T0	4/19/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	H9EJP-4	96	2.25%	4266
1	3 mo.	T0	4/19/21	*Rh. intraradices*-like	IN4F5-4	3	0.07%	4266
1	3 mo.	T0	19/21	*Rhizophagus* sp.	IQ9C0-21	5	0.12%	4266
1	3 mo.	T0	4/19/21	*Rhizophagus* sp. uncultured (Ha-N2)	IG97K-19	1783	41.80%	4266
1	3 mo.	T0	4/19/21	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IEDCP-19	135	3.16%	4266
1	3 mo.	T1	4	Ac. laevis-like	IANDZ-4	3	0.11%	2669
1	3 mo.	T1	4/19/21	*Acaulospora* sp.	IMOMV-19	22	0.82%	2669
1	3 mo.	T1	4/19/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	H3JLH-4	36	1.35%	2669
1	3 mo.	T1	4/19/21	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	H85TX-19	38	1.42%	2669
1	3 mo.	T1	4/9/10/19/21	*Acaulospora* sp. uncultured (N4-Ha)	IFB77-9	773	28.96%	2669
1	3 mo.	T1	4/10	*Archaeospora* sp.	H5Q7K-10	6	0.22%	2669
1	3 mo.	T1	4/9/10/19/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	H487Z-21	40	1.50%	2669
1	3 mo.	T1	4/19	**CONTAMINATION - *Cryptococcus neoformans*-like**	H9NAK-4	3	0.11%	2669
1	3 mo.	T1	4/19	*Gl. macrocarpum*	H1NIS-4	3	0.11%	2669
1	3 mo.	T1	4/19	*Rh. clarus*-like	H7JNO-4	6	0.22%	2669
1	3 mo.	T1	4/19/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IFBFP-4	85	3.18%	2669
1	3 mo.	T1	4/19/21	*Rh. irregularis*-like	IMRTZ-4	676	25.33%	2669

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
1	3 mo.	T1	4/19/21	*Rhizophagus* sp.	IIQID-21	100	3.75%	2669
1	3 mo.	T1	4/9/10/19/21	*Rhizophagus* sp. uncultured (Ha-N2)	IRMWZ-4	785	29.41%	2669
1	3 mo.	T1	4/19/21	*Rhizophagus* sp. 1 uncultured (N3-Cm)	H5DP5-4	93	3.48%	2669
1	3 mo.	T2	4/9/10/19/21	*Acaulospora* sp. uncultured (Ha-N4)	H0374-4	3837	84.52%	4540
1	3 mo.	T2	10	*Archaeospora* sp.	H3YKR-10	575	12.67%	4540
1	3 mo.	T2	10	*Ar. trappei*-like W5337/Att1456-7; W5340/Att1452-6	H4RWO-10	106	2.33%	4540
1	3 mo.	T2	9/10/19	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IB8I5-10	6	0.13%	4540
1	3 mo.	T2	10/19	*Gl. macrocarpum*	H619Z-19	3	0.07%	4540
1	3 mo.	T2	9/19	*Rh. irregularis*	H0FMF-19	10	0.22%	4540
1	3 mo.	T2	10/19	*Rhizophagus* sp. 1 uncultured (N3-Cm)	H963P-10	3	0.07%	4540
1	3 mo.	T3	10/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	H13AX-21	226	5.91%	3821
1	3 mo.	T3	4/9/10/21	*Acaulospora* sp. uncultured (Ha-N4)	IBKHY-21	446	11.67%	3821
1	3 mo.	T3	9/10	*Archaeospora* sp. uncultured (N6-Ha)	H326H-10	857	22.43%	3821
1	3 mo.	T3	4/9/10/19/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	H1ZRY-9	2292	59.98%	3821
1	3 mo.	T3	4/9/10/21	*Rh. irregularis*	ITU0D-10	7	0.18%	3821
1	3 mo.	T3	9/10/19	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IG47Q-10	5	0.13%	3821
1	3 mo.	T4	9/10/19	*Ac. brasiliensis*-like	H042K-9	19	0.24%	7992
1	3 mo.	T4	9/10/21	*Acaulospora* sp.	IGJJ5-21	1330	16.64%	7992
1	3 mo.	T4	9/19	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	IQ388-9	32	0.40%	7992
1	3 mo.	T4	9	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	H2JAQ-9	3	0.04%	7992
1	3 mo.	T4	9/10/19/21	*Acaulospora* sp. uncultured (Ha-N4)	ICPIL-21	1869	23.39%	7992
1	3 mo.	T4	19/21	*Archaeospora* sp.	IF24T-19	785	9.82%	7992
1	3 mo.	T4	4/9/19/10/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IJVON-10	2429	30.39%	7992
1	3 mo.	T4	10/19	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	H5U6E-19	347	4.34%	7992
1	3 mo.	T4	9	*Gl. macrocarpum*	IQN0K-9	6	0.08%	7992
1	3 mo.	T4	4/9	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IFNFS-4	149	1.86%	7992

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
1	3 mo.	T4	4/9	*Rhizophagus* sp.	IPMMW-9	299	3.74%	7992
1	3 mo.	T4	4/9	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	IF5OE-9	662	8.28%	7992
1	3 mo.	T4	9	*Rhizophagus* sp. 1 uncultured (N3-Cm)	ICF50-9	62	0.78%	7992
2	6 mo.	T0	9	*Acaulospora* sp.	JVSRI-9	6	0.11%	5361
2	6 mo.	T0	4/10	*Acaulospora* sp. environmental (Po)	JSH6J-4	7	0.13%	5361
2	6 mo.	T0	4/9/10	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	JR3VX-9	1330	24.81%	5361
2	6 mo.	T0	4/19/21	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	JF1GV-19	2104	39.25%	5361
2	6 mo.	T0	4/10/21	*Acaulospora* sp. uncultured (N4-Ha)	JMHLN-21	916	17.09%	5361
2	6 mo.	T0	4/9/10	*Archaeospora* sp.	JZ3PR-9	4	0.07%	5361
2	6 mo.	T0	10	*Archaeospora* sp. uncultured (N3-Cm)	JO6O0-10	18	0.34%	5361
2	6 mo.	T0	4/10	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	JUETP-4	26	0.48%	5361
2	6 mo.	T0	4/10	*Gl. macrocarpum*	JHEU1-10	4	0.07%	5361
2	6 mo.	T0	4/10	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	JSAUC-4	48	0.90%	5361
2	6 mo.	T0	4	*Rh. intraradices*-like	JQSG1-4	3	0.06%	5361
2	6 mo.	T0	4/9/10	*Rhizophagus* sp.	JGR16-4	172	3.21%	5361
2	6 mo.	T0	4/10/19	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	JTFKQ-4	681	12.70%	5361
2	6 mo.	T0	4/10/19	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JTPCC-4	42	0.78%	5361
2	6 mo.	T1	9/21	*Ac. brasiliensis*-like	JH6JJ-9	15	0.95%	1583
2	6 mo.	T1	4/9/19/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	JC6QY-9	25	1.58%	1583
2	6 mo.	T1	4/9/10/19/21	*Acaulospora* sp. uncultured (Ha-N4)	JCQGD-4	106	6.70%	1583
2	6 mo.	T1	4/9	*Ambispora* sp.	JYYA9-4	23	1.45%	1583
2	6 mo.	T1	4/9/10/19/21	*Archaeospora* sp. uncultured (Cm-N3&N6)	JR2P5-10	351	22.17%	1583
2	6 mo.	T1	4/9/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	JFTE2-21	14	0.88%	1583
2	6 mo.	T1	9	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	I57IS-9	4	0.25%	1583
2	6 mo.	T1	4/9/19	*Di. epigaea*	I9GBD-4	11	0.69%	1583
2	6 mo.	T1	4/9/21	*Gl. macrocarpum*	JZ0Q4-4	24	1.52%	1583

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
2	6 mo.	T1	9/19/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	I6BKF-9	39	2.46%	1583
2	6 mo.	T1	9/21	*Rh. clarus*	JLUWA-21	5	0.32%	1583
2	6 mo.	T1	4/9/21	*Rh. irregularis*	JS2W0-9	890	56.22%	1583
2	6 mo.	T1	4/9/10/19/21	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JUZOU-9	76	4.80%	1583
2	6 mo.	T2	4	*Acaulospora* brasiliensis-like	JHJB6-4	12	0.21%	5638
2	6 mo.	T2	4/9/19	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	JOI5E-4	19	0.34%	5638
2	6 mo.	T2	4/9/19	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	JBYXR-19	128	2.27%	5638
2	6 mo.	T2	4/9/10/19/21	*Acaulospora* sp. uncultured (Ha-N4)	I5T3T-9	3865	68.55%	5638
2	6 mo.	T2	10/19/21	*Archaeospora* sp. uncultured (N6-Ha)	I4UF8-19	617	10.94%	5638
2	6 mo.	T2	4/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	I9Q0Y-4	16	0.28%	5638
2	6 mo.	T2	4/9	*Glomeraceae* sp.	I5C0G-4	12	0.21%	5638
2	6 mo.	T2	4/9/19	*Gl. macrocarpum*	JFRWY-19	8	0.14%	5638
2	6 mo.	T2	4/19	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	I6RHS-4	63	1.12%	5638
2	6 mo.	T2	4/9/19/21	*Rh. irregularis*	I61DK-4	823	14.60%	5638
2	6 mo.	T2	4/19	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JNYFN-4	75	1.33%	5638
2	6 mo.	T3	9/21	*Ac. brasiliensis*-like	JTA43-9	22	0.40%	5504
2	6 mo.	T3	9/19/21	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	JMWK0-21	34	0.62%	5504
2	6 mo.	T3	9/21	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	JDRNO-21	4	0.07%	5504
2	6 mo.	T3	4/9/19/21	*Acaulospora* sp. uncultured (Ha-N4)	JKB9S-4	315	5.72%	5504
2	6 mo.	T3	4/10/19	*Archaeospora* sp. uncultured (N3-Cm)	I4UHX-4	2054	37.32%	5504
2	6 mo.	T3	4/9/10/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	JWHUA-10	841	15.28%	5504
2	6 mo.	T3	9/19/21	*Gl. macrocarpum*	JCDRI-9	13	0.24%	5504
2	6 mo.	T3	9/10/21	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	JA2A8-10	361	6.56%	5504
2	6 mo.	T3	9/10/21	*Rhizophagus* sp.	JKV6Y-9	372	6.76%	5504
2	6 mo.	T3	9/21	*Rhizophagus* sp. uncultured (Ha-N2)	I44N5-21	1386	25.18%	5504
2	6 mo.	T3	9/19/21	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JVOMR-9	102	1.85%	5504

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
2	6 mo.	T4	19	*Ac. cavernata*-like	I6JGL-19	1305	25.21%	5176
2	6 mo.	T4	9/10/21	*Acaulospora* sp. uncultured (Ha-N4)	I7WW7-10	1250	24.15%	5176
2	6 mo.	T4	19/21	*Archaeospora* sp.	I9F49-19	65	1.26%	5176
2	6 mo.	T4	9/10/21	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	I62E0-21	1212	23.42%	5176
2	6 mo.	T4	4	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	I5L5N-4	171	3.30%	5176
2	6 mo.	T4	4/10	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	I4000-10	1162	22.45%	5176
2	6 mo.	T4	4/10/21	*Rh. irregularis*	JUSNE-21	5	0.10%	5176
2	6 mo.	T4	9	*Rhizophagus* sp.	I8UUG-9	3	0.06%	5176
2	6 mo.	T4	9/19	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JXNOY-19	3	0.06%	5176
3	13 mo.	T0	51/214	*Acaulospora* sp.	HD9WL-51	61	0.56%	10963
3	13 mo.	T0	214	*Archaeospora* sp. uncultured (N6-Ha)	HMG8W-214	15	0.14%	10963
3	13 mo.	T0	80	*Gigaspora* sp.	HM8HJ-80	3	0.03%	10963
3	13 mo.	T0	51/214	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	G7C55-51	310	2.83%	10963
3	13 mo.	T0	51/80/214	*Rh. irregularis*	G01YT-80	36	0.33%	10963
3	13 mo.	T0	51/80/214/236/237	*Rhizophagus* sp.	G054L-214	7079	64.57%	10963
3	13 mo.	T0	51/214/236/237	*Rhizophagus* sp. 1 uncultured (N3-Cm)	G0SD0-236	2943	26.84%	10963
3	13 mo.	T0	51/80/214/237	*Ce. gilmorei*-like	G4KON-80	516	4.71%	10963
3	13 mo.	T1	197	*Acaulospora* sp. uncultured (Ha-N4)	G5137-197	62	0.53%	11788
3	13 mo.	T1	29	*Archaeospora* sp. uncultured (N3-Cm)	HMKRS-29	10	0.08%	11788
3	13 mo.	T1	175	*Archaeospora* sp. uncultured (N6-Ha)	HJFFY-175	28	0.24%	11788
3	13 mo.	T1	175/188/197/259	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	HH9A6-175	68	0.58%	11788
3	13 mo.	T1	197	*Glomus* sp. environmental (Po)	GVJ5A-197	5	0.04%	11788
3	13 mo.	T1	29/188	*Rh. clarus*-like	GWKK8-29	89	0.76%	11788
3	13 mo.	T1	29/175/188/197/259	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	G4Q9I-188	6481	54.98%	11788
3	13 mo.	T1	29/175	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	HO52L-175	442	3.75%	11788
3	13 mo.	T1	29/175/188/197/259	*Rhizophagus* sp. 1 uncultured (N3-Cm)	G1Z7E-197	3323	28.19%	11788

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
3	13 mo.	T1	29/175/188/259	*Ce. pellucida*-like	HHCPN-259	1280	10.86%	11788
3	13 mo.	T2	250	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	HMO0X-250	3	0.02%	19317
3	13 mo.	T2	205/250	*Acaulospora* sp. uncultured (N4-Ha)	GU3I1-250	8	0.04%	19317
3	13 mo.	T2	210	*Ar. schenkii*-like	HMEFY-210	3	0.02%	19317
3	13 mo.	T2	205/210/251	*Archaeospora* sp. uncultured (N2-Ha)	HBYVY-251	164	0.85%	19317
3	13 mo.	T2	131	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	HFV94-131	104	0.54%	19317
3	13 mo.	T2	131/205/250/251	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	GU96Z-131	31	0.16%	19317
3	13 mo.	T2	205/250	*Rh. irregularis*-like	GW3RG-250	52	0.27%	19317
3	13 mo.	T2	131/205/210/250/251	*Rhizophagus* sp.	HA7FU-205	2498	12.93%	19317
3	13 mo.	T2	210	*Rhizophagus* sp. environmental (Po)	HFI5U-210	90	0.47%	19317
3	13 mo.	T2	131/205/250/251	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	HDK2Q-251	1024	5.30%	19317
3	13 mo.	T2	131/205/210/250/251	*Rhizophagus* sp. 1 uncultured (N3-Cm)	GZNM2-250	12313	63.74%	19317
3	13 mo.	T2	131/210/250/251	*Ce. pellucida/gilmorei*-like	G7ASH-251	3027	15.67%	19317
3	13 mo.	T3	77	*Ac. brasiliensis*-like	HC26X-77	71	0.67%	10650
3	13 mo.	T3	77	*Archaeospora* sp. uncultured (N3-Cm)	HPKCE-77	5	0.05%	10650
3	13 mo.	T3	277	*Archaeospora* sp. uncultured (N6-Ha)	G8L5H-277	37	0.35%	10650
3	13 mo.	T3	77/151/186	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	HJBXK-151	212	1.99%	10650
3	13 mo.	T3	77	*Claroideoglomus* sp.	HMD8G-77	9	0.08%	10650
3	13 mo.	T3	77	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	GVMX9-77	13	0.12%	10650
3	13 mo.	T3	151/186	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HCJSA-151	261	2.45%	10650
3	13 mo.	T3	77/186/277/286	*Rh. irregularis*	G05MB-77	460	4.32%	10650
3	13 mo.	T3	77/151/186/277/286	*Rhizophagus* sp. 1 uncultured (N3-Cm)	G1K0B-277	6140	57.65%	10650
3	13 mo.	T3	77/186	*Ce. gilmorei*-like	HK3U7-186	3442	32.32%	10650
3	13 mo.	T4	221/263	*Ac. brasiliensis/alpina*-like	H6Z1M-221	6	0.04%	16562
3	13 mo.	T4	1/221	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	H7264-221	8	0.05%	16562
3	13 mo.	T4	1/31/43/61	*Acaulospora* sp. uncultured (N4-Ha)	G0MQM-1	7	0.04%	16562

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
3	13 mo.	T4	1/31/43/112/221	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	G17AS-1	3220	19.44%	16562
3	13 mo.	T4	1/31/43/61/112	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	G7MO5-112	393	2.37%	16562
3	13 mo.	T4	1/31/43/61/112/221	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	G0GJQ-43	54	0.33%	16562
3	13 mo.	T4	1/31/43/61/112/221	*Rhizophagus* sp. 1 uncultured (N3-Cm)	GWXF0-61	9951	60.08%	16562
3	13 mo.	T4	1/61/112/221	*Ce. pellucida/gilmorei*-like	G3QMU-61	2923	17.65%	16562
4	16 mo.	T0	214/237	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	IU35Q-237	676	6.03%	11214
4	16 mo.	T0	51/80/237	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IPO7J-80	1397	12.46%	11214
4	16 mo.	T0	51/80/214/236/237	*Rhizophagus* sp.	IPCQP-80	3456	30.82%	11214
4	16 mo.	T0	51/237	*Rhizophagus* sp. environmental (Po)	IBWF3-237	24	0.21%	11214
4	16 mo.	T0	80	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	ISP8G-80	28	0.25%	11214
4	16 mo.	T0	51/80/214/236/237	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IF6K5-237	3462	30.87%	11214
4	16 mo.	T0	51/80/214/236/237	*Ce. pellucida/gilmorei*-like	IIY6U-214	2171	19.36%	11214
4	16 mo.	T1	197	*Glomeromycota* sp.	H416Y-197	8	0.06%	13916
4	16 mo.	T1	29/175/188/197/259	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	H0GR7-29	7690	55.26%	13916
4	16 mo.	T1	29/175/259	*Rhizophagus irregularis*	ISSJ7-175	76	0.55%	13916
4	16 mo.	T1	29/175/188/197/259	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IQCXC-259	1525	10.96%	13916
4	16 mo.	T1	29/175/188/197/259	*Ce. gilmorei*-like	INR8N-259	4617	33.18%	13916
4	16 mo.	T2	131/205/210/250	*Glomeromycota* sp.	IMGX6-250	28	0.29%	9598
4	16 mo.	T2	131/205/210/250/251	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IA2UF-131	4129	43.02%	9598
4	16 mo.	T2	131/205/210/251	*Rh. irregularis*	H35H8-131	7	0.07%	9598
4	16 mo.	T2	251	*Rhizophagus* sp. environmental (Po)	IUG8A-251	25	0.26%	9598
4	16 mo.	T2	131/205/210/250/251	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IHDDN-210	4107	42.79%	9598
4	16 mo.	T2	131/205/210/250/251	*Ce. gilmorei*-like	IN1NB-205	1302	13.57%	9598
4	16 mo.	T3	77/186	*Ac. brasiliensis/alpina*-like	H3MIC-77	67	0.53%	12749
4	16 mo.	T3	77	*Acaulospora* sp.	IMRL0-77	78	0.61%	12749
4	16 mo.	T3	151	*Ar. schenkii*-like	H8K2X-151	21	0.16%	12749
4	16 mo.	T3	72	*Archaeospora* sp. uncultured (N6-Ha)	IDJDZ-72	83	0.65%	12749

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
4	16 mo.	T3	151	*Gl. macrocarpum*-like	IOKNI-151	524	4.11%	12749
4	16 mo.	T3	72/77/151/277	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IM7G8-151	2385	18.71%	12749
4	16 mo.	T3	72/77/151/277	*Rhizophagus* sp.	INLLD-72	3286	25.77%	12749
4	16 mo.	T3	72/77	*Rhizophagus* sp. uncultured (Ha-N2/Rh. irregularis-like)	H8HDC-77	553	4.34%	12749
4	16 mo.	T3	72/77/151/186/277	*Rhizophagus* sp. 1 uncultured (N3-Cm)	H1B3M-77	4377	34.33%	12749
4	16 mo.	T3	72/186/277	*Ce. pellucida/gilmorei*-like	H86K7-186	1375	10.79%	12749
4	16 mo.	T4	61	*Ac. brasiliensis*-like	H556I-61	3	0.02%	16831
4	16 mo.	T4	61	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-roots, rhizosphere afforestation)	ISTYN-61	3	0.02%	16831
4	16 mo.	T4	61	*Acaulospora* sp. uncultured (N4-Ha)	H8XY2-61	4	0.02%	16831
4	16 mo.	T4	43/61/112	*Glomeraceae* sp.	IPDCW-61	574	3.41%	16831
4	16 mo.	T4	43/112	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	H27AT-112	576	3.42%	16831
4	16 mo.	T4	61	*Rh. irregularis*	H5GAZ-61	17	0.10%	16831
4	16 mo.	T4	43/61/112	*Rhizophagus* sp.	H3UGF-61	13034	77.44%	16831
4	16 mo.	T4	61	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IS33N-61	3	0.02%	16831
4	16 mo.	T4	43/112	*Ce. gilmorei*-like	IC9IS-43	2617	15.55%	16831

Table A10: 454 sequence reads of *Heliocarpus americanus* in the nursery and field phase. *: Sequence reads occurring only once (singletons) or twice (doubletons) were excluded from the analysis. The 454 sequences are listed after the related AMF species, with the according sample point, time, treatment, number of replicate or plot, number of sequence reads, percentage and total reads. The Ecuadorian AMF cultures used for inoculum are marked in dark gray, the uncultured Ecuadorian sequences achieved from the Nursery experiment No. 1 (Urgiles *et al.* 2009) in light grey, environmental sequences from *Podocarpus oleifolius* in medium grey and contaminations were written in bold. 454 reads alike to former sequences from Ecuadorian material were marked with the according tree species and/or sample code, Cm: *Cedrela montana*, Ha: *Heliocarpus americanus*, Tc: *Tabebuia chrysantha*, Po: *Podocarpus oleifolius*. The treatments are as follows **T0:** control, **T1:** high fertilization, **T2:** heat-killed AMF inoculum + low fertilization, **T3:** AMF inoculum + low fertilization and **T4:** AMF inoculum only.

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
1	3 mo.	T0	15	*Ac. brasiliensis*	GHYD6-15	95	1.58%	6002
1	3 mo.	T0	8/15	*Acaulospora* sp.	F1WYE-15	9	0.15%	6002
1	3 mo.	T0	1/8/14/15	*Acaulospora* sp. uncultured (N4-Ha)	FTVIZ-15	2393	39.87%	6002
1	3 mo.	T0	1/8/20	*Archaeospora* sp. uncultured (N6-Ha)	F0QMQ-20	813	13.55%	6002
1	3 mo.	T0	1/8/14/15/20	*Gl. macrocarpum*	GG6DA-8	2682	44.69%	6002
1	3 mo.	T0	8/20	*Rhizophagus* sp. environmental (Po)	GIU7N-8	7	0.12%	6002
1	3 mo.	T0	8	*De. heterogama*	GJY31-8	3	0.05%	6002
1	3 mo.	T1	1/3	*Ac. brasiliensis*-like	FVVSK-1	35	0.43%	8069
1	3 mo.	T1	15	*Ac. laevis*-like	FR016-15	34	0.42%	8069
1	3 mo.	T1	1/3/8/15/20	*Acaulospora* sp. uncultured (N4-Ha)	F14TF-1	5933	73.53%	8069
1	3 mo.	T1	15/20	*Archaeospora* sp. uncultured (N2-Ha; N3-Cm)	F4OAV-20	14	0.17%	8069
1	3 mo.	T1	1/8/20	*Ar. trappei*-like	FSAV8-8	44	0.55%	8069
1	3 mo.	T1	1/8/15	*Glomeraceae* sp.	FW29H-8	57	0.71%	8069
1	3 mo.	T1	1/8/15/20	*Gl. macrocarpum*	GI98J-8	1952	24.19%	8069
1	3 mo.	T2	1/8/14/15	*Acaulospora* sp. uncultured (N4-Ha)	F88MZ-1	2482	63.09%	3934
1	3 mo.	T2	1	*Ambispora* sp.	F6NM1-1	8	0.20%	3934
1	3 mo.	T2	1/14/15	*Ar. schenkii*-like	FQUWX-14	73	1.86%	3934
1	3 mo.	T2	1	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	GBY5Q-1	6	0.15%	3934
1	3 mo.	T2	1/8/14/15/20	*Gl. macrocarpum*	F0KA0-8	1346	34.21%	3934
1	3 mo.	T2	8/15/20	*Rhizophagus* sp.	FYFP2-15	8	0.20%	3934
1	3 mo.	T2	1/14	*Rhizophagus* sp. 1 uncultured (N3-Cm)	GEVB6-14	7	0.18%	3934
1	3 mo.	T2	1/8	*De. dipapillosa*-like	FQ54Z-1	4	0.10%	3934
1	3 mo.	T3	20	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	GCML1-20	56	0.59%	9466
1	3 mo.	T3	1/8/20	*Acaulospora* sp. uncultured (N4-Ha)	GDS7W-20	3272	34.57%	9466
1	3 mo.	T3	15	*Archaeospora* sp. uncultured (N3-Cm)	F9T7W-15	367	3.88%	9466
1	3 mo.	T3	1/8/14/20	*Archaeospora* sp. uncultured (N6-Ha)	F2A5B-8	18	0.19%	9466

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
1	3 mo.	T3	1/8	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	F2DXN-1	278	2.94%	9466
1	3 mo.	T3	1	*Claroideoglomus* sp.	F9BRP-1	6	0.06%	9466
1	3 mo.	T3	15	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	F4DVV-15	171	1.81%	9466
1	3 mo.	T3	1/8/14/20	*Gl. macrocarpum*	GBVLN-14	2204	23.28%	9466
1	3 mo.	T3	8	*Glomus* sp.	FS3PG-8	6	0.06%	9466
1	3 mo.	T3	15/20	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	FWK19-15	1604	16.94%	9466
1	3 mo.	T3	1/15	*Rhizophagus* sp. environmental (Po)	F0L4Q-15	1406	14.85%	9466
1	3 mo.	T3	1/14	*Rhizophagus* sp. 1 uncultured (N3-Cm)	F3H0C-1	74	0.78%	9466
1	3 mo.	T3	14	*De. heterogama*-like	FV706-14	4	0.04%	9466
1	3 mo.	T4	15	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	FTLGT-15	13	0.25%	5168
1	3 mo.	T4	15/20	*Acaulospora* sp. uncultured (N4-Ha)	FP01H-20	488	9.44%	5168
1	3 mo.	T4	14/15/20	*Ar.schenkii*-like	FZWR2-14	101	1.95%	5168
1	3 mo.	T4	1/8/15	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	F6HAD-15	543	10.51%	5168
1	3 mo.	T4	1/20	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	GF2RK-1	541	10.47%	5168
1	3 mo.	T4	8	*Fu. coronatum*-like	F4CEZ-8	4	0.08%	5168
1	3 mo.	T4	1/15	*Glomeraceae* sp.	F4ZPJ-1	25	0.48%	5168
1	3 mo.	T4	1/8/14/15/20	*Gl. macrocarpum*	GC8X7-20	1764	34.13%	5168
1	3 mo.	T4	1/15	*Rhizophagus* sp.W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	GESGT-15	1662	32.16%	5168
1	3 mo.	T4	1	*Rh. irregularis*	GHUYK-1	17	0.33%	5168
1	3 mo.	T4	1/15/20	*Rhizophagus* sp.	F2OAE-20	10	0.19%	5168
2	6 mo.	T0	1/14/15/20	*Acaulospora* sp. uncultured (N4-Ha)	G6ICR-14	2800	38.62%	7250
2	6 mo.	T0	1/8/15/20	*Archaeospora* sp.	GW4LX-1	697	9.61%	7250
2	6 mo.	T0	1/8/14/15/20	*Glomeraceae* sp.	GWJZD-15	9	0.12%	7250
2	6 mo.	T0	1/8/14/15/20	*Gl. macrocarpum*	HK0R3-20	3744	51.64%	7250
2	6 mo.	T1	1/8	*Ac. brasiliensis*	HO7T1-8	118	2.33%	5062
2	6 mo.	T1	15	*Ac. laevis*-like	HJ0H9-15	24	0.47%	5062

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
2	6 mo.	T1	14	*Acaulospora* sp. uncultured (Cm-N1/N3/N5)	HINSW-14	87	1.72%	5062
2	6 mo.	T1	1/8/14/15	*Acaulospora* sp. uncultured (N4-Ha)	HKCH6-8	904	17.86%	5062
2	6 mo.	T1	1/8/14/15	*Archaeospora* sp. uncultured (N3-Cm)	HLDI6-15	196	3.87%	5062
2	6 mo.	T1	1/8/14/15/20	*Gl. macrocarpum*	G7HLH-14	3682	72.74%	5062
2	6 mo.	T1	1	*Glomus* sp. environmental (Po)	G716R-1	16	0.32%	5062
2	6 mo.	T1	1/8/15	*Rhizophagus* sp.	G29UD-8	35	0.69%	5062
2	6 mo.	T2	1/8/14/15/20	*Acaulospora* sp. uncultured (N4-Ha)	G1QT9-1	418	5.32%	7852
2	6 mo.	T2	1/8/14/15/20	*Archaeospora* sp.	HI4T7-1	1441	18.35%	7852
2	6 mo.	T2	1/8	*Archaeospora* sp. uncultured (N3-Cm)	GWRN4-8	27	0.34%	7852
2	6 mo.	T2	1/8/14/15/20	*Gl. macrocarpum*	G2LCO-8	5937	75.61%	7852
2	6 mo.	T2	8/15	*Glomus* sp.	HAY8E-15	21	0.27%	7852
2	6 mo.	T2	1/15	*Rhizophagus* sp. 1 uncultured (N3-Cm)	GXMBH-15	8	0.10%	7852
2	6 mo.	T3	1/8/15/20	*Acaulospora* sp. uncultured (N4-Ha)	GYNT1-15	1121	18.92%	5924
2	6 mo.	T3	14/15/20	*Archaeospora* sp. uncultured (N3-Cm)	G5EWS-14	240	4.05%	5924
2	6 mo.	T3	1/8/14/20	*Archaeospora* sp. uncultured (N6-Ha)	G3TLX-8	1961	33.10%	5924
2	6 mo.	T3	1/8/14	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	G1A97-14	8	0.14%	5924
2	6 mo.	T3	8/14/15	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	HMYVA-14	39	0.66%	5924
2	6 mo.	T3	1/8/14/15/20	*Gl. macrocarpum*	G0DCN-20	2419	40.83%	5924
2	6 mo.	T3	8/15	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	HHFKO-15	109	1.84%	5924
2	6 mo.	T3	15	*Rhizophagus* sp. environmental (Po)	G01BT-15	4	0.07%	5924
2	6 mo.	T3	1	*Rhizophagus* sp. 1 uncultured (N3-Cm)	HHQKL-1	23	0.39%	5924
2	6 mo.	T4	1/4/20	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	HFM8A-20	439	7.61%	5765
2	6 mo.	T4	1/4/8/20	*Acaulospora* sp. uncultured (N4-Ha)	HH6AN-8	1212	21.02%	5765
2	6 mo.	T4	1/4/8/14	*Ar. trappei*-like W5337/Att1456-7 (Ha-N2); W5340/Att1452-6 (Ha-N6)	G3UER-4	42	0.73%	5765
2	6 mo.	T4	4/8/20	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	HKVC2-20	24	0.42%	5765
2	6 mo.	T4	20	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	GYZ0F-20	168	2.91%	5765

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
2	6 mo.	T4	20	*Di. epigaea*	GZYTJ-20	38	0.66%	5765
2	6 mo.	T4	1/4/8/14/20	*Gl. macrocarpum*	HG15I-14	3776	65.50%	5765
2	6 mo.	T4	1/4/8/14/20	*Glomus* sp.	HCNT0-1	41	0.71%	5765
2	6 mo.	T4	1	*Rh. irregularis*	G6J8F-1	10	0.17%	5765
2	6 mo.	T4	1	*Rhizophagus* sp.	GW2IH-1	9	0.16%	5765
2	6 mo.	T4	1	*Rhizophagus* sp. 1 uncultured (N3-Cm)	G63J9-1	6	0.10%	5765
3	12 mo.	T0	32/134/153/224/242	*Acaulospora* sp.	IEKXW-224	423	35.25%	1200
3	12 mo.	T0	134/153	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	H9CT0-134	8	0.67%	1200
3	12 mo.	T0	32/134/153/224/242	*Acaulospora* sp. uncultured (N4-Ha)	ISCCW-32	202	16.83%	1200
3	12 mo.	T0	32/134/224/242	*Ar. schenkii*-like	ID1N6-242	95	7.92%	1200
3	12 mo.	T0	32/242	*Archaeospora* sp. uncultured (N3-Cm)	IEAR9-32	6	0.50%	1200
3	12 mo.	T0	32/134/153/224/242	*Gl. macrocarpum*	IGX5X-224	384	32.00%	1200
3	12 mo.	T0	32	*Glomus* sp. (FR750291+2)	IEPMN-32	4	0.33%	1200
3	12 mo.	T0	32	*Glomus* sp. environmental (Po)	IINHW-32	5	0.42%	1200
3	12 mo.	T0	32/134/153/242	*Rhizophagus* sp.	H7CY8-32	60	5.00%	1200
3	12 mo.	T0	32	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IGG49-32	13	1.08%	1200
3	12 mo.	T1	19/48	*Ac. colliculosa*-like	ITNJN-19	43	1.76%	2444
3	12 mo.	T1	19/209	*Acaulospora* sp.	H1W8Q-209	718	29.38%	2444
3	12 mo.	T1	19	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	H9WG6-19	42	1.72%	2444
3	12 mo.	T1	19/48/135/190/209	*Acaulospora* sp. uncultured (N4-Ha)	IP26H-209	135	5.52%	2444
3	12 mo.	T1	19/48/135	*Ar. schenkii*-like	IM9LF-48	76	3.11%	2444
3	12 mo.	T1	135	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	INBXW-135	6	0.25%	2444
3	12 mo.	T1	19/48/135/190/209	*Gl. macrocarpum*	IAQR3-48	1150	47.05%	2444
3	12 mo.	T1	19/135	*Glomus* sp. environmental (Po)	I644P-19	11	0.45%	2444
3	12 mo.	T1	48/135	*Rhizophagus* sp.	IRSMV-135	84	3.44%	2444
3	12 mo.	T1	19/48/135/209	*Rhizophagus* sp. 1 uncultured (N3-Cm)	ID1N2-209	179	7.32%	2444
3	12 mo.	T2	139/255	*Acaulospora* sp.	H93ST-255	146	4.70%	3109

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
3	12 mo.	T2	139/217/255	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	IFGKO-217	528	16.98%	3109
3	12 mo.	T2	25/45/139/217	*Acaulospora* sp. uncultured (N4-Ha)	H9YD8-45	149	4.79%	3109
3	12 mo.	T2	45/139/217	*Ar. schenkii*-like	IEMK5-217	19	0.61%	3109
3	12 mo.	T2	45/139	*Archaeospora* sp. uncultured (N3-Cm)	IAS29-139	4	0.13%	3109
3	12 mo.	T2	217	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IJM4J-217	10	0.32%	3109
3	12 mo.	T2	217	*Glomeromycota* sp.	IM52Z-217	12	0.39%	3109
3	12 mo.	T2	45/139/217/255	*Gl. macrocarpum*	H7V3X-217	1173	37.73%	3109
3	12 mo.	T2	25/139/217	*Glomus* sp. environmental (Po)	H9LKZ-139	221	7.11%	3109
3	12 mo.	T2	139/255	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IK81G-139	57	1.83%	3109
3	12 mo.	T2	25/45/139/217/255	*Rhizophagus* sp.	IITJQ-139	565	18.17%	3109
3	12 mo.	T2	45/217	*Rhizophagus* sp. 1 uncultured (N3-Cm)	IHQ5M-45	225	7.24%	3109
3	12 mo.	T3	16/265	*Ac. brasiliensis*-like	I8HOI-265	25	0.60%	4144
3	12 mo.	T3	16	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	IS6O2-16	24	0.58%	4144
3	12 mo.	T3	16/93/130/249/265	*Acaulospora* sp. uncultured (N4-Ha)	I6R11-249	156	3.76%	4144
3	12 mo.	T3	16/93/265	*Archaeospora* sp.	H76S4-93	760	18.34%	4144
3	12 mo.	T3	130	*Archaeospora* sp. uncultured (N3-Cm)	IAAR4-130	10	0.24%	4144
3	12 mo.	T3	16/93/130/249/265	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IROTS-93	852	20.56%	4144
3	12 mo.	T3	16/130/249	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	IPGGD-130	9	0.22%	4144
3	12 mo.	T3	16/93/130/249/265	*Gl. macrocarpum*	II8HM-93	2126	51.30%	4144
3	12 mo.	T3	16/130/249/265	*Glomus* sp.	H878V-16	76	1.83%	4144
3	12 mo.	T3	93/130/249	*Rhizophagus* sp.W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IGL04-249	74	1.79%	4144
3	12 mo.	T3	249/265	*Rhizophagus* sp.	IBL3C-249	32	0.77%	4144
3	12 mo.	T4	195/229/241	*Ac. brasiliensis*-like	H2NUB-195	56	0.78%	7134
3	12 mo.	T4	229/241	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	H95TK-241	11	0.15%	7134
3	12 mo.	T4	62/195/220/229/241	*Acaulospora* sp. uncultured (N4-Ha)	IVXMG-229	630	8.83%	7134
3	12 mo.	T4	241	*Archaeospora* sp. uncultured (N3-Cm)	IV273-241	3	0.04%	7134

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
3	12 mo.	T4	62/195/229/241	*Archaeospora* sp. uncultured (N6-Ha)	ISVOA-241	26	0.36%	7134
3	12 mo.	T4	62/195/229/241	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	IM7ZY-195	102	1.43%	7134
3	12 mo.	T4	195/229/241	*Claroideoglomus* sp.	H8SLF-195	17	0.24%	7134
3	12 mo.	T4	195/241	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	IWF0M-195	341	4.78%	7134
3	12 mo.	T4	62/195/229/241	*Di. epigaea*-like	H845L-229	50	0.70%	7134
3	12 mo.	T4	62/195/220/229	*Glomeraceae* sp.	ID26Q-195	4216	59.10%	7134
3	12 mo.	T4	62/195/220/229/241	*Gl. macrocarpum*	H5RM8-229	736	10.32%	7134
3	12 mo.	T4	62/241	*Glomus* sp. environmental (Po)	H14QC-241	41	0.57%	7134
3	12 mo.	T4	229/241	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	IQ49N-229	49	0.69%	7134
3	12 mo.	T4	62	*Rh. irregularis*-like	ILZVY-62	45	0.63%	7134
3	12 mo.	T4	62/195/241	*Rhizophagus* sp.	H10V3-195	784	10.99%	7134
3	12 mo.	T4	62/229/241	*Rhizophagus* sp. uncultured (N2-Ha)	I9JF7-229	27	0.38%	7134
4	15 mo.	T0	242	*Ac. brasiliensis*-like	JJB57-242	17	1.09%	1565
4	15 mo.	T0	32/134	*Acaulospora* sp.	JJHEH-134	3	0.19%	1565
4	15 mo.	T0	32/134/153/224/242	*Acaulospora* sp. uncultured (N4-Ha)	JF07D-242	104	6.65%	1565
4	15 mo.	T0	134/242	*Archaeospora* sp.	JW3AG-134	933	59.62%	1565
4	15 mo.	T0	32/134/153/224/242	*Glomeraceae* sp.	JAVT9-32	153	9.78%	1565
4	15 mo.	T0	32/134/153/242	*Gl. macrocarpum*	JKXMI-134	300	19.17%	1565
4	15 mo.	T0	32/242	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JIS20-32	55	3.51%	1565
4	15 mo.	T1	19/48/135/209	*Ac. brasiliensis*-like	JLKY7-19	44	5.66%	778
4	15 mo.	T1	209	*Ac. laevis*-like	JDYGE-209	27	3.47%	778
4	15 mo.	T1	19/48/135/190/209	*Acaulospora* sp. uncultured (N4-Ha)	J0XN2-135	226	29.05%	778
4	15 mo.	T1	19/48/135/209	*Archaeospora* sp.	JWMVB-48	11	1.41%	778
4	15 mo.	T1	19/48/209	*Archaeospora* sp. uncultured (N3-Cm)	JVDHJ-48	61	7.84%	778
4	15 mo.	T1	48/209	*Claroideoglomus* sp.	J0G0U-209	10	1.29%	778
4	15 mo.	T1	19/48/135/190/209	*Gl. macrocarpum*	JVNYE-209	369	47.43%	778
4	15 mo.	T1	48/209	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JLNOI-48	30	3.86%	778

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
4	15 mo.	T2	45	*Acaulospora* sp.	JPSBV-45	11	0.50%	2210
4	15 mo.	T2	25/45/139/217	*Acaulospora* sp. uncultured (N4-Ha)	J2I90-45	292	13.21%	2210
4	15 mo.	T2	25/45	*Ar. schenkii*-like	JQCAB-45	9	0.41%	2210
4	15 mo.	T2	45	*Archaeospora* sp. uncultured (N3-Cm)	JZF9M-45	25	1.13%	2210
4	15 mo.	T2	25/45/139/217/255	*Gl. macrocarpum*	JWAZ3-139	954	43.17%	2210
4	15 mo.	T2	45/255	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	JZMAD-255	53	2.40%	2210
4	15 mo.	T2	25/45/139/217/255	*Rhizophagus* sp.	JA91U-255	823	37.24%	2210
4	15 mo.	T2	45/139/217	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JMDKJ-217	43	1.95%	2210
4	15 mo.	T3	16/265	*Ac. brasiliensis*-like	JJ1FD-265	70	3.72%	1880
4	15 mo.	T3	16	*Ac. lacunosa*-like	J0K3O-16	5	0.27%	1880
4	15 mo.	T3	16/93/130/249/265	*Acaulospora* sp. uncultured (N4-Ha)	I9XP3-93	303	16.12%	1880
4	15 mo.	T3	16/93/249/265	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	JLRXX-93	134	7.13%	1880
4	15 mo.	T3	93/249/265	*Claroideoglomus* sp.	JO478-93	174	9.26%	1880
4	15 mo.	T3	249	*Diversispora* sp.W5349/Att1449-5 (Ha-N4)	JL0V7-249	3	0.16%	1880
4	15 mo.	T3	93/265	*Di. epigaea*-like	JD08A-265	5	0.27%	1880
4	15 mo.	T3	16	*Gigaspora* sp.	JX7I5-16	26	1.38%	1880
4	15 mo.	T3	16/93/130/249/265	*Gl. macrocarpum*	JAUBI-16	984	52.34%	1880
4	15 mo.	T3	16/93/130/249/265	*Glomus* sp.	JMD8H-265	72	3.83%	1880
4	15 mo.	T3	93/130/249	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	JMAMI-249	88	4.68%	1880
4	15 mo.	T3	130	*Rh. irregularis*	JK5SM-130	3	0.16%	1880
4	15 mo.	T3	16/130/265	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JL7JP-130	8	0.43%	1880
4	15 mo.	T3	93	*Ce. nodosa*-like	JO2PU-93	5	0.27%	1880
4	15 mo.	T4	195/229/241	*Ac. colliculosa*-like	J1RPC-195	42	6.25%	672
4	15 mo.	T4	241	*Acaulospora* sp.	J03VK-241	8	1.19%	672
4	15 mo.	T4	229	*Acaulospora* sp. nov. W5350/Att1450-1 (Cm-Rhizosphere afforestation)	JHDNN-229	27	4.02%	672
4	15 mo.	T4	62/195/220/229/241	*Acaulospora* sp. uncultured (N4-Ha)	I9WFC-241	104	15.48%	672
4	15 mo.	T4	241	*Ar. schenkii*-like	J27MH-241	5	0.74%	672

Sample point	Time	Treat-ment	Repl./Plot	AMF	Ref seq	No. of seq	Percentage	Total reads*
4	15 mo.	T4	62/195/229/241	*Cl. etunicatum*-like W5333/Att1449-10 (Ha-N4); W5335/Att1451-6 (Cm-N5); W5348/Att1456-11 (Ha-N2)	JOHZA-195	31	4.61%	672
4	15 mo.	T4	195/241	*Diversispora* sp. W5349/Att1449-5 (Ha-N4)	JU2CD-241	8	1.19%	672
4	15 mo.	T4	220/229/241	*Diversispora* sp.	JXJEX-229	7	1.04%	672
4	15 mo.	T4	195/220/229/241	*Glomeraceae* sp.	JHOND-241	108	16.07%	672
4	15 mo.	T4	62/195/220/229/241	*Gl. macrocarpum*	JUVT7-241	187	27.83%	672
4	15 mo.	T4	241	*Glomus* sp. environmental (Po)	JCDZA-241	9	1.34%	672
4	15 mo.	T4	229/241	*Rhizophagus* sp. W5335/Att1451-8 (Cm-N5); W5336/Att1456-1 (Ha-N2)	JCLYP-241	36	5.36%	672
4	15 mo.	T4	62/229/241	*Rhizophagus* sp.	JDJCH-229	59	8.78%	672
4	15 mo.	T4	229/241	*Rhizophagus* sp. 1 uncultured (N3-Cm)	JNRRV-229	41	6.10%	672

Table A11: 454 sequence reads of *Tabebuia chrysantha* in the nursery and field phase. *: Sequence reads occurring only once (singletons) or twice (doubletons) were excluded from the analysis. The 454 sequences are listed after the related AMF species, with the according sample point, time, treatment, number of replicate or plot, number of sequence reads, percentage and total reads. The Ecuadorian AMF cultures used for inoculum are marked in dark gray, the uncultured Ecuadorian sequences achieved from the Nursery experiment No. 1 (Urgiles *et al.* 2009) in light gray, environmental sequences from *Podocarpus oleifolius* in medium gray. 454 reads alike to former sequences from Ecuadorian material were marked with the according tree species and/or sample code, Cm: *Cedrela montana*, Ha: *Heliocarpus americanus*, Tc: *Tabebuia chrysantha*, Po: *Podocarpus oleifolius*. The treatments are as follows **T0:** control, **T1:** high fertilization, **T2:** heat-killed AMF inoculum + low fertilization, **T3:** AMF inoculum + low fertilization and **T4:** AMF inoculum only.

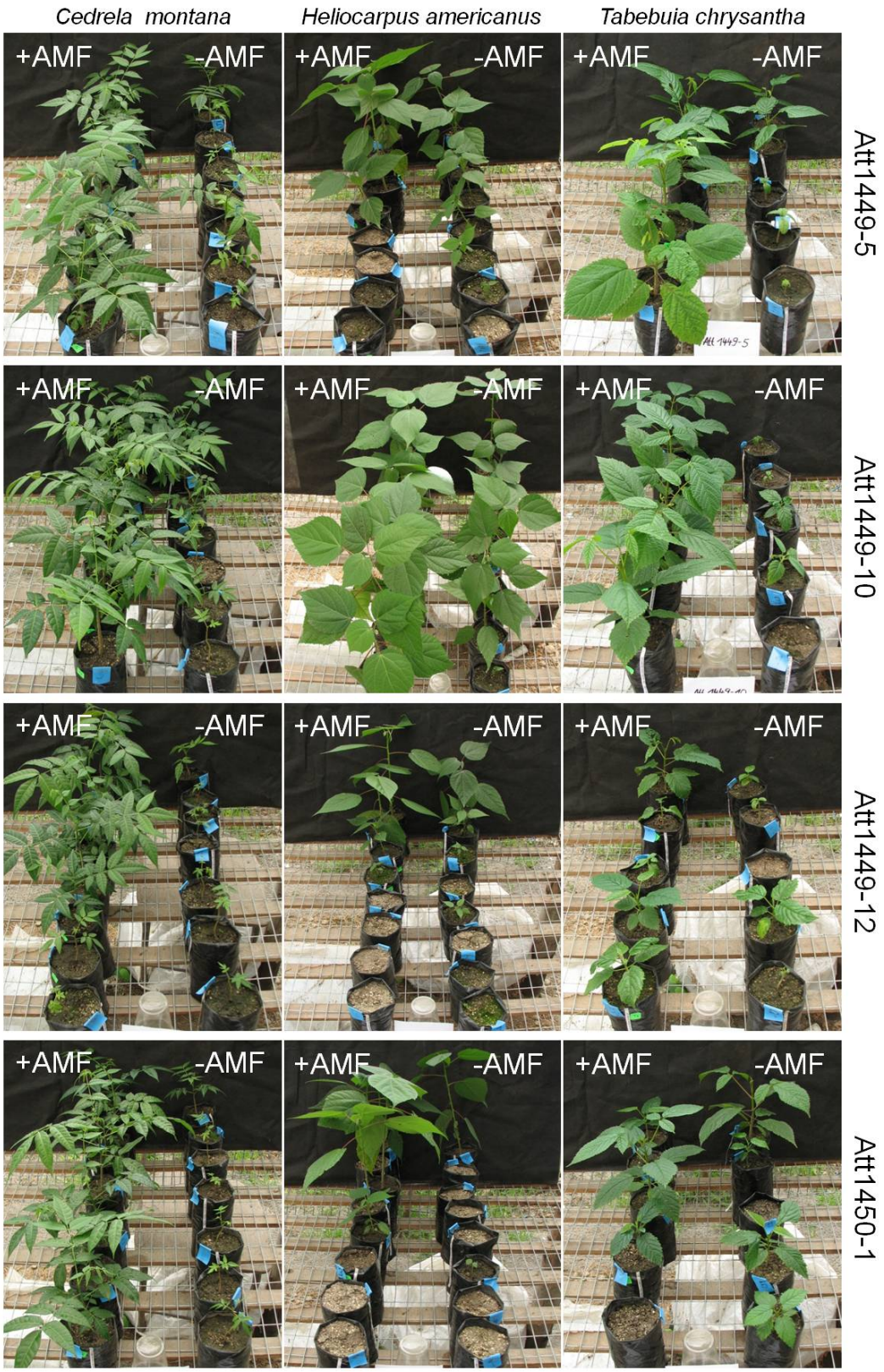


Fig. A3-1: Plant growth performance of tree seedlings in the nursery experiment No4, after the inoculation with individual AMF species (after 5 months). The treatments are labeled as follows +AMF: living AMF, –AMF: heat-killed AMF, both treatments received a low fertilization dose. Att1449-5: *Diversispora* sp., Att1449-10: *Claroideoglomus etunicatum*-like, Att1449-12: *Ambispora* sp., Att1450-1: *Acaulospora* sp. nov.

Fig. A3-2: Plant growth performance of tree seedlings in the nursery experiment No4, after the inoculation with individual AMF species (after 5 months). The treatments are labeled as follows **+AMF:** living AMF, **–AMF:** heat-killed AMF, both treatments received a low fertilization dose. **Att1451-8:** *Rhizophagus* sp., **Att1451-18:** *Claroideoglomus etunicatum*-like*, **Att1456-7:** *Archaeospora trappei*-like. The *Cl. etunicatum*-like multispore culture is marked with ***** because it most likely contains more than one AMF species.

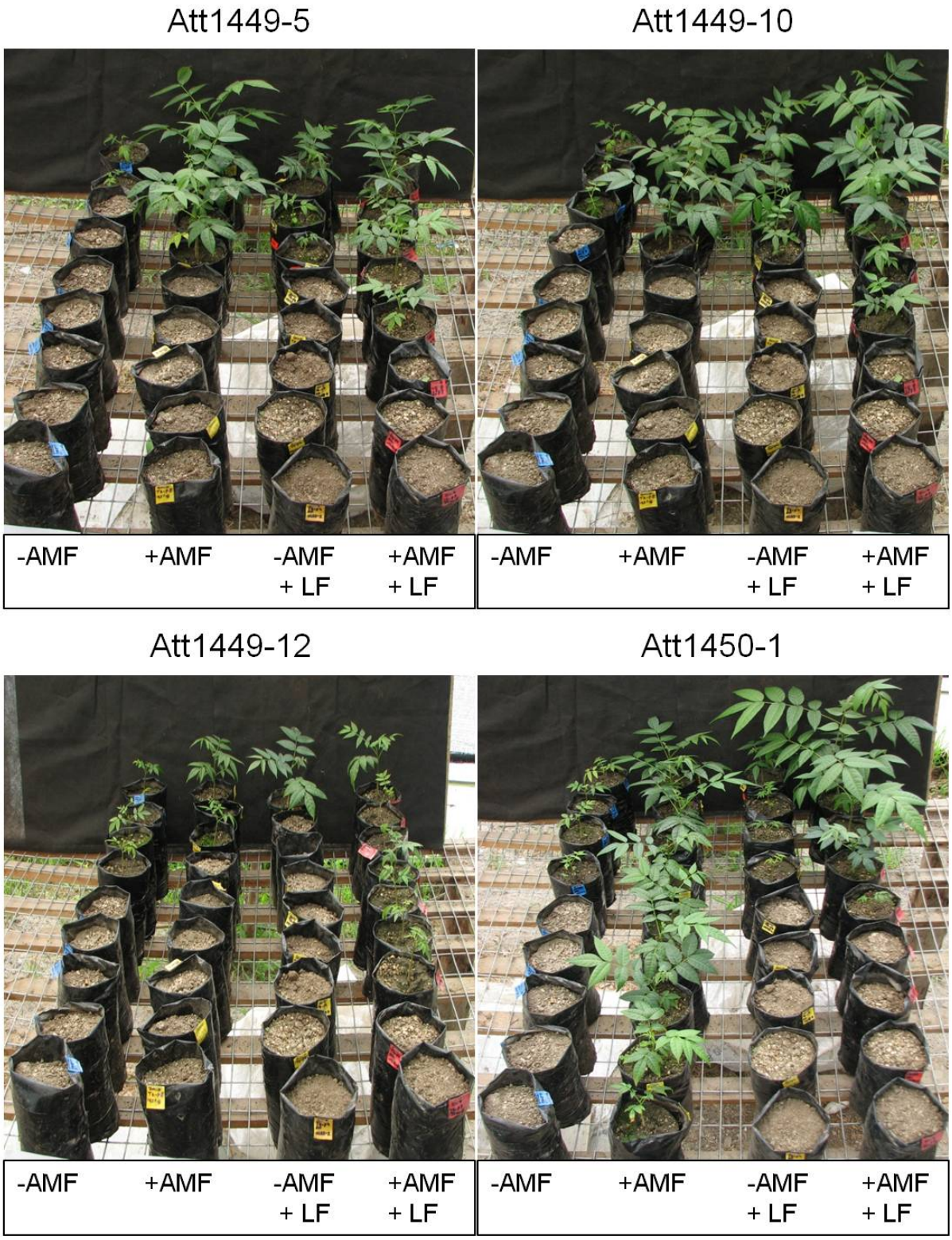


Fig. A4-1: Plant growth performance of *Cedrela montana* in the nursery experiment No4A, after the inoculation with individual AMF species (after 5 months). The treatments are labeled as follows **–AMF:** heat-killed AMF, **+AMF:** living AMF, **-AMF + LF:** heat-killed AMF + low fertilization, **+AMF + LF:** living AMF + low fertilization. **Att1449-5:** *Diversispora* sp., **Att1449-10:** *Claroideoglomus etunicatum*-like, **Att1449-12:** *Ambispora* sp., **Att1450-1:** *Acaulospora* sp. nov.

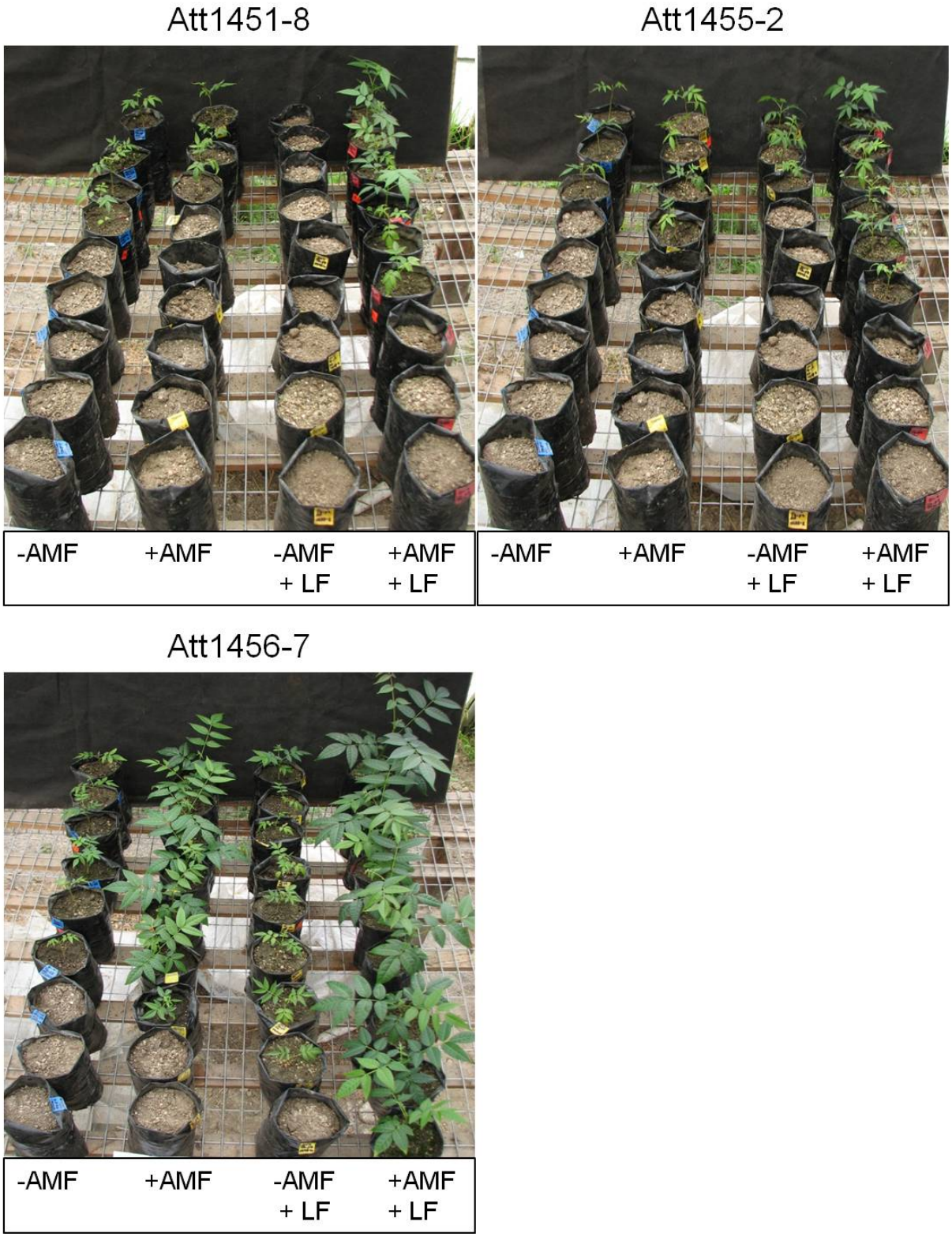


Fig. A4-2: Plant growth performance of *Cedrela montana* in the nursery experiment No4A, after the inoculation with individual AMF species (after 5 months). The treatments are labeled as follows **–AMF:** heat-killed AMF, **+AMF:** living AMF, **-AMF + LF:** heat-killed AMF + low fertilization, **+AMF + LF:** living AMF + low fertilization. **Att1451-8:** *Rhizophagus* sp., **Att1455-2:** *Dentiscutata savannicola*, **Att1456-7:** *Archaeospora trappei*-like.

8.2 Manuals

8.2.1 Amplicon Library Preparation Method Manual - GS FLX Titanium Series

Amplicon Library Preparation Method Manual

GS FLX Titanium Series

October 2009

1. Workflow

The procedure to prepare Amplicon libraries is shown in Figure 1. It consists of a PCR amplification, performed using special Fusion Primers for the Genome Sequencer FLX System (see Sections 2 and 4.1) The method provides for the preparation of just a few or of a large number of Amplicons at a time, in individual PCR tubes or in 96-well plates.

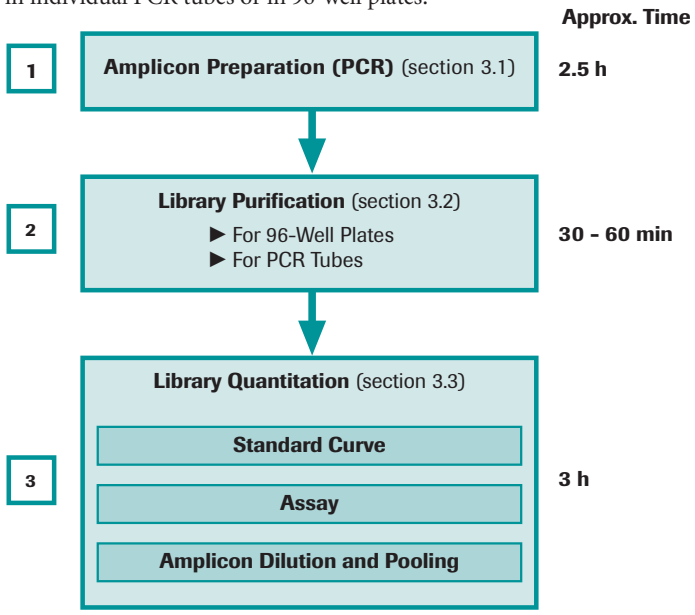


Figure 1: Workflow of the Amplicon library preparation method.

2. Before You Begin

Experimental set up for sequencing an Amplicon library can be complex. See the relevant sections of the *Genome Sequencer System Research Applications Guide* and the *Genome Sequencer System Introduction Manual*. All materials used to create the Amplicons that will constitute the library must be obtained from 3rd party vendors, including the forward (A) and reverse (B) fusion primers.

This procedure requires 5 – 20 ng (genomic DNA) or 1 – 2 ng (plasmid DNA or similar) of starting DNA material, in 1 µl of molecular biology grade water.

PCR optimization: Amplification of any given target sequence may require individual testing and optimization. See Appendix, Section 4.1.

3. Procedure

3.1 Amplicon Preparation (PCR)

1. Prepare the PCR Master Mix. Table 1 gives the volumes for 1, 8 or 96 Amplicons. Make the quantity appropriate for the number of Amplicons included in your experimental design.

Reagent	1 Amplicon		8 Amplicons		96 Amplicons	
Forward Primer (10 µM)	1	µl	8.8	µl	105.6	µl
Reverse Primer (10 µM)	1	µl	8.8	µl	105.6	µl
dNTP mix (10 mM each)	0.5	µl	4.4	µl	52.8	µl
FastStart 10 × Buffer #2	2.5	µl	22	µl	264	µl
FastStart HiFi Polymerase (5 U/µl)	0.25	µl	2.2	µl	26.4	µl
Molecular Biology Grade Water	18.75	µl	165	µl	1980	µl
Total	**24**	**µl**	**211.2**	**µl**	**2534.4**	**µl**

Table 1: Composition of the PCR Master Mix.
The columns for 8 and 96 Amplicons make 10% extra mix; the totals have been rounded.

2. Dilute the DNA sample(s) to the appropriate concentration, in molecular biology grade water.
   a. Genomic DNA: dilute to 5 – 20 ng/µl
   b. Plasmid DNA: dilute to 1 – 2 ng/µl
3. Dispense 24 µl of PCR Master Mix for the number of Amplicons you are preparing. Depending on the number of Amplicons you are preparing, you can do this in PCR tubes or in 96-well PCR plates. (See Appendix, Section 4.2, for recommended plate layout.)
4. To each 24 µl of PCR Master Mix, add 1 µl of a diluted DNA sample.
5. Seal the plate carefully and place it in a thermocycler.
6. Run the PCR program; the conditions below are guidelines only (see Note on PCR Optimization in the Before You Begin section).
   - 1 ×: 94°C, 3 min
   - 25 to 35 ×:
     - 94°C, 15 sec
     - 55 – 65°C, 45 sec
     - 72°C, 1 min
   - 1 ×: 72°C, 8 min
   - 4°C on hold

3.2 Library Purification

3.2.1 For 96-Well Plates

It is recommended to process one plate at a time. See Appendix, Section 4.2, for recommended plate layout.

1. Set a heat block to 40°C.
2. Prepare 25 ml of 70% ethanol, by adding 17.5 ml of 100% ethanol to 7.5 ml of Molecular Biology Grade Water, and vortex.
3. Centrifuge the plate with PCR products (your Amplicons) for **30 sec at 900 × *g***.
4. Pipet **22.5 µl** of molecular biology grade water into each well of a new 96-well, round bottom, polypropylene (PP) plate.
5. Carefully transfer **22.5 µl** of each PCR product from the PCR plate to each well of the PP plate.
6. Vortex the AMPure bead bottle for 20 seconds, or until the beads are completely resuspended.
7. Add **72.0 µl** of AMPure beads to each well and mix thoroughly by pipetting up and down **at least 12 times**, until the mixture is homogeneous.
8. Incubate for **10 min** at room temperature.
9. Place the plate on the 96-well magnetic ring stand and incubate for **5 min** at room temperature, until the supernatant is clear.
10. With the plate still on the magnetic ring stand, carefully remove and discard the supernatant without disturbing the beads.
11. Remove the plate from the magnetic ring stand and add **100 µl** of 70% ethanol (freshly prepared in step 2) to each well.
12. Tap the plate **10 times** to agitate the solution. The pellet may not resuspend completely; this is acceptable.
13. Place the plate on the magnetic ring stand and incubate for **1 min**.
14. With the plate still on the magnetic ring stand, carefully remove and discard the clear supernatant without disturbing the beads.
15. Repeat steps 11– 14. Remove as much of the supernatant as possible.
16. Place the plate and magnetic ring stand together on a heat block set at 40°C until all pellets are completely dry (**10 – 20 min**). Do not leave the plate on the heat block longer than necessary to avoid overdrying.
17. Carefully remove the plate from the heat block, keeping it on the magnetic ring stand to ensure that the pellets are stable during transfer.

▶▶▶

18. Add **20 µl** of 1× TE to each well. Remove from the ring stand. Tap the plate gently until all pellets are resuspended.
19. Place the plate over the magnetic ring stand and move it in a circular motion to dislodge the pellet ring. Tap the plate again until all pellets are dispersed. This ensures efficient elution of the PCR products from the beads.
20. Place the plate on the magnetic ring stand and incubate for **2 min**.
21. Transfer the supernatant from each well into a fresh 96-well PCR plate. It is difficult to avoid any transfer of pellet from some of the wells; this is acceptable.
22. Seal the plate and store at -20°C until ready to proceed to the quantitation step, Section 3.3.

■

3.2.2 For PCR Tubes

1. Set a heat block to 37°C.
2. Prepare 70% ethanol in the amount needed (400 µl per Amplicon). For 10 ml, add 7 ml of 100% ethanol to 3 ml Molecular Biology Grade Water, and vortex.
3. Briefly centrifuge the PCR tubes.
4. Pipet **22.5 µl** of molecular biology grade water into 1.5 ml tubes (one tube per Amplicon).
5. Transfer **22.5 µl** of each PCR product from the PCR tubes to each 1.5 ml tube.
6. Vortex the AMPure bead bottle for 20 seconds, or until the beads are completely resuspended.
7. Add **72.0 µl** of AMPure beads to each tube, and mix thoroughly by vortexing for **5 sec**.
8. Incubate for **10 min** at room temperature.
9. Place the tubes in an Magnetic Particle Collector (MPC) and incubate for **5 min** at room temperature.
10. With the tubes still in the MPC, carefully remove and discard the supernatant without disturbing the beads.
11. Remove the tubes from the MPC and add **200 µl** of 70% ethanol (freshly prepared in step 2) to each tube.
12. Vortex the tubes for **5 sec**. The pellet may not resuspend completely; this is acceptable.
13. Place the tubes on the MPC and incubate **1 min**.
14. With the tubes still on the MPC, carefully remove and discard the supernatant without disturbing the beads.

▶▶▶

15. Repeat steps 11 – 14. Remove as much of the supernatant as possible.
16. Place the open tubes on a heat block set at 37°C until the pellet is completely dry (about **5 min**). Do not leave the tubes on the heat block longer than necessary to avoid overdrying.
17. Remove the tubes from the MPC.
18. Add **10 µl** of 1× TE to each tube. Vortex **5 sec** or until the pellet is completely resuspended.
19. Place the tubes in the MPC and incubate for **2 min** at room temperature.
20. With the tubes still in the MPC, carefully transfer the supernatants to a set of fresh screw cap o-ring 1.5 ml tubes.
21. Store the purified Amplicons individually at -20°C until ready to proceed to the quantitation step, Section 3.3.

3.3 Library Quantitation

Library quantitation is done by fluorometry using the Quant-iT PicoGreen dsDNA Assay Kit.

- It is recommended to carry out the assay in duplicates (both samples and standard curve). The method provides sufficient diluted standards for two standard curves (one plate in duplicate). If you have more than 88 samples to assay, prepare more standards accordingly.
- Be aware that different make/models of fluorometers have different dynamic ranges. Depending on the equipment used, the standard curve may not be linear over the full range of the assay described below. Make sure to use only the linear portion of the curve to assess the concentration of your libraries.

3.3.1 Standard curve

1. Thaw the DNA standard provided with the PicoGreen reagent (100 ng/µl)
2. Label eight 1.5 ml microcentrifuge tubes 1 – 8, and transfer 1× TE into them as follows:
   a. Tube 1: **594 µl**
   b. Tubes 2 – 8: **300 µl**
3. Transfer **6 µl** of DNA standard to Tube 1 (100× dilution: 1 ng/µl) and vortex for **10 sec**.
4. Transfer **300 µl** from Tube 1 to Tube 2 and vortex for **10 sec**.
5. Transfer **300 µl** from Tube 2 to Tube 3 and vortex for **10 sec**.
6. Continue the dilution series by transferring **300 µl** from one tube into the next and vortexing **10 sec**, until **Tube 7**. Tube 8 constitutes the "no DNA control".

▶▶▶

7. Transfer **100 µl** of each DNA standard dilution to the wells of column 12 of two 96-well black fluorometer plates (for duplicate measurements). The amounts of DNA per standard well are as listed in Table 2.

Tube #	Well	DNA Concentration
Tube 1	A12	100.00 ng/well
Tube 2	B12	50.00 ng/well
Tube 3	C12	25.00 ng/well
Tube 4	D12	12.50 ng/well
Tube 5	E12	6.25 ng/well
Tube 6	F12	3.13 ng/well
Tube 7	G12	1.56 ng/well
Tube 8	H12	0.00 ng/well

Table 2: DNA concentration of the 8-point standard curve for the fluorometric assay of Amplicon libraries.

3.3.2 Assay

1. Transfer **99 µl** of 1× TE Buffer to the remaining 88 wells (or as needed) of each of the 96-well black fluorometer plates.
2. Transfer **1.0 µl** of each purified Amplicon DNA sample (from Section 3.2.1 or 3.2.2) to the appropriate wells of the fluorometer plates. Make sure to use a fresh tip for each sample.
3. Mix by pipetting up and down 4 times, using a multichannel pipettor set to 100 µl. Again, make sure to use a fresh tip for each sample.
4. Carry out the assay as described by the manufacturer of the Quant-iT PicoGreen dsDNA Assay Kit, adding 100 µl of a 1:200 dilution of PicoGreen reagent to each well. Mix carefully by pipetting up and down 4 times. Use a fresh tip for each well.
5. Verify that the R^2 value of the standard curve is at least 0.98.
   a. If it is not, check if the top point of the curve is below the curve; your fluorometer may not have a sufficient dynamic range for the top point. If that is the case, eliminate the top point and recalculate.
   b. If the R^2 value is below 0.98 and it is not due to a dynamic range issue, repeat the assay, pipetting carefully. See an example standard curve in Appendix, Section 4.3.
6. Verify that the sample readings fall within the range of the standard curve.
   a. If any sample readings exceed the highest standard curve value, dilute and re-measure these samples, and take the additional dilution factor into account when calculating final concentration.
   b. If any sample readings fall below 5 ng/ul, it is recommended to verify the size and purity of the Amplicon before proceeding. If necessary, repeat the purification or preparation of these Amplicons.

3.3.3 Amplicon Dilution and Pooling

1. Calculate the concentration of each Amplicon in molecules/µl, using the following equation:

$$\text{Molecules/µl} = \frac{\text{sample conc [ng/µl]} \times 6.022 \times 10^{23}}{656.6 \times 10^{9} \times \text{amplicon length [bp]}}$$

2. Dilute each Amplicon (separately) to 1×10^9 molecules/µl, in 1× TE Buffer. This is easily done by adding 1 µl of each Amplicon sample in the volume of TE calculated as follows:

$$\left(\frac{\text{molecules/µl (from step 1)}}{10^9} - 1 \right) \text{µl}$$

3. If multiple Amplicons are to be sequenced together, *i.e.* within a region of a PicoTiterPlate device (which is typical), mix an equal volume (*e.g.* 10 µl) of each of these diluted Amplicons to prepare Amplicon pools.

4. Dilute each Amplicon pool to 10^7 molecules/µl by adding 2 µl of the Amplicon pool from step 3 to 198 µl Molecular Biology Grade Water. Store the 1×10^9 molecules/µl stock and the diluted aliquots at -20°C.

4. Appendix

4.1 PCR / Primers Optimization

Amplification of any given target sequence may require individual testing and optimization. Addition of the adaptors and MIDs defined in the GS FLX Titanium chemistry may introduce additional possibilities for primer duplex and hairpin formation, and it is recommended to test these both in a prediction algorithm such as that hosted by Integrated DNA Technologies (IDT; see at **http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/**) and by experimentation.

The PCR conditions given in this method are a suggested starting point but will not be optimal for every Amplicon. The method uses the Roche FastStart High Fidelity Polymerase, which in our hands has routinely provided robust amplification of fragments in the 200 – 600 bp size range from a variety of targets with variable %GC content. Be sure to include appropriate positive and negative controls in your optimization tests.

The optimal annealing temperature can be predicted from the melting temperature of the gene-specific part of the fusion primers for each target sequence, but, again, empirical optimization may be necessary.

4.2 PCR Plate Layout

It is convenient to use only columns 1 – 11 of a 96-well plate when preparing and purifying your Amplicons, for a total of 88 Amplicons per plate. This way, you can use the same plate layout for the quantitation assay (where column 12 is used for the standard curve) as for the amplification and the library purification, minimizing the risk of confusion in the identity of the Amplicons.

4.3 Example Library Quantitation Standard Curve

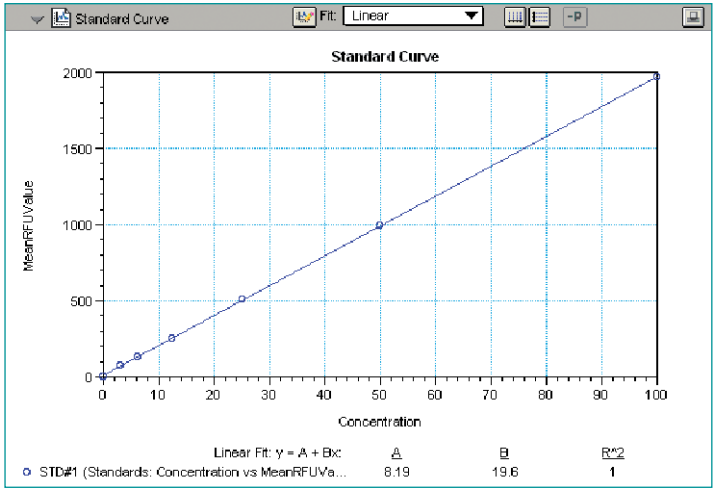


Figure 2

Table of Contents

1. Workflow 2

2. Before You Begin 2

3. Procedure 2

3.1 Amplicon Preparation (PCR) 2
3.2 Library Purification 3
3.2.1 For 96-Well Plates 3
3.2.2 For PCR Tubes 3
3.3 Library Quantitation 4
3.3.1 Standard curve 4
3.3.2 Assay 4
3.3.3 Amplicon Dilution and Pooling 5

4. Appendix 5

4.1 PCR/Primers Optimization 5
4.2 PCR Plate Layout 5
4.3 Example Library Quantitation Standard Curve 5

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8.2.2 TCB No. 005-2009 – Using Multiplex Identifer (MID) Adaptors for the GS FLX Titanium Chemistry – Extended MID Set

TCB No. 005-2009 April 2009

Technical Bulletin

Genome Sequencer FLX System

Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Extended MID Set

Summary

This bulletin describes the use of a set of up to 151 Multiplex Identifiers (MID) with the GS FLX Titanium chemistry. This document is intended to be an extension of Technical Bulletin 09004: *Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set*. Libraries prepared with these Adaptors may be multiplexed in emulsion PCR (emPCR) in order to enable sample identification following sequencing on the Genome Sequencer FLX Instrument. This bulletin enables much deeper multiplexing for users who wish to employ the 10-base barcode strategy provided by the MID Adaptors.

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Note: This bulletin assumes that the user is familiar with the contents of *TCB-09004: Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set.* Please consult this document for specific instructions regarding the preparation of MID Adaptors as well as for the handling of libraries made with the same.

Introduction

The information contained in this document is provided to enable users of the GS FLX Titanium sequencing chemistry to employ Multiplex Identifier (MID)-containing adaptors for General (*e.g.* Shotgun) library preparation. Please note that the *GS FLX Titanium General Library Preparation Method Manual* and the GS FLX Titanium General Library Preparation Kit can be used to prepare a library without reference to the information contained in this bulletin. If you are preparing standard, non-MID libraries, you do not need this document.

This document describes the preparation and use of up 151 Multiplex Identifier (MID) Adaptors. This extends the Basic MID Set of ten Adaptors for users requiring greater multiplexing capabilities with the GS FLX Titanium sequencing chemistry. These MID Adaptors may be used as a replacement for the Adaptors provided in the GS FLX Titanium General Library Preparation Kit. These Adaptors include a 10-nucleotide sequence tag on Adaptor A which is unique for each MID. When different libraries are prepared with different MIDs, they can be amplified by emPCR and sequenced together, in a multiplex fashion; the sequencing reads can be deconvoluted by the data analysis software after the sequencing Run, such that the reads from each of the pooled libraries are identified by their MID tag and correctly assigned.

Extended Multiplex Identifier Set Design

A robust set of ten decamer Multiplex Identifier (MID) sequences (Basic MID Set, described previously in *TCB-09004: Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set.*) was designed to facilitate library multiplexing in the 454 Sequencing system. A length of ten bases is sufficient to ascertain that, for the large number of reads involved and the design parameters considered, the chances of mis-assigning reads is extremely low. By relaxing some of the design requirements, an even greater number of 10-base MIDs can be devised for library multiplexing purposes. Relaxing the requirements for shotgun libraries is not expected to result in a significant loss of reads, because sequencing is highly accurate just beyond the key sequence, at the beginning of sequencing reads. This larger set of MID Adaptors is know as the Extended MID Set:

- The Extended MID Set is listed in Table 1. Each MID sequence is at least 4 changes (insertion, deletion, substitution) away from the other members of the Extended MID Set. This means that for any of these MIDs, it is possible to either detect up to 2 errors and correct 1 error, or alternatively, detect 3 errors and correct none.

- The first ten sequences in the list (highlighted in bold text in Table 1) represent the Basic Set MIDs. To this set have been added 141 MIDs to comprise the final set of 151.

- The Extended Set MIDs are sorted according to the number of reagent flows needed to sequence each, with lower number meaning fewer flows. As a result, the lower numbered entries of the Extended Set MIDs should be preferred over the higher numbered Adaptors, because they can be sequenced using fewer reagent flows thereby maximizing the number of remaining flows for sequencing the library fragment.

Table 1. 10-base Extended Multiplex Identifier (MID) Set Sequences

MID-1	**ACGAGTGCGT**		MID-40	TACGCTGTCT
MID-2	**ACGCTCGACA**		MID-41	TAGTGTAGAT
MID-3	**AGACGCACTC**		MID-42	TCGATCACGT
MID-4	**AGCACTGTAG**		MID-43	TCGCACTAGT
MID-5	**ATCAGACACG**		MID-44	TCTAGCGACT
MID-6	**ATATCGCGAG**		MID-45	TCTATACTAT
MID-7	**CGTGTCTCTA**		MID-46	TGACGTATGT
MID-8	**CTCGCGTGTC**		MID-47	TGTGAGTAGT
MID-10	**TCTCTATGCG**		MID-48	ACAGTATATA
MID-11	**TGATACGTCT**		MID-49	ACGCGATCGA
MID-13	CATAGTAGTG		MID-50	ACTAGCAGTA
MID-14	CGAGAGATAC		MID-51	AGCTCACGTA
MID-15	ATACGACGTA		MID-52	AGTATACATA
MID-16	TCACGTACTA		MID-53	AGTCGAGAGA
MID-17	CGTCTAGTAC		MID-54	AGTGCTACGA
MID-18	TCTACGTAGC		MID-55	CGATCGTATA
MID-19	TGTACTACTC		MID-56	CGCAGTACGA
MID-20	ACGACTACAG		MID-57	CGCGTATACA
MID-21	CGTAGACTAG		MID-58	CGTACAGTCA
MID-22	TACGAGTATG		MID-59	CGTACTCAGA
MID-23	TACTCTCGTG		MID-60	CTACGCTCTA
MID-24	TAGAGACGAG		MID-61	CTATAGCGTA
MID-25	TCGTCGCTCG		MID-62	TACGTCATCA
MID-26	ACATACGCGT		MID-63	TAGTCGCATA
MID-27	ACGCGAGTAT		MID-64	TATATATACA
MID-28	ACTACTATGT		MID-65	TATGCTAGTA
MID-29	ACTGTACAGT		MID-66	TCACGCGAGA
MID-30	AGACTATACT		MID-67	TCGATAGTGA
MID-31	AGCGTCGTCT		MID-68	TCGCTGCGTA
MID-32	AGTACGCTAT		MID-69	TCTGACGTCA
MID-33	ATAGAGTACT		MID-70	TGAGTCAGTA
MID-34	CACGCTACGT		MID-71	TGTAGTGTGA
MID-35	CAGTAGACGT		MID-72	TGTCACACGA
MID-36	CGACGTGACT		MID-73	TGTCGTCGCA
MID-37	TACACACACT		MID-74	ACACATACGC
MID-38	TACACGTGAT		MID-75	ACAGTCGTGC
MID-39	TACAGATCGT		MID-76	ACATGACGAC

MID-77	ACGACAGCTC
MID-78	ACGTCTCATC
MID-79	ACTCATCTAC
MID-80	ACTCGCGCAC
MID-81	AGAGCGTCAC
MID-82	AGCGACTAGC
MID-83	AGTAGTGATC
MID-84	AGTGACACAC
MID-85	AGTGTATGTC
MID-86	ATAGATAGAC
MID-87	ATATAGTCGC
MID-88	ATCTACTGAC
MID-89	CACGTAGATC
MID-90	CACGTGTCGC
MID-91	CATACTCTAC
MID-92	CGACACTATC
MID-93	CGAGACGCGC
MID-94	CGTATGCGAC
MID-95	CGTCGATCTC
MID-96	CTACGACTGC
MID-97	CTAGTCACTC
MID-98	CTCTACGCTC
MID-99	CTGTACATAC
MID-100	TAGACTGCAC
MID-101	TAGCGCGCGC
MID-102	TAGCTCTATC
MID-103	TATAGACATC
MID-104	TATGATACGC
MID-105	TCACTCATAC
MID-106	TCATCGAGTC
MID-107	TCGAGCTCTC
MID-108	TCGCAGACAC
MID-109	TCTGTCTCGC
MID-110	TGAGTGACGC
MID-111	TGATGTGTAC
MID-112	TGCTATAGAC
MID-113	TGCTCGCTAC
MID-114	ACGTGCAGCG
MID-115	ACTCACAGAG

MID-116	AGACTCAGCG
MID-117	AGAGAGTGTG
MID-118	AGCTATCGCG
MID-119	AGTCTGACTG
MID-120	AGTGAGCTCG
MID-121	ATAGCTCTCG
MID-122	ATCACGTGCG
MID-123	ATCGTAGCAG
MID-124	ATCGTCTGTG
MID-125	ATGTACGATG
MID-126	ATGTGTCTAG
MID-127	CACACGATAG
MID-128	CACTCGCACG
MID-129	CAGACGTCTG
MID-130	CAGTACTGCG
MID-131	CGACAGCGAG
MID-132	CGATCTGTCG
MID-133	CGCGTGCTAG
MID-134	CGCTCGAGTG
MID-135	CGTGATGACG
MID-136	CTATGTACAG
MID-137	CTCGATATAG
MID-138	CTCGCACGCG
MID-139	CTGCGTCACG
MID-140	CTGTGCGTCG
MID-141	TAGCATACTG
MID-142	TATACATGTG
MID-143	TATCACTCAG
MID-144	TATCTGATAG
MID-145	TCGTGACATG
MID-146	TCTGATCGAG
MID-147	TGACATCTCG
MID-148	TGAGCTAGAG
MID-149	TGATAGAGCG
MID-150	TGCGTGTGCG
MID-151	TGCTAGTCAG
MID-152	TGTATCACAG
MID-153	TGTGCGCGTG

Note: While the Extended MID Set has been designed to the best of our ability using all current knowledge, not all sequences have yet been thoroughly tested in library construction. It is possible that one or more MIDs on the list may not perform as expected. Our experience to date with 10-base MIDs has shown that approximately one in ten sequences performs below expectations, because of unexpected dimerization/ligation events or unanticipated PCR amplification artifacts. Please note that MID-9 and MID-12 are only used for Standard Series, not Titanium Series methods, and are intentionally excluded herein.

Obtaining and Preparing MID Adaptors

1. For each different MID desired, you must obtain the required oligonucleotides and prepare a new Adaptors mix. Each MID Adaptors mix is comprised of an Adaptor A and an Adaptor B. Each adaptor is comprised of two oligonucleotides that are annealed in an equimolar ratio and these adaptors are then combined to make an MID Adaptors mix.

2. All oligonucleotides should be obtained according to the processes and specifications outlined in the document *Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set.* Importantly, oligonucleotide synthesis specifications for the Extended MID Set are identical to those for the Basic MID Set:

    a. Each oligonucleotide should have phosphorothioate modifications in both the first four and last four bases of the oligomers.
    b. The Adaptor B long oligonucleotide 'Ti-MID-B' must be synthesized with a 5-prime biotin-TEG moiety.
    c. All oligonucleotides must be purified using HPLC.

3. The full sequences of the 306 oligonucleotides that would be required to synthesize all possible Extended MID Set Adaptors A are not provided in this document. However, one can easily design and synthesize the Adaptor A for the particular MID(s) of interest as follows:

    a. Consult the Appendix for a graphical depiction of the structure of the MID-1 Adaptor A (as well as the common Adaptor B which is used with all MID Adaptors mixes).
    b. The highlighted portion of the 'Ti-MID1-A' and 'Ti-MID1-Aprime' oligonucleotides indicates the region of each containing the 10-base MID sequence.
    c. Replace the highlighted portion of the "A" oligonucleotide with the 10-base MID sequence from Table 1 corresponding to the MID of interest. For example, for MID-13:
        1. The 10-base sequence for MID-13 from Table 1 is: CATAGTAGTG
        2. Therefore, the oligonucleotide 'Ti-MID13-A' sequence would be:

        5′-C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCATAGT*A*G*T*G-3′

    d. Replace the highlighted portion of the "Aprime" oligonucleotide with the reverse complement of the 10-base MID barcode from Table 1 corresponding to the MID of interest. Continuing the example of MID-13:
        1. The 10-base sequence for MID-13 is: CATAGTAGTG and the reverse complement of the same is: CACTACTATG.
        2. Therefore, the oligonucleotide 'Ti-MID13-Aprime' sequence would be :

        5′-C*A*C*T*A*CTATGCTGAGTCG*G*A*G*A-3′

e. It is critical to verify your design by examining the structure of the adaptor that would result from annealing of the two oligonucleotides (including verification of proper Watson-Crick complementary base pairing) as shown in the Appendix for MID-1. Note that you must reverse the left-to-right sequence orientation of the 'Aprime' adaptor to the 3-prime to 5'prime direction in this exercise:

MID-13 Adaptor A:

```
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGCATAGTAGTG-3′  Ti-MID13-A
                  3′-AGAGGCTGAGTCGTATCATCAC-5′  Ti-MID13-Aprime
```

4. Once ordered and received, oligonucleotides should be annealed and prepared for the Extended MID Set Adaptors mixes according to the procedures given in the document *Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set.*

5. Consult the document *Using Multiplex Identifier (MID) Adaptors for the GS FLX Titanium Chemistry - Basic MID Set* for instructions on library preparation, emPCR and sequencing.

Appendix

Example: MID Oligonucleotide Sequences for Ordering

OLIGO NAME	OLIGO SEQUENCE (5-prime to 3-prime orientation)
Ti-MID1-A	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGACGAGT*G*C*G*T
Ti-MID1-Aprime	A*C*G*C*ACTCGTCTGAGTCG*G*A*G*A
Ti-MID-B	/5BioTEG/C*C*T*A*TCCCCTGTGTGCCTTGGCAGTC*T*C*A*G
Ti-MID-Bprime	C*T*G*A*GACT*G*C*C*A

- Phosphorothioate bonds are indicated with an asterisk (*)
- A 5'-biotin-TEG moiety is indicated by '/5BioTEG/'
- Inverse (white on black) text denotes the portion of each nucleotide containing the 10-base MID sequence. Note that the highlighted sequence in the "Aprime" oligonucleotide is the reverse complement of that in the associated "A" oligonucleotide.

Purification: All oligonucleotides must be ordered with HPLC purification and with the modifications (i.e. phosphorothioate bonds and 5'-biotin-TEG) shown.

Examples of Annealed MID Adaptors

MID-1 Adaptor A:

```
5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGT-3′  Ti-MID1-A
                    3′-AGAGGCTGAGTCTGCTCACGCA-5′  Ti-MID1-Aprime
```

MID Adaptor B (Common):

```
5′Biotin-TEG-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3′  Ti-MID-B
                              3′- ACCGTCAGAGTC-5′  Ti-MID-Bprime
```

- Phosphorothioate-modified bases are not shown in this figure for ease of sequence alignment
- Sequencing Key is indicated in GREEN. 10 bp MID sequence is shown in YELLOW.

Curriculum vitae

Personal Data	Born 15.07.1980 in Zwickau,
	German,
	Single

Education:

Studies

from Apr. 2007	Dissertation within the working group Schüßler at the Ludwig-Maximilians-University Munich, Dept. Biology I, topic: “Arbuscular mycorrhizal fungi for reforestation of native tropical trees in the Andes of South Ecuador”
Oct. 1999 - Feb. 2006	TU-Bergakademie Freiberg, study of Applied Natural Science („Angewandte Naturwissenschaften“)
17.01.2006	Diploma examination, grade 2.7
Mar. 2005 - Sep. 2005	Diploma thesis in the working group environmental microbiology of the TU-Bergakademie Freiberg, topic: “Analysis of the annual cycle of iron oxidizers in the Wettinquelle (Bad Brambach) using nucleic acid sequence based amplification (NASBA)“
Oct. 2001 – Feb. 2006	Main studies: concentration biotechnology, grade 2.8
18.09.2001	Prediploma
Oct. 1999 – Sep. 2001	Basic studies: Applied natural science

Further education

Oct. 2004 – Mar. 2005	Evening course “Communication Skills Intermediate“
Apr. 2003 – Jul. 2003	Attendance of “Databases for natural scientists“
Apr. 2002 – Sep. 2002	Certificate of „Umfassende Sachkunde“ gemäß § 5 der Chemikalienverbotsordnung (test of technical knowledge in accordance with §5 of Germany's Federal Order Prohibiting Certain Chemicals)
Oct. 2000 – Sep. 2001	Unicert III (English), rating good
Oct. 1999 – Sep. 2000	Unicert II (English), rating good

Publication list

Journals:

Krüger C, Walker C, Schüßler A (2014) Redescription, epitypification and DNA-barcoding of *Scutellospora savannicola* and its transfer to *Dentiscutata*. in preparation.

Urgiles N, **Krüger C**, Strauß A, Schüßler A (2014). Native arbuscular mycorrhizal fungi can increase seedling growth of the Ecuadorian potential crop tree species (*Tabebuia chrysantha*, *Cedrela montana* and *Heliocarpus americanus*) in the nursery by more than 300%. in preparation.

2012

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 Phytologist 193: 970-84.

2009

Krüger M, Stockinger H, **Krüger C**, Schüßler A (2009) DNA-based species-level detection of arbuscular mycorrhizal fungi: one PCR primer set for all AMF. New Phytologist 183: 212-223.

DFG RU816 Newsletters:

2013

Schüßler A, **Krüger C**, Urgiles N (March 2013) Mycorrhizal fungi shape tree performances in nursery. TMF newsletter No. 19: page 18. doi: 10.5678/lcrs/for816.cit.1233.

2010

Krüger C and Schüßler A (July 2010) Mycorrhiza for reforestation. TMF newsletter No. 9: page 6.

2009

Schüßler A, **Krüger C** (October 2009) Arbuscular mycorrhiza for tree seedling survival. TMF newsletter No. 7: pages 6-7.

Symposia and Conferences

Talks:

7th International Conference on Mycorrhizas (ICOM7), 'Arbuscular mycorrhizal fungi for reforestation' New Delhi (India), 06.-11. January 2013; awarded with the **Biosynterra Award for the best oral presentation** by a graduate student (emphasis on applied research and innovative/industrial applications with mycorrhizas)

GTÖ 2011, 'Application of native arbuscular mycorrhizal fungi for reforestation with native tree species in South Ecuador', Frankfurt/Main (Germany), 21.-24. February 2011

Poster presentations:

GTÖ 2011, 'Characterisation of some native arbuscular mycorrhizal (AM) fungi forming symbioses with trees native to South Ecuador', Frankfurt/Main (Germany), 21.-24. February 2011

Status Symposium 2009 of the DFG Research Unit 816 (FOR816), 'B1.1 AM for Reforestation: Tracing AM fungi – markers, DNA barcodes and methods', Loja (Ecuador), 7.-8. October 2009

6th International Conference on Mycorrhizas (ICOM6), 'Arbuscular mycorrhiza (AM) for reforestation', Belo Horizonte (Brazil), 9.-14. August 2009

Workshop 'Mycorrhizas in Tropical Forests', 'Isolation and molecular characterisation of South-Ecuadorian AMF for reforestation attempts', Loja (Ecuador), 22.-25. September 2008

Bi-national Symposium 2008 of the DFG Research Unit 816 (FOR816), 'Molecular characterisation of South-Ecuadorian arbuscular mycorrhizal fungi', Loja (Ecuador), 11.-12. September 2008

Status Symposium 2007 of the DFG Research Unit 816 (FOR816), 'Molecular phylogeny of AM fungi and DNA barcoding to identify AM fungal communities within roots', Loja (Ecuador), 20.-21. September 2007

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den _____ _____

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Erklärung

Hiermit erkläre ich,

dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

dass ich mich anderweitig einer Doktorprüfung ohne Erfolg nicht unterzogen habe.

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