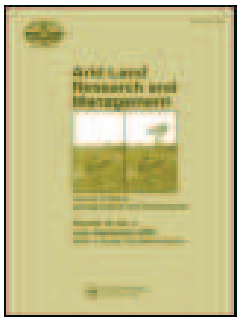

Arid Land Research and Management

ISSN: 1532-4982 (Print) 1532-4990 (Online) Journal homepage: http://www.tandfonline.com/loi/uasr20

Effect of native and allochthonous arbuscular mycorrhizal fungi on Casuarina equisetifolia growth and its root bacterial community

Nathalie Diagne, Ezékiel Baudoin, Sergio Svistoonoff, Christine Ouattara, Diegane Diouf, Aboubacry Kane, Cheikh Ndiaye, Kandioura Noba, Didier Bogusz, Claudine Franche & Robin Duponnois

To cite this article: Nathalie Diagne, Ezékiel Baudoin, Sergio Svistoonoff, Christine Ouattara, Diegane Diouf, Aboubacry Kane, Cheikh Ndiaye, Kandioura Noba, Didier Bogusz, Claudine Franche & Robin Duponnois (2017): Effect of native and allochthonous arbuscular mycorrhizal fungi on Casuarina equisetifolia growth and its root bacterial community, Arid Land Research and Management, DOI: 10.1080/15324982.2017.1406413

To link to this article: https://doi.org/10.1080/15324982.2017.1406413

ARID LAND RESEARCH AND MANAGEMENT
https://doi.org/10.1080/15324982.2017.1406413

Effect of native and allochthonous arbuscular mycorrhizal fungi on *Casuarina equisetifolia* growth and its root bacterial community

Nathalie Diagne[a,b], Ezékiel Baudoin[c], Sergio Svistoonoff[b,c], Christine Ouattara[b,d], Diegane Diouf[b,d], Aboubacry Kane[b,d], Cheikh Ndiaye[b], Kandioura Noba[d], Didier Bogusz[e], Claudine Franche[e], and Robin Duponnois[c]

[a]National Center for Agronomical Research, Senegalese Institute for Agricultural Research (CNRA/ISRA), Bambey, Senegal; [b]Joint International Laboratory on the Adaptation of Plants and associated microorganisms to environmental Stresses (LAPSE), Research Center of Bel-Air, Dakar, Senegal; [c]IRD, French National Research Institute for Sustainable Development, Tropical & Mediterranean Symbioses Laboratory (LSTM), 34398 Montpellier, France; [d]Department of Plant Biology, Cheikh Anta Diop University (UCAD), Dakar, Senegal; [e]IRD, French National Research Institute for Sustainable Development, (UMR), 34394 Montpellier, Cedex 5, France

ABSTRACT

Exotic trees are often planted to recover degraded lands. Inoculation with mycorrhizal fungi can improve their survival. Plant growth is partly dependent on the strain used, but little attention has been paid to the selection of mycorrhizal fungi. The aim of this study was to determine whether the growth of *Casuarina equisetifolia* L. (Johnson) is affected by two different mycorrhizal inocula generated using fungal spores retrieved from an Australian site (allochthonous soil) and a Senegalese site (native soil) under *C. equisetifolia* trees. Comparative experiments were conducted with plants in a Senegalese soil, previously sterilized or not, and grown in a greenhouse. At harvest, parameters related to plant growth and mycorrhization were evaluated and soil bacterial communities were compared. Tree growth was significantly influenced by both types of inoculants. In unsterilized soil, plants inoculated with the native inoculant were taller than plants inoculated with the allochthonous inoculant and control plants. The frequency of mycorrhization with both inoculants was higher in unsterilized soil. The strongest effects of the mycorhizosphere on the soil microbiome were obtained with the allochthonous inoculum, and analysis of the taxonomic composition revealed mycorrhizal communities specific to each inoculum. These results suggest that the development of *C. equisetifolia* and its root bacterial community are dependent on the composition of the mycorrhizal inoculum. The functional consequences of this rhizosphere effect in terms of soil fertility should be further studied to better guide reforestation operations.

ARTICLE HISTORY
Received 2 May 2017
Accepted 14 November 2017

KEYWORDS
Arbuscular mycorrhizal fungi; bacterial community; *Casuarina*; rhizosphere

Introduction

Many reforestation programs have used exotic trees to recover degraded lands, particularly fast-growing species originating from Australia (Bâ et al. 2010; Diagne et al. 2012). The

CONTACT Nathalie Diagne nathaliediagne@gmail.com National Center for Agronomical Research, Senegalese Institute for Agricultural Research (CNRA/ISRA), Bambey, Senegal BP 53 Bambey, Senegal.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uasr.
Supplemental data for this article can be accessed on the publisher's website.

Australian species were chosen for their ability to grow in harsh environments characterized by strong abiotic stresses (drought, salinity) (National Research Council 1984). Among them are several species of the Casuarinaceae family that have been widely planted in the tropics. In Senegal, the first *Casuarina equisetifolia* plantations were established in the Niayes region in 1925 and today these plantations play essential economic and ecological roles by improving soil fertility, stabilizing coastal sand dunes, and acting as windbreaks, thereby limiting land erosion (Diagne et al. 2013). The ecological success of plants in constrained environments is partly influenced by their ability to associate with bacterial and fungal symbionts (Sprent and Parsons 2000). Plants belonging to the Casuarinaceae family have the capacity to support dual symbioses with soil nitrogen-fixing bacteria (*Frankia* spp. as the actinorhizal microsymbionts) and arbuscular mycorrhizal fungi (AMF). The productivity, diversity, and stability of plant covers are known to be favored by mycorrhizal diversity and similar conclusions have been drawn concerning nitrogen-fixing symbionts (van der Heijden et al. 1998; Spehn et al. 2002). These fungal and bacterial symbionts basically influence the plant cover through their functional complementarity, which mainly improves hydromineral nutrition (van der Heijden, Bardgett, and Van Straalen 2008; Santi, Bogusz, and Franche 2013, Sadhana 2014). For instance, Dostálek et al. (2013) recently confirmed the essential role of AMF in dry grasslands with low nutrient availability. These authors observed that the suppression of the AMF community with fungicides resulted in a significant decrease in plant species richness, particularly within the canopy of perennial plants.

Thanks to these positive effects, controlled inoculations of symbiotic microorganisms are often used in reforestation programs to increase the survival rate and performance of tree saplings, but the outcome of such inoculations greatly depends on the identity of symbiotic strains (Klironomos 2003). For instance, under drought stress, *Dactylis glomerata* L. (Stebbins & Zohary) Rivas Mart & Izco plants performed better in terms of root dry weight and survival rate when they were inoculated with *Rhizophagus intraradices* (Thaxt. Gerd. & Trappe) than with *Funneliformis mosseae* (T.H. Nicolson & Gerd.) (Kyriazopoulos et al. 2014). Under low temperature conditions, Chen et al. (2014) described variable responses to inoculation with four AMF species, including *Claroideoglomus etunicatum* (W.N. Becker & Gerd.), *R. intraradices,* (N.C. Schenck & G.S. Sm.) *Acaulospora scrobiculata* (Trappe), and *Corymbiglomus tortuosum* (N.C. Schenck & G.S. Sm.). The highest malondialdehyde and soluble sugar contents were obtained with *C. etunicatum* and *R. intraradices*. In addition, functional complementarity between mycorrhizal strains has been reported in several studies and concluded that most benefits are obtained with AMF inocula combining several species/genera rather than with monoxenic inoculants (Koide 2000; Smith, Jakobsen, and Smith 2000).

In the case of such multispecies inoculation, the taxonomic and functional diversity of the mycorrhizal strains can be modulated by sampling soils of distinct origins. Inoculation with native, locally adapted AMF strains has been shown to increase the effects of phytostimulation compared to inoculation with allochthonous strains (Middleton et al. 2015). Estrada et al. (2013) describe greater effectiveness of native AMF strains in enhancing the establishment and growth of the halophyte *Asteriscus maritimus* L. (Greuter) under saline conditions. Its survival rate was 30% when mycorrhizal strains originating from an allochthonous bank were used, whereas survival reached 100% when inoculations were performed using local AMF strains. The better results obtained with local strains could be

due to coadaptation between the native AMF and plant communities, as suggested by Middleton et al. (2015), who found that locally adapted AMF performed better in restoring native prairies compared to commercially propagated AMF.

Finally, distinct AMF inoculants have distinct effects not only on plant survival rate and performance but also on soil microbes. Mycorrhizae exhibit a rhizodeposition pattern that is distinct from noncolonized roots, resulting in different rhizosphere microbiota (Marschner, Crowley, and Higashi 1997; Andrade, Mihara, and Linderman 1998). Soil bacterial communities are also sensitive to the physiological activity of extraradical hyphae (hyphosphere effect) (Andrade et al. 1997; Toljander et al. 2007; Uroz et al. 2007). Overall, distinct mycorrhizal communities are reported to sustain distinct soil microbial communities (Rillig et al. 2006; Nuccio et al. 2013).

Yet, up to now, little attention has been paid to the origin of the mycorrhizal strains used as inoculants in *C. equisetifolia* reforestation programs or to their effects on the soil microbial communities (Diagne et al. 2012). We conducted an experiment to assess the effect of AMF inocula from an Australian site (allochthonous soil) or a Senegalese site (native soil) on plant growth, root mycorrhization, and soil bacterial communities. We show that local AMF are better than allochthonous AMF at improving *C. equisetifolia* growth and cause less alteration to the soil microbial communities. We hypothesize that native AMF have the potential to develop coadaptation mechanisms to strengthen the interaction with soil bacteria communities and to stimulate *C. equisetifolia* growth in contrast to allochthonous AMF, which could enter in competition with the local soil bacterial communities.

Materials and methods

Origin and elaboration of AMF inoculants

Composite soil samples (0–25 cm) were collected during the dry season under *C. equisetifolia* trees in Senegal (14°43′W, 17°26′N) and in Australia (Noah Beach, Queensland, 16°18′SE, 145°14′E). These soils were used in trap cultures with *C. equisetifolia* to check the presence of compatible AMF and to produce the inoculants. Fifty grams of soil from each geographical origin was used to inoculate *C. equisetifolia* seedlings growing in a sandy sterile soil. Seeds of *C. equisetifolia* subsp. *equisetifolia* were collected in Notto Gouye Diama (14°58′56″N, 17°00′55″W, Senegal). Some roots of 3-month-old plants were cleared with KOH and stained with trypan blue (Phillips and Hayman 1970, see description of root mycorrhizal communities below) to confirm the presence of internal arbuscular mycorrhizal structures (e.g., hyphae, vesicles). The soils of both trap cultures contained spores, fragments of extramatrical hyphae, and of mycorrhized roots. They were used as inoculants, and named COA and COS for their Australian and Senegalese origin, respectively. Inoculants consisted of 10 g of each type of soil containing roughly 47 spores per gram of soil and root fragments with at least 85% of colonized root length.

Greenhouse experimental design

Seeds of *C. equisetifolia* were surface sterilized with 95% sulfuric acid for 3 min. Seeds were then rinsed several times in sterile distilled water and further disinfected in a 5% (w/v) sodium hypochlorite solution for 40 min. After rinsing several times in sterilized distilled water, the seeds were left to germinate on a sterilized sandy soil. Two-week-old seedlings

were then individually transplanted in pots (24 cm in height, 12 cm in diameter) filled with an arable sandy soil collected in Sangalkam, Senegal (14°46′52″N, 17°13′40″W). The soil was crushed, sieved to 2 mm, mixed, and air-dried. Its physical–chemical characteristics were pH (H_2O) 5.3, 3.6% clay; 0% fine silt; 0.8% coarse silt; 55.5% fine sand; 34.9% coarse sand; 0.17% carbon; 0.02% nitrogen; 39 mg kg^{-1} of total phosphorus; 4.8 mg kg^{-1} of soluble phosphorus (Olsen). Plants were grown in unsterilized and sterilized soils (2 kg units, autoclaved twice for 20 min at 120°C with 1 day at room temperature in-between). Three treatments were done in the unsterilized and sterilized soils: noninoculated plants, plants inoculated with COS, and plants inoculated with COA. Eight replicates were carried out for each treatment.

Mycorrhizal inoculations were performed using 10 g of COS and COA inoculants per pot as described by Duponnois et al. (2003). Uninoculated control treatments were implemented using the same COA and COS inoculant materials previously sterilized twice by autoclaving. The pots were transferred to the greenhouse (28°C day, 18°C night, 10 h photoperiod), arranged in a randomized complete block design and watered regularly with distilled water for 6 months. At harvest, plant growth (dry biomasses, height, foliar N and P contents) and root mycorrhization (colonization indices, taxonomic diversity) were measured. Soil bacterial communities were also characterized on the basis of their genetic structures and the diversity of their catabolic potential. After determining plant height and fresh weight, the entire root systems were gently washed in tap water. Fresh root systems were longitudinally divided into two equal parts and weighed. The first half was used to reveal inner root mycorrhizal structures using trypan blue staining (see below). The second half was dried for 1 week at 50°C, with its shoots and then weighed again. The dry weight of the total root system was inferred from this measurement. Molecular analyses of mycorrhizal diversity were performed on the dried root material. In each replicate, foliar N–P contents were determined on dried shoots at the IRD Laboratory LAMA-US 191, certified ISO 9001:2008 by Euro Quality System (http://www.lama.ird.sn). Briefly, the dried shoots were ground, turned to ash (500°C), digested with 2 ml HCl 6N and 10 ml HNO_3, and then analyzed by colorimetry to evaluate P content. For N contents (Kjeldahl method), ground tissues were digested with 36N H_2SO_4 containing 50 g L^{-1} salicylic acid.

Description of root mycorrhizal communities

Root mycorrhizal colonization was quantified in each replicate pot. Fresh roots were cleared in 10% KOH for 1 h at 90°C, rinsed in tap water, and stained with trypan blue in lactophenol (0.05%) for 30 min at 90°C (Phillips & Hayman 1970). Stained roots were cut into 1-cm-long fragments. Single batches of 20 root fragments per root replicate were mounted on slides in 90% glycerol and observed under a stereomicroscope (magnification × 40). The percentage of mycorrhized root fragments [colonization index = (number of mycorrhized root fragments/total number of root fragments) × 100] was assessed from these slides and the intensity and frequency of mycorrhization were determined according to Trouvelot, Kough, and Gianinazzi-Pearson (1986).

To determine the taxonomic composition of the root mycorrhizal communities (only for COS and COA treatments in the unsterilized soil), equal amounts of dried root subsamples of the eight replicates were ground with a mortar and pestle and mixed in liquid nitrogen. Root DNA was extemporaneously extracted with the PureLink Plant Total

DNA Purification Kit (Invitrogen, St. Aubin, France) on 50 mg of ground biomass in duplicate. Duplicate DNA eluates were pooled and further quantified by fluorescence with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) on an infinite M200 microtiter plate reader (Tecan, Lyon, France). The molecular diversity of the root AMF communities was studied by sequencing fragments of the 25S rDNA gene amplified from root DNA extracts using AMF-specific primers. Nested PCR amplifications were performed using the GoTaq® DNA Polymerase kit (Promega, Lyon, France) and the GeneAmp® PCR System 9700 (Thermo Fischer Scientific, Illkirch, France). The eukaryotic primers LR1 and NDL22 were used for the first PCR (van Tuinen et al. 1998; Farmer et al. 2007; Pivato et al. 2007). The AMF-specific primers FLR3 and FLR4 were used in the second PCR using the LR1/NDL22 amplicons (van Tuinen et al. 1998; Trouvelot et al. 1999; Gollotte, van Tuinen, and Atkinson 2004). Amplicons were retrieved and purified from agarose gels with a PureLink Quick Gel Extraction Kit (Invitrogen). Purified PCR products were quantified at 260 nm with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). Purified PCR products (20 ng) were cloned in a pGEM-T Easy vector (Promega) using XL2-Blue ultracompetent cells (Agilent Technologies, Massy, France) according to the manufacturer's instructions. For each of the two libraries, 47 white clones were randomly selected and DNA from the cell aliquot was extracted in 50 µl of sterile water by seven thermal shocks (96°C for 2 min and 4°C for 10 s). Cellular debris were pelleted at 3000× *g*, and supernatants were used as template DNA in a PCR amplification using the pUC/M13 17-mer vector primers (Promega). PCR products of the expected size were sequenced by Genoscreen (Sanger technology, http://www.genoscreen.fr).

Phylogenetic analysis

Raw sequences were manually trimmed between the FLR3/FLR4 priming sites and grouped in OTU using Usearch software at 97% similarity (Edgar 2010) (Supporting information Tables 1 and 2). OTUs were analyzed by running Megablast against the database Nucleotide collection (nr/nt) to identify their taxonomic affiliation and to check for the presence of sequences not specific to Glomeromycetes. Phylogenetic analyses were performed on specific sequences along with the GenBank sequences with the highest BLAST scores and representatives of different clades of Glomeromycota (Supporting information Table 3). Phylogenies were constructed using the Phylogeny.fr online platform with default parameters (Dereeper et al. 2008). Ninety-three sequences were deposited in NCBI GenBank under accession numbers KX245446-KX245491 (COA treatment), KX245492-KX245538 (COS treatment); (Supporting information Tables 4 and 5). The phylogeny was edited with FigTree software. Branches with less than 50% bootstrap support were collapsed to polytomies.

Description of soil bacterial communities

The genetic structure of soil bacterial communities (COS, COA, and control treatments with the unsterilized soil) was determined using the RISA fingerprinting method based on the length polymorphism of the 16S–23S ribosomal intergenic spacers (IGS). Four replicates out of eight were randomly chosen and individually processed for extraction of soil DNA. Once the roots were retrieved, the remaining bulk soil was homogenized and total soil DNA extracts were obtained from triplicate soil aliquots (500 mg) according

to Porteous, Seidler, and Watrud (1997), pooled, sequentially purified on PVPP and Sepharose 4B columns (Edel-Hermann et al. 2004), and quantified by fluorescence with the Quant-iT™ PicoGreen® dsDNA Assay Kit. Bacterial IGS were PCR amplified from 5 ng soil DNA per reaction according to Ranjard et al. (2001), using a GeneAmp® PCR System 9700 and the GoTaq® DNA Polymerase kit. Amplicons were purified using the MinElute kit (Qiagen, Courtaboeuf, France) and quantified with a Nanodrop ND-1000 spectrophotometer. Purified RISA amplicons were electrophoretically resolved using the Agilent DNA 1000 Chips kit on an Agilent 2100 Bioanalyzer (Agilent Technologies) according to the manufacturer's standard instructions. Electropherograms were encoded in numerical matrices based on peak positions and heights using the default parameters of the Bioanalyzer software to delineate individual peaks. RISA matrices were standardized by replacing the height of each peak by its relative height calculated as the height of the peak divided by the sum of the heights of all the peaks.

The patterns of in situ catabolic potential (ISCP) of soil microbial communities were individually evaluated for each pot replicate as described in Dabire et al. (2007) after plant harvest (unsterilized soil experiment). Among the 32 substrates tested, five were carbohydrates (mannose, glucose, sucrose, maltose, starch), three were alcohols (meso-inositol, sorbitol, mannitol), 11 were amino acids (arginine, asparagine, cysteine, glutamic acid (ac.), histidine, serine, lysine, tyrosine, leucine, proline, glutamine), 12 were organic acids (gallic ac., ascorbic ac., citric ac., fumaric ac., malic ac., quinic ac., succinic ac., tartaric ac., malonic ac., keto-glutaric ac., oxalic ac., pantholeic ac.), and one was an amide (glucosamine).

Statistical analysis

The percentages of the mycorrhizal colonization were arcsin transformed prior to statistical analysis. Data were treated with one-way analysis of variance (ANOVA). Means were compared using the Tukey's HSD test ($p < 0.05$) using XLSTAT (v2010.5.04) software. Standardized RISA matrices were compared by ascending hierarchical clustering analysis using Euclidean distances and the Ward's clustering method. The ISCP patterns were compared using the between-group analysis (Culhane et al. 2002).

Results

The effect of inoculant types on C. equisetifolia *growth*

Plant height, shoot biomass, root biomass, and foliar N contents all increased significantly in response to COA and COS inoculations, irrespective of soil disinfection (Table 1). Moreover, the root-to-shoot ratio decreased significantly in response to both inoculations whatever the soil pretreatment is. Surprisingly, the foliar P contents decreased significantly in response to both types of inoculation only in unsterilized soil. Similar trends were observed with the sterilized soil. In addition, a few significant differences were observed between COS and COA inoculants when applied to the unsterilized soil (Table 1). Indeed, the native COS inoculant proved to be more efficient than the COA inoculant in increasing shoot height. Interestingly, there was less decrease in foliar P with the COS inoculant. Overall, the soil sterilization treatment had a significant effect only on shoot height (increase), root biomass (decrease), and the R:S ratio (decrease), whatever the inoculant (Table 2). The inoculation factor had an overall significant effect on all the parameters, whereas the interactions with the soil sterilization factor were systematically nonsignificant.

Table 1. Effects of the indigenous (COS) and allochthonous (COA) mycorrhizal inoculants on the growth of *Casuarina equisetifolia,* its foliar N and P contents, and its mycorrhization.

Soil types	Inoculation treatments	Height (cm)	Shoot biomass (g dry weight)	Root biomass (g dry weight)	*R/S*[*]	Kjeldahl N (%)	Total P ($mg \cdot kg^{-1}$)	Mycorrhization frequency (%)	Mycorrhization intensity (%)
Sterilized soil	Control	39.34 (2.82)[a]	1.62 (0.36)[a]	0.49 (0.07)[a]	0.30 (0.02)[a]	0.75 (0. 09)[a]	1574 (323)[a]	0	0
	COA inoculum	69. 15 (2.45)[b]	5.09 (0.31)[b]	0.83 (0.06)[b]	0.16 (0.018)[b]	1.79 (0.07)[b]	780 (264)[a]	35.00 (11.53)[a]	6.94 (5.80)[a]
	COS inoculum	74.07 (2.45)[b]	6.08 (0.31)[b]	0.69 (0.06)[b]	0.11 (0.018)[b]	1.51 (0.07)[b]	921 (264)[a]	46.87 (9.98)[a]	16.06 (5.02)[a]
Unsterilized soil	Control	34.9 (2.96)[a]	1.86 (0.27)[a]	0.65 (0.08)[a]	0.36 (0.02)[a]	0.72 (0.12)[a]	1844 (50)[c]	24.13 (7.70)[a]	7.79 (1.89)[a]
	COA inoculum	54.51 (2.96)[b]	4.50 (0.27)[b]	0.96 (0.08)[b]	0.21 (0.02)[b]	1.33 (0.09)[b]	450 (41)[a]	55.71 (8.06)[b]	11.27 (2.76)[a]
	COS inoculum	65.42 (2.76)[c]	5.03 (0.26)[b]	1.26 (0.08)[b]	0.25 (0.02)[b]	1.68 (0.09)[b]	933(41)[b]	50.00 (7.54)[b]	11.25 (2.52)[a]

Notes: For a given soil type, data in the same column followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$, $n = 8$).
[*]Root to shoot biomass ratio.

Table 2. Effects of the inoculation and soil sterilization factors on the growth of *Casuarina equisetifolia,* its foliar N and P contents, and its mycorrhization.

Factors	Factor Treatments	Height (cm)	Shoot biomass (g dry weight)	Root biomass (g dry weight)	*R/S**	Kjeldahl N (%)	Total P (mg · kg^{-1})	Mycorrhization frequency (%)	Mycorrhization intensity (%)
Sterilization	Sterilized soil	61.40 (3.27)^{b}	4. 26 (0.38)^{a}	0.67 (0.06)^{a}	0.19 (0.01)^{a}	1.35 (0.15)^{a}	1091 (203)^{a}	27.29 (5.69)^{a}	7.66 (2.22)^{a}
	Unsterilized soil	51.49 (3.20)^{a}	3.76 (0.39)^{a}	0.95 (0.06)^{b}	0.27 (0.01)^{b}	1.24 (0.14)^{a}	1076(191)^{a}	43.28 (5.82)^{a}	10.10 (2.22)^{a}
Inoculation	Uninoculated	36.97 (2.33)^{a}	1.74 (0.23)^{a}	0.52 (0.07)^{a}	0.33 (0.02)^{a}	0.73 (0.09)^{a}	1709(145)^{b}	12.06 (6.14)^{a}	3.89 (2.50)^{a}
	COA inoculant	62.32 (2.25)^{b}	4.79 (0.22)^{b}	0.89 (0.07)^{b}	0.19 (0.02)^{b}	1.56 (0.08)^{b}	615 (132)^{a}	45.35 (6.14)^{b}	9.10 (2.50)^{b}
	COS inoculant	69.20 (2.18)^{c}	5.55 (0.21)^{b}	0.97 (0.06)^{b}	0.18 (0.01)^{b}	1.59 (0.08)^{b}	927(132)^{a}	48.44 (5.74)^{b}	13.65 (2.33)^{b}
Sterilization	$p = 0.019$	$p = 0.170$	$p = 0.006$	$p = 0.000$	$p = 0.420$	$p = 0.853$	$p = 0.925$	$p = 0.763$	
Inoculation	$p < 0.0001$	$p < 0.0001$	$p = 0.000$	$p < 0.0001$	$p < 0.0001$	$p = 0.000$	$p < 0.0001$	$p = 0.006$	
Interaction	$p = 0.887$	$p = 0.874$	$p = 0.892$	$p = 0.944$	$p = 0.847$	$p = 0.864$	$p = 0.881$	$p = 0.931$	

Notes: Data represent the mean of 24 replicates (3 × 8) for the factor "Sterilization" and 16 replicates (2 × 8) for the factor "Inoculation."
For a given factor, data in the same column followed by the same letter are not significantly different according to the Tukey's HSD test ($p < 0.05$).
*Root to shoot biomass ratio.

Effect of the type of inoculant on C. equisetifolia *mycorrhizal communities*

When the unsterilized soil was used, the mycorrhization frequency index increased significantly and similarly following both inoculation treatments, unlike the mycorrhization intensity index (Table 1). When the sterilized soil was used, no mycorrhizal structures were observed on the roots in the control treatment and again no significant differences in either index were observed between COS and COA treatments (Table 1). The overall effect of the soil sterilization factor was nonsignificant for both mycorrhization indices unlike that of the inoculation factor (Table 2).

In all, 46 and 47 partial 25S rDNA sequences specific to Glomeromycota were obtained to describe the root mycorrhizal communities of *C. equisetifolia* in unsterilized soil when inoculated with COS and COA, respectively. These 93 sequences were split into 11 different OTUs, four for COA and seven for COS. Phylogenetic analysis revealed that all the OTUs belong to the *Glomeraceae* family except for COS-OTU5 which is closely related to *Claroideoglomus* (Figure 1). All the sequences related to the allochthonous COA inoculant clustered in a single clade (0.74 bootstrap value) divided in two subclusters, while the sequences obtained with the COS inoculant split into several other clades and subclusters outside the COA clade.

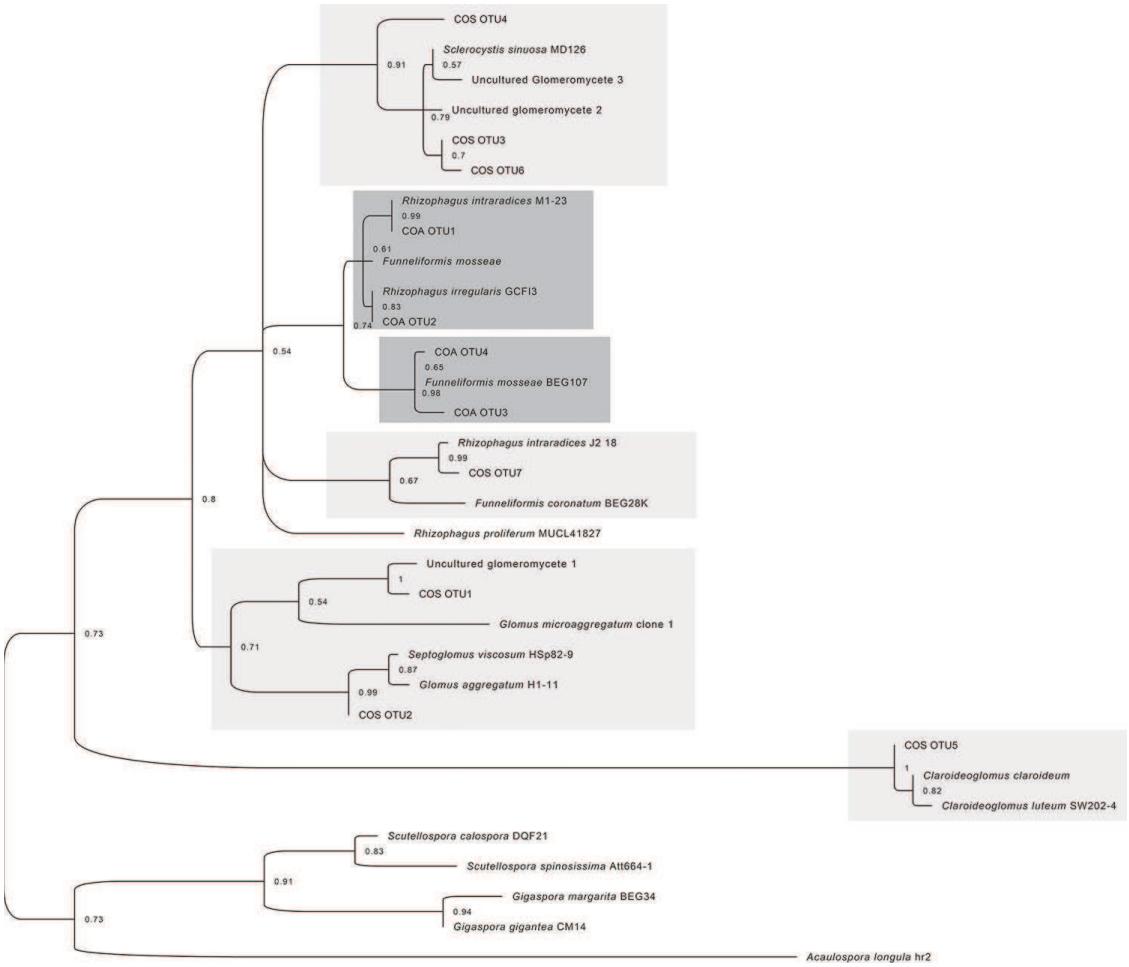

Figure 1. Maximum likelihood phylogeny of AMF based on the 25S RNA. Statistical support for branches was evaluated using the approximate likelihood ratio test. COA, inoculum from Australia; COS, inoculum from Senegal.

Effect of type of inoculant on soil microbial communities

The RISA banding patterns used to fingerprint the genetic structure of the soil bacterial communities not only displayed visible differences (especially band intensity) between inoculation treatments but also replicates (Figure 2a), which were compared by cluster analysis (Figure 2b). Intratreatment variability of COS and COA replicates clustered

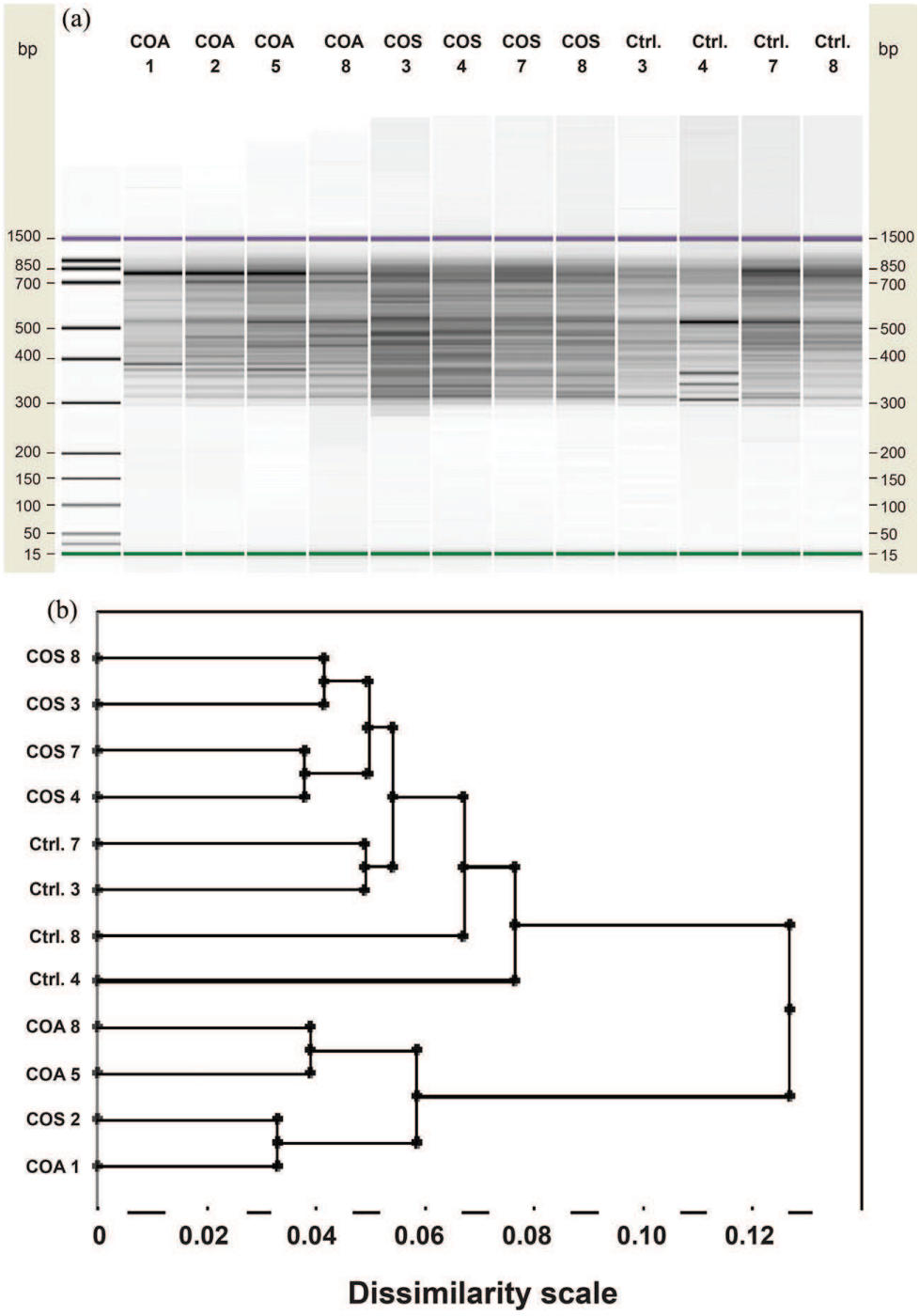

Figure 2. (a) In silico rebuilt scan of the electrophoretic profiles of RISA amplicons obtained from soil DNA extracts. (b) Hierarchical cluster analysis of the RISA fingerprints. Composite inoculum origins: COA (Australia), COS (Senegal), and Ctrl. (uninoculated). Numbers to the right of the inoculum codes show the replicate identity numbers.

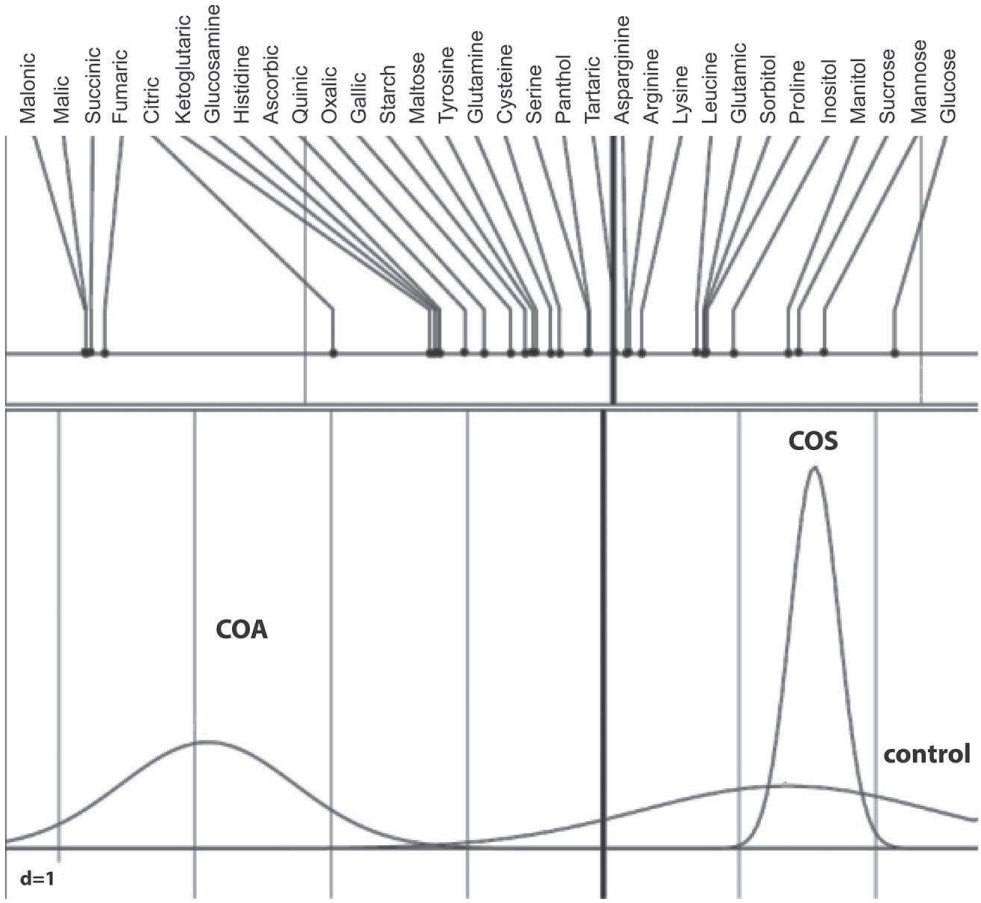

Figure 3. Graphic representation of Between-Group Analysis (BGA) showing the patterns of in situ catabolic potential (ISCP) of microbial communities in the three treatments. Treatment codes: Ctrl. (noninoculated control), COA (allochthonous inoculant), and COS (indigenous inoculant).

separately was lower and was also distinct from the uninoculated treatment. The control treatment however was closer to the COS cluster than the COA one, showing that the composition of the soil bacterial community was more sensitive to the allochthonous inoculant.

Between-group analysis of the catabolic potentials revealed distinct effects of the COA and COS inoculants (Figure 3). On the first horizontal axis, the COA treatment is segregated from the other two treatments and substrates preferentially catabolized in the soils inoculated with COA are malonic acid, malic acid, succinic acid, fumaric acid, citric acid, ketoglutaric acid, glucosamine, histidine, ascorbic acid, and quinic acid. However, the COS and control treatments could not be differentiated on the first axis. Microbial communities in these soils preferentially oxidized asparagine, arginine, lysine, leucine, glutamic acid, sorbitol, proline, inositol, mannitol, sucrose, mannose, and glucose. However, according to the second vertical axis, the overall level of respiration recorded with soils inoculated with COS was much higher than that obtained with control soils or soils inoculated with COA.

Discussion

Arbuscular mycorrhizal fungi are soil microorganisms that are widely reported to improve plant growth (Smith and Read 2008). This growth stimulation is linked to the fact that AM

fungi extend the absorbing network beyond the nutrient depletion zones of the rhizosphere, thereby allowing mycorrhizae access to a larger volume of soil than noncolonized roots (Nakmee, Techapinyawat, and Ngamprasit 2016). By extending the root absorbing area, AMF increase the total absorption surface of the inoculated plants, thereby improving plant access to nutrients (Smith and Read 2008). However, distinct AMF inoculants have distinct effects on plant growth and coadaptation between native AMF and plant communities has been described (Middleton et al. 2015).

In our experiment, both mycorrhizal inoculants improved plant growth, especially in the unsterilized soil. The native AMF inoculum promoted more shoot growth than the allochthonous inoculum in the unsterilized soil. More importantly, it also significantly mitigated the unexpected decrease in foliar P. This suggests that the native inoculum could be more efficient in stimulating the growth of *C. equisetifolia* in situ than the allochthonous inoculum. This result is in accordance with that of Requena et al. (2001), who found that native AMF species were more efficient in promoting *Anthyllis cytisoides* L plant growth. Similar tendencies were identified by Estrada et al. (2013), who reported that native AMF strains improved *A. maritimus* L. growth and adaptation under saline conditions. Klironomos (2003) also reported that plant communities benefit more from a locally adapted AMF community.

Mycorrhizal frequency and intensity are important parameters for assessing plant colonization, particularly the formation of fungal structures such as arbuscules, vesicles, and hyphae (Smith and Read 2008). Colonization of plant roots by AMF has been shown to have a variety of effects on plant growth and biomass allocation (Bohra and Vyas 2013). In the present study, mycorrhization frequency and intensity along with plant biomass were significantly improved in response to both AMF inoculants to the same extent in both soil types. However, inoculated plants displayed significantly lower foliar P levels than uninoculated seedlings. Thus, the controlled mycorrhization of *C. equisetifolia* in sterilized and unsterilized soils significantly improved its biomass and mycorrhization, whereas foliar P levels decreased. This counterintuitive result is hard to account for, as mycorrhizal symbionts are usually reported to improve plant P nutrition, and this abrupt P decline clearly did not penalize plant growth. Interestingly, this dramatic decrease in P contents was significantly mitigated with the indigenous AMF inoculum only in the unsterilized soil. This difference could be interpreted as a positive outcome of the interaction between the soil microflora and mycorrhizal symbionts. As *C. equisetifolia* has been established in Senegal since 1920, the native AMF preferentially associated with this tree species may be more prone to stimulate the activity of soil bacteria by the hyphosphere effect including mycorrhizal helper bacteria “MHB” (Frey-Klett et al. 2005; Tarka and Frey-Klett 2008). These MHB are known to stimulate the establishment and functioning of the mycorrhizal symbiosis (Garbaye 1994; Duponnois and Kisa 2006).

The taxonomic composition of the root mycorrhizal community can vary according to the locality (Bohra and Vyas 2013). Brundrett (1991) demonstrated that edaphic conditions can have a substantial effect on the characteristics of an AMF population. In our study, the taxonomic composition of the root mycorrhizal community of the COS-inoculated seedlings did not share any OTU with that of the COA-inoculated seedlings. The variability observed in the mycorrhizal fungi community could be due to the range of AMF strains that are able to form mycorrhiza in *C. equisetifolia* plants. Similar results were found by He and Critchley (2008), who showed that *C. equisetifolia* forms a relationship with many

AMF genera including *Acaulospora* (e.g., *Acaulospora laevis* Gerdemann and Trappe), *Rhizophagus* (e.g., *Rhizophagus fasciculatum* (Thaxt.) Gerd. & Trappe) *Diversispora* (e.g., *Diversispora versiformis*). These taxonomic differences are likely related to the contrasting functional efficiencies of P uptake and transfer that were apparently more efficient with the native inoculant. Depending on the origin of the AMF inoculum, *C. equisetifolia* roots harbored completely distinct mycorrhizal communities. Arbuscular mycorrhizae alter the diversity and function of root bacterial communities (Marschner et al. 2001; Marschner and Timonen 2005). Marschner, Crowley, and Higashi (1997) and Andrade, Mihara, and Linderman (1998) found that mycorrhizal fungi modify plant physiology and hence root exudation, leading to modifications in the structure of bacterial communities. In our study, the genetic structures of soil bacterial communities also diverged. The resulting shift in the composition of the soil bacterial communities was greater in plants inoculated with COA than in uninoculated plants. This greater responsiveness of soil bacteria to the allochthonous inoculant is possibly related to the rhizodeposition pattern of *C. equisetifolia* that may have been altered by COA and/or by the hyphosphere effect associated with the extramatrical hyphae of COA strains. As the composition of soil bacterial communities shifted in response to AMF inoculations, some functional attributes may also have been affected (Marschner and Timonen, 2005; Dabire et al. 2007). Comparisons of the soil catabolic signatures clearly confirmed that the ability of soil bacterial community to oxidize particular organic compounds changed after inoculation. As already observed with RISA fingerprints, the strongest effects were observed with the allochthonous COA inoculant for which a preferential oxidation of 10 organic compounds was identified compared to bacteria in the control and COS-inoculated soils. Interestingly, eight of these discriminating substrates were carboxylic acids. By contrast, bacteria inhabiting control and COS-inoculated soils preferentially oxidized amino acids and carbohydrates, while their oxidation levels were highest in the COS-inoculated soil. This different catabolic potential could reflect the availability of hyphae and root exudates in the soil. However, this would then mean that the COA-inoculated soil was enriched particularly by carboxylic acids, and local acidification of the soil should have triggered increased solubility of some nutrients such as P. However, this theory was undermined by the comparison of foliar P levels that were lowest in the COA-inoculated soil. Nevertheless, as soil bacteria mediate most biogeochemical processes including organic matter mineralization, such distinct catabolic signatures could translate altered capacities of bacteria living in the COA-inoculated soil to thrive on some organic molecules, which could, in turn, modify the decomposition rate of soil organic matter and the associated supply of mineral nutrients for plant growth.

Conclusion

AMF associated with *C. equisetifolia* in Australia differed genetically from those associated with the same plant introduced in Senegal. Both types of inoculum stimulated *C. equisetifolia* growth in the Senegalese soil, but the highest score was obtained with the native Senegalese inoculum. Mycorrhizal inoculation also modified the structure of soil bacterial communities and the diversity of its catabolic potential. However, the strongest shifts were induced by the allochthonous inoculant. This study illustrates the importance of selecting appropriate arbuscular mycorrhizal inoculants to optimize plant growth while limiting their impact on the resident soil bacterial communities. The potential of

native AMF to improve plant performance makes the native AMF inoculum a promising biological tool that can be used to recover degraded lands. What is more, these native AMF can be used together with selected *Frankia* strains in Senegalese reforestation programs.

Acknowledgment

The authors thank Chris McMillan (Word Vision Senegal), Elaine Gross (IRD), and Daphne Goodfellow for critical reading of the paper.

Funding

This work was supported in part by the Institut de Recherche pour le Développement (IRD) through a PhD grant and le Ministère de l'Enseignement Supérieur et de la Recherche du Sénégal through a national PhD grant for Nathalie DIAGNE.

References

Andrade, G., K. L. Mihara, and R. G. Linderman. 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. *Plant Soil* 202:89–96.

Andrade, G., K. L. Mihara, R. G. Linderman, and G. J. Bethlenfalvay. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. *Plant Soil* 192:71–79.

Bâ, A. M., A. G. Diédhiou, Y. Prin, A. Galiana, and R. Duponnois. 2010. Management of ectomycorrhizal symbionts associated to useful exotic tree species to improve reforestation performances in tropical Africa. *Annals of Forest Science* 67:301–01. doi:10.1051/forest/2009108

Bohra, S., and A. Vyas. 2013. Distribution of arbuscular mycorrhizal fungi associated with landscape tree growth in Indian Thar Desert. *International Journal of Plant, Animal and Environmental Sciences* 3:98–102. doi:10.3923/ijss.2007.119.127

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. *Advances in Ecological Research* 21:171–313.

Culhane, A. C., G. Perrier, E. C. Considin, T. G. Cotter, and D. G. Higgins. 2002. Between-group analysis of microarray data. *Bioinformatics* 18:1600–08. doi:10.1093/bioinformatics/18.12.1600

Chen, X., F. Song, F. Liu, C. Tian, S. Liu, H. Xu, and X. Zhu. 2014. Effect of different arbuscular mycorrhizal fungi on growth and physiology of maize at ambient and low temperature regimes. *Science World Journal* 2014:1–7. doi:10.1155/2014/956141

Dabire, A. P., V. Hien, M. Kisa, A. Bilgo, K. S. Sangare, C. Plenchette, A. Galiana, Y. Prin, and R. Duponnois. 2007. Responses of soil microbial catabolic diversity to arbuscular mycorrhizal inoculation and soil disinfection. *Mycorrhiza* 17:537–45. doi:10.1007/s00572-007-0126-5

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J. F. Dufayard, S. Guindon, V. Lefort, M. Lescot et al. 2008. Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36:465–69. doi:10.1093/nar/gkn180

Diagne, N., D. Diouf, S. Svistoonoff, A. Kane, K. Noba, C. Franche, D. Bogusz, and R. Duponnois. 2013. *Casuarina* in Africa: Distribution, role and importance of arbuscular mycorrhizal, ectomycorrhizal fungi and *Frankia* on plant development. *Journal of Environmental Management* 128:204–09. doi:10.1016/j.jenvman.2013.05.009

Diagne, N., D. Diouf, S. Svistoonoff, A. Kane, K. Noba, L. Laplaze, C. Franche, D. Bogusz, and R. Duponnois. 2012. Arbuscular and ectomycorrhiza fungi: Useful biological tool to promote establishment of exotic trees in arid and semi-arid African areas. In *Fungi: Type, environmental impact and role in disease*, ed. A. Paz Silva and M. Sol, 215–34. Nova Sciences Publisher, Lugo, Spain.

Duponnois, R., S. Diedhiou, J. L. Chotte, and M. O. Sy. 2003. Relative importance of the endomycorrhizal and/or ectomycorrhizal associations in *Allocasuarina* and *Casuarina* genera. *Canadian Journal of Microbiolology* 50:691–96. doi:10.1139/w03-038

Duponnois, R., and M. Kisa. 2006. The possible role of trehalose in the mycorrhiza helper effect. *Canadian Journal of Botany* 84:1005–08. doi:10.1139/b06-053

Dostálek, T., H. Pánková, Z. Münzbergová, and J. Rydlová. 2013. The effect of AMF suppression on plant species composition in a nutrient poor dry grassland. *PLoS One* 8:e80535. doi:10.1371/journal.pone.0080535

Edel-Hermann, V., C. Dreumont, A. Pérez-Piqueres, and C. Steinberg. 2004. Terminal restriction fragment length polymorphism analysis of ribosomal RNA genes to assess changes in fungal community structure in soils. *FEMS Microbiology Ecology* 47:397–404. doi:10.1016/s0168-6496(04)00002-9

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–61. doi:10.1093/bioinformatics/btq461

Estrada, B., R. Aroca, C. Azcón-Aguilar, J. M. Barea, and J. M. Ruiz-Lozano. 2013. Importance of native arbuscular mycorrhizal inoculation in the halophyte *Asteriscus maritimus* for successful establishment and growth under saline conditions. *Plant Soil* 370:175–85. doi:10.1007/s11104-013-1635-y

Farmer, M. J., X. Li, G. Feng, B. Zhao, O. Chatagnier, S. Gianinazzi, V. Gianinazzi-Pearson, and D. van Tuinen. 2007. Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China. *Applied Soil Ecology* 35:599–609. doi:10.1016/j.apsoil.2006.09.012

Frey-Klett, P., M. Chavatte, M. L. Clausse, S. Courrier, C. Le Roux, J. Raaijmakers, M. G. Martinotti, J. C. Pierrat, and J. Garbaye. 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. *New Phytologist* 165:317–28. doi:10.1111/j.1469-8137.2004.01212.x

Garbaye, J. 1994. Helper bacteria: A new dimension to the mycorrhizal symbiosis. *New Phytologist* 128:197–210. doi:10.1111/j.1469-8137.1994.tb04003.x

Gollotte, A., D. van Tuinen, and D. Atkinson. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species. *Agrostis capillaris* and *Lolium perennein* a field experiment. *Mycorrhiza* 14:111–17. doi:10.1007/s00572-003-0244-7

He, X. H., and C. Critchley. 2008. *Frankia* nodulation, mycorrhization and interactions between *Frankia* and mycorrhizal fungi in casuarina plants. In *Mycorrhiza – State of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics*, ed. A Varma, 3rd ed., 767–81. Springer Verlap. Heidelberg, Germany.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–301. doi:10.1890/02-0413

Koide, R. T. 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. *New Phytologist* 147:233–35. doi:10.1046/j.1469-8137.2000.00710.x

Kyriazopoulos, A. P., M. Orfanoudakis, E. M. Abraham, Z. M. Parissi, and N. Serafidou. 2014. Effects of arbuscular mycorrhiza fungi on growth characteristics of *Dactylis glomerata* L. under drought stress conditions. *Notulae Botanicae Horti Agrobotanici* 42:132–37.

Marschner, B., D. E. Crowley, and R. M. Higashi. 1997. Root exudation and physiological status of root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.). *Plant Soil* 189:11–20. doi:10.1016/0038-0717(96)00072-7

Marschner, P., and S. Timonen. 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Applied Soil Ecology* 28:23–36. doi:10.1016/j.apsoil.2004.06.007

Marschner, P., C. H. Yang, R. Lieberei, and D. E. Crowley. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biology and Biochemistry* 33:1437–1445.

Middleton, L., S. Richardson, L. Koziol, C. E. Palmer, Z. Yermakov, J. A. Henning, P. A. Schultz, and J. D. Bever. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere* 6:1–16. doi:10.1890/ES15-00152.1

Nakmee, P. S., S. Techapinyawat, and S. Ngamprasit. 2016. Comparative potentials of native arbuscular mycorrhizal fungi to improve nutrient uptake and biomass of *Sorghum bicolor* Linn. *Agriculture and Natural Resources* 50:173–78. doi:10.1016/j.anres.2016.06.004

National Research Council. 1984. *Casuarinas: Nitrogen-fixing trees for adverse sites*. Washington, DC, USA: Office of International Affairs.

Nuccio, E. E., A. Hodge, J. Pett-Ridge, D. J. Herman, P. K. Weber, and M. K. Firestone. 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen

cycling during litter decomposition. *Environmental Microbiology* 15:1870–81. doi:10.1111/1462-2920.12081

Phillips, J. M., and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British mycological Society* 55:158–61. doi:10.1016/s0007-1536(70)80110-3

Pivato, B., S. Mazurier, P. Lemanceau, S. Siblot, G. Berta, C. Mougel, and D. van Tuinen. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytolologist* 176:97–210. doi:10.1111/j.1469-8137.2007.02151.x

Porteous, L. A., R. J. Seidler, and L. S. Watrud. 1997. An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. *Molecular Ecology* 6:787–91. doi:10.1046/j.1365-294x.1997.00241.x

Ranjard, L., F. Poly, J. C. Lata, C. Mougel, J. Thioulouse, and S. Nazaret. 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: Biological and methodological variability. *Applied Environmental Microbiology* 67:4479–87. doi:10.1128/aem.67.10.4479-4487.2001

Requena, N., E. Perez-Solis, C. Azcón-Aguilar, P. Jeffries, and J. M. Barea. 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied Environmental Microbiology* 67:495–98. doi:10.1128/aem.67.2.495-498.2001

Rillig, M. C., D. L. Mummey, P. W. Ramsey, J. N. Klironomos, and J. E. Gannon. 2006. Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. *FEMS Microbiology Ecology* 57:389–95. doi:10.1111/j.1574-6941.2006.00129.x

Santi, C., D. Bogusz, and C. Franche. 2013. Biological nitrogen fixation in non-legume plants. *Annals of Botany* 111:743–67. doi:10.1093/aob/mct048

Sadhana, B. 2014. Arbuscular mycorrhizal fungi (AMF) as a biofertilizer a review. *International Journal of Current Microbiology and Applied Sciences* 3:384–400.

Spehn, E. M., M. Scherer-Lorenzen, B. Schmid, A. Hector, M. C. Caldeira, P. G. Dimitrakopoulos, J. A. Finn, A. Jumpponen, G. O'Donnovan, J. S. Pereira, et al. 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos* 98:205–18. doi:10.1034/j.1600-0706.2002.980203.x

Sprent, J. I., and R. Parsons. 2000. Nitrogen fixation in legume and non- legume trees. *Field Crops Research* 65:183–96. doi:10.1016/s0378-4290(99)00086-6

Smith, F. A., I. Jakobsen, and S. E. Smith. 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytology* 147:357–66. doi:10.1046/j.1469-8137.2000.00695.x

Smith, S. E., and D. J. Read. 2008. *Mycorrhizal symbiosis*, 3rd ed. Academy Press. London.

Tarka, M. T., and P. Frey-Klett. 2008. Mycorrhiza helper bacteria. In *Mycorrhiza*, ed. A. Varma, 113–32. Berlin: Springer-Verlag Heidelberg.

Toljander, J. F., B. D. Lindahl, L. R. Paul, M. Elfstrand, and R. D. Finlay. 2007. Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiology Ecology* 61:295–304. doi:10.1111/j.1574-6941.2007.00337.x

Trouvelot, A., J. L. Kough, and V. Gianinazzi-Pearson. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Proceedings of the 1st ESM, INRA Press, Paris, 217–21.

Trouvelot, S., D. van Tuinen, M. Hijri, and V. Gianinazzi-Pearson. 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. *Mycorrhiza* 8:203–06. doi:10.1007/s005720050235

Uroz, S., C. Calvaruso, M. P. Turpault, J. C. Pierrat, C. Mustin, and P. Frey-Klett. 2007. Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied Environmental Microbiology* 73:3019–27. doi:10.1128/aem.00121-07

van der Heijden, M. G. A., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:72–75. doi:10.1038/23932

van Tuinen, D., E. Jacquot, B. Zhao, A. Gollotte, and V. Gianinazzi-Pearson. 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology* 7:879–87. doi:10.1046/j.1365-294x.1998.00410.x