

RESEARCH ARTICLE

Impact of alien pines on local arbuscular mycorrhizal fungal communities—evidence from two continents

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One sentence summary: Effect of alien pine trees on native AM fungal diversity is context dependent, differing among continents with different biogeographic history.

Editor: Petr Baldrian

ABSTRACT

The introduction of alien plants can influence biodiversity and ecosystems. However, its consequences for soil microbial communities remain poorly understood. We addressed the impact of alien ectomycorrhizal (EcM) pines on local arbuscular mycorrhizal (AM) fungal communities in two regions with contrasting biogeographic histories: in South Africa, where no native EcM plant species are present; and in Argentina, where EcM trees occur naturally. The effect of alien pines on AM fungal communities differed between these regions. In South Africa, plantations of alien EcM pines exhibited lower AM fungal richness and significantly altered community composition, compared with native fynbos. In Argentina, the richness and composition of local AM fungal communities were similar in plantations of alien EcM pines and native forest. However, the presence of alien pines resulted in slight changes to the phylogenetic structure of root AM fungal communities in both regions. In pine clearcut areas in South Africa, the richness and composition of AM fungal communities were intermediate between the native fynbos and the alien pine plantation, which is consistent with natural regeneration of former AM fungal communities following pine removal. We conclude that the response of local AM fungal communities to alien EcM pines differs between biogeographic regions with different histories of species coexistence.

Keywords: biogeography; arbuscular mycorrhiza; ectomycorrhiza; alien species; afforestation

INTRODUCTION

Biological invasion is one of the major threats associated with global change due to its impact on biodiversity and ecosystem services (Walther *et al.* 2009; Pyšek and Richardson 2010; Simberloff *et al.* 2013). In invaded communities, existing eco-

logical interactions may be disrupted and new relationships form (Schweiger *et al.* 2010). Notably, global change and species invasion can modify plant–soil interactions (van der Putten *et al.* 2013), inducing changes to chemical and biological soil properties (Jandová *et al.* 2014) including soil microbial

communities (van der Putten, Klironomos and Wardle 2007; Bever et al. 2010).

Mycorrhizal fungi are important components of the soil microbial community and contribute to the maintenance of ecosystem productivity and diversity (van der Heijden, Bardgett and van Straalen 2008). Mycorrhizal associations may change during plant invasion, and these can play an important role in determining either the success of invaders or the resistance of native communities (Richardson et al. 2000; Pringle et al. 2009; van der Putten 2012; Johnson et al. 2013; Traveset and Richardson 2014). It is known that herbaceous alien plants can change the diversity and taxon composition of native arbuscular mycorrhizal (AM) fungal communities (Mummey and Rillig 2006; Stinson et al. 2006; Callaway et al. 2008; Zhang et al. 2010; Moora et al. 2011; Lekberg et al. 2013). An important question that remains to be addressed is how alien ectomycorrhizal (EcM) trees influence local AM fungal communities. Replacement of AM plant communities with EcM vegetation may alter many ecosystem functions (Dickie et al. 2014) but only limited evidence exists about direct negative effects of EcM host plants on native AM fungal communities (Nunez and Dickie 2014). Cumming and Kelly (2007) recorded a decrease in AM fungal spore abundance under arriving EcM pines, while Remigi et al. (2008) reported changes to AM fungal spore community composition under invasive EcM *Acacia* trees. As far as we know, no information exists concerning the impact of alien EcM trees on the molecular diversity and composition of native AM fungal communities in the roots of AM plants and/or in the soil.

Various tree species that associate with EcM fungi have been widely used in forestry programs in the southern hemisphere, with fast-growing pines constituting more than half of the reported hosts (Vellinga, Wolfe and Pringle 2009). Several alien pine species are considered problematic in the southern hemisphere due to their disproportionate effect on various ecosystem processes (Higgins and Richardson 1998; Richardson and Rejmanek 2004; Dehlin et al. 2008). The impact of EcM trees, such as pines, on AM fungi may be via changes in soil chemistry (Tyndall 2005; Cumming and Kelly 2007) and litter (Piotrowski, Morford and Rillig 2008; Becklin, Pallo and Galen 2012). EcM fungi could also reduce mineralization rates and decrease nutrient availability to AM plants (Dickie et al. 2014). Biogeographically, the introduction of exotic EcM trees may occur in two fundamentally different contexts: in ecosystems where EcM plants are naturally absent, thus resulting in a novel combination of functional groups, or in ecosystems where EcM plants are already a natural component of the local community. The distinction might be important because AM fungal communities that have historically coexisted with native EcM hosts and fungi might be more robust to introduction of new EcM hosts. Despite the global distributions of many AM fungal taxa, fungal community composition is influenced by local ecological conditions (Davison et al. 2015). Because the presence of EcM trees may result in specific changes to both local biotic and abiotic conditions (Dickie et al. 2014), the long-term presence of EcM trees might select for particular AM fungal taxa that tolerate the conditions the best. Under such circumstances, one might not expect the introduction of another EcM tree to have a strong additional effect on AM fungal communities. Conversely, in vegetation without EcM trees, introducing a new EcM species may result in a cascade of changes to local conditions that importantly influence AM fungal communities.

South Africa serves as example of an area lacking native EcM plants (Allsopp and Stock 1993; Traveset and Richardson 2014). However, exotic pines have been introduced to large parts of the country (Richardson 1998; van Wilgen and Richardson

2012), typically in association with EcM fungal inoculum, which can persist in soil for a long time (Pringle et al. 2009; Nunez and Dickie 2014). In South African fynbos, the introduction of alien pines has resulted in transformation of these species-rich shrublands into species-poor woodlands or forests. Alien pines disrupt the natural regeneration cycle of fynbos shrubs, which cannot be reinstated without human intervention in the form of pine removal (Rundel, Dickie and Richardson 2014). By contrast, several areas in the southern part of South America represent regions where EcM fungi, usually in association with *Nothofagus* spp. host trees, are naturally present (Nouhra et al. 2012). Exotic pines have also been introduced to large parts of Argentina (Andenmatten and Letourneau 1997; Simberloff, Relva and Nuñez 2002; Nunez, Horton and Simberloff 2009; Hess and Austin 2014). In north-western Patagonia, pines have been planted into mixed stands of native *Araucaria araucana*, *Austrocedrus chilensis*, *Nothofagus antarctica* and *N. dombeyi*, and in some cases have become invasive. Introduced pines are in rare cases able to associate with some of the locally existing EcM fungi (Barroetavena, Cazares and Rajchenberg 2007; Hayward, Horton and Nunez 2015), but in most cases are colonized by exotic EcM fungi that are introduced intentionally or accidentally along with the alien hosts (Nunez, Horton and Simberloff 2009; Hayward, Horton and Nunez 2015; Hayward et al. 2015).

We compared the impact of alien EcM pines on the molecular richness and composition of local AM fungal communities in two biogeographically different situations – in South African fynbos vegetation and in Argentinian temperate *Araucaria* forest. While these regions differ in many respects, from the perspective of native AM fungi confronted by a widespread new EcM plant host there is one notable difference—the EcM symbiosis (and EcM fungi) is historically absent in South Africa, while in Argentinian temperate forest it has been historically present. By definition, such biogeographic-scale natural experiments are difficult or impossible to replicate; however, they provide valuable information that complements controlled experiments. We expected changes to AM fungal diversity, notably, decreases in richness and turnover of composition, to be greatest in South Africa, where alien EcM hosts represent a novel addition to ecosystems; nonetheless we also expected changes to occur in Argentina due to the significant change in tree stand composition brought about by the introduction of pines. In South Africa, it was also possible to study the effect of clearcutting alien pine plantations, which could permit regeneration of original AM fungal communities if AM fungal propagules can persist in soil during the period of pine predominance or if they can disperse from surrounding AM vegetation.

MATERIALS AND METHODS

Study areas and sampling

Our study sites were located in fynbos shrubland in South Africa and in temperate forest in Argentina. In South Africa, the study was conducted in the Jonkershoek Valley, Western Cape, South Africa (33° 60'S, 18° 58' E), ~60 km east of Cape Town. The surrounding mountains and portions of the upper valley form part of a biodiversity nature reserve, while the valley and inner mountain slopes have been planted with *Pinus radiata* D. Don. The Jonkershoek Valley has been the subject of long-term research focusing on the impacts of exotic timber plantations (predominantly pines) on biodiversity, fire dynamics, invasion control and hydrology. Natural (mountain fynbos), afforested and clear-cut areas all occur under the same

geological (Table Mountain Group sandstone and quartzite) and climatic (Mediterranean climate with hot, dry summers and cool, wet winters) conditions—see Garcia-Quijano et al. (2007) for more information about the study area. According to Allsopp and Stock (1993), about 62% of the flora of the Cape Floristic Region form AM, whilst there are no indigenous EcM plant species.

In Argentina, the study area was located in Moquehue, north-western Patagonia (38° 58'S, 71° 21'W), ~300 km west of the city of Neuquen. It forms part of the Southern Andes, near Lanin National park, about 1200 m above sea level. Native vegetation is dominated by temperate *A. araucana* and *N. antarctica* forest, growing on allophanic soils. Ponderosa pine (*P. ponderosa* Dougl. ex Laws.) was first experimentally introduced for forestry purposes in the Patagonian Andes in 1927 (Cozzo 1987) and has now become a widely used conifer species in afforestation programs in region (Andenmatten and Letourneau 1997). In some places, it has become invasive (Sarasola et al. 2006). Native vegetation, including the dominant tree *A. araucana*, is predominantly AM (Diehl, Mazzarino and Fontenla 2008), with the exception of the subdominant tree *N. antarctica*, which is EcM (Nouhra et al. 2012). *Nothofagus* is a genus of Gondwanan origin and is distributed across the southern hemisphere (Swenson, Hill and McLoughlin 2001).

We targeted AM fungal communities in both plant roots and soil in order to gather the most complete information possible about the occurrence of AM fungal taxa in ecosystems. Plant roots and rhizosphere soil have recently been shown to host partially overlapping subsets of the local AM fungal taxon pool (Saks et al. 2014; Varela-Cervero et al. 2015). Three habitat types with different status with respect to the alien pines were sampled in South Africa in January 2010: fynbos (natural vegetation); pine plantation (*P. radiata*, either 18- or 20-year-old); and 3-year-old clearcut in pine plantation. These habitats were located in initially homogeneous conditions with respect to climate and soil. Two sites, ~0.5 km apart, were sampled in each habitat (Table 1). The sites of about 50 × 50 m were located in visually homogeneous plant communities. In each site, we aimed to sample four locally dominant herbaceous plant species, but fewer species were sampled in the plantation and clearcut because of low local herbaceous plant diversity. Altogether, we sampled 13 host plant species (Table 1). Because vegetation composition in fynbos, pine plantation and clearcut was very different, we were unable to sample overlapping plant species among the native and disturbed habitats (except *Gerbera crocea*); *Hypochaeris radiata* was sampled from all plantation and clearcut sites.

Two habitat types with different status with respect to alien pines were sampled in Argentina in January 2013: native temperate *A. araucana* forest with *N. antarctica* understory, and 25-year-old pine (*P. ponderosa*) plantation in former *Araucaria* forest. Following the protocol used in South Africa, two sites ~0.5 km apart were sampled in both habitat types. In each site, four locally dominant herbaceous plant species were selected for root sampling. There were no clearcut areas available in this study region.

At each study site, five (South African sites) or ten (Argentinian sites) haphazardly chosen individuals of each plant species were sampled. Whole root systems of each plant were excavated and kept in individual plastic bags until they were processed for storage within 48 h from sampling. Soil and any other material adhering to the roots were carefully removed by hand, without wetting the roots. Individual roots were cut with scissors from the root system (total length >20 cm), wrapped in tissue paper, placed in a zip-lock bag containing silica gel and stored airtight at room temperature until analyzed. In addition,

at each site, soil samples were collected from nine points on a regularly spaced 30 × 30 m sampling grid (Table 1). Each soil sample consisted of 10 g of soil collected from the 2–10 cm topsoil, where most of the roots of herbaceous plant species and most AM fungal biomass are located. Soil samples were packed in paper envelopes inside zip-lock bags with silica gel and stored airtight at room temperature prior to analysis.

Molecular analyses

DNA was extracted from c. 70 mg of dried roots with PowerSoil 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) with modifications described by Saks et al. (2014), and from 5 g of dried soil with PowerMax Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) with the following modifications, analogous to the protocol used for DNA extraction from roots. First, in order to increase DNA yield, bead solution tubes were shaken at a higher temperature (60°C as suggested by the manufacturer) for 10 min at 100 rpm in a shaking incubator. Second, before adding the final elution buffer, the samples were allowed to dry for 10 min at room temperature under the fume hood. Glomeromycota sequences were amplified from root or soil DNA extracts using the nuclear small subunit ribosomal RNA (SSU rRNA) gene primers NS31 and AML2 (Simon, Lalonde and Bruns 1992; Lee, Lee and Young 2008), linked to 454-sequencing adaptors A and B, respectively, and following the 454-sequencing approach of Davison et al. (2012) and Öpik et al. (2013). This marker region provided us with a larger comparative sequence dataset of AM fungi than would be available for any other marker used for detection and identification of AM fungi from environmental samples (Öpik et al. 2010, 2014).

Sequence reads were included in subsequent analyses only if they carried the correct barcode and forward primer sequences, and were ≥ 170 bp long (excluding the bar-code and primer sequence). Potential chimeras (8834 reads) were detected and removed from the data using UCHIME (Edgar et al. 2011) in reference database mode (MaarjAM; Öpik et al. 2010) and the default settings. After stripping the barcode and primer sequences, we used the MaarjAM database of published Glomeromycota SSU rRNA gene sequences for taxonomic assignment of the obtained reads (status April 2014). The MaarjAM database contains representative sequences covering the NS31/AML2 amplicon from published environmental Glomeromycota sequence groups and known taxa. As of April 2014 it contained a total of 6064 records that could be associated with SSU sequence-based phylotypes, or so-called virtual taxa (VT, cf. Öpik et al. 2009, 2014). VT are molecular (DNA sequence-based) taxa that are delimited phylogenetically at approximately species level, on the basis of AM fungal specimen-originating and environmental sequences (Öpik et al. 2009, 2014). The MaarjAM database maintains the VT nomenclature and assigns VT identity to published AM fungal sequences, thereby, permitting easy comparison of datasets and providing stable molecular species proxies for further AM fungal community surveys. Samples gaining fewer than 7 hits were removed from the data matrix since these contain little information with which to assess diversity, and VT represented by a single hit were also omitted since these may result from PCR or sequencing errors. A set of representative sequence reads has been deposited in the EMBL nucleotide collection (accession numbers LN824227–LN827521; the set consists of 1–2 randomly selected reads representing each VT from each host or soil sample from each site). AM fungal sequence data from plant root samples from fynbos plots JFx and JFy have been published in

Table 1. Location and details of sampling; sample source (root or soil), number of samples (i.e. samples yielding >6 hits, N), number of AM fungal molecular taxa (VT) and number of Glomeromycota sequences (AMF reads) per study site.

Country	Habitat/Sampling date	Plot	Coordinates	Plant family	Host species	Source	N	AMF VT	AMF reads
Argentina	A. araucana forest 05/02/2013	AAx	38° 57' 36" S 71° 21' 30" W 1168 m	Araucariaceae	<i>A. araucana</i>	Root	10	42	8622
				Berberidaceae	<i>Berberis microphylla</i>	Root	10	51	8332
				Poaceae	<i>Festuca pallescens</i>	Root	10	45	7133
				Rosaceae	<i>Potentilla chilensis</i>	Root	8	45	4239
			Soil	9	38	4310			
	AAy	38° 59' 22" S 71° 22' 40" W 1376 m	Ranunculaceae	<i>Anemone multifida</i>	Root	10	39	6979	
			Araucariaceae	<i>A. araucana</i>	Root	10	51	5996	
			Poaceae	<i>Festuca pallescens</i>	Root	10	58	9316	
			Rosaceae	<i>Potentilla chilensis</i>	Root	10	46	7405	
			Soil	9	36	3302			
	Pine (<i>P. ponderosa</i>) plantation 05/02/2013	AAw	38° 59' 22" S 71° 22' 40" W 1376 m	Ranunculaceae	<i>Anemone multifida</i>	Root	10	47	7219
				Araucariaceae	<i>A. araucana</i>	Root	10	50	6869
				Poaceae	<i>Festuca pallescens</i>	Root	10	46	9344
				Rosaceae	<i>Potentilla chilensis</i>	Root	10	43	8540
			Soil	9	36	4194			
	AAz	38° 57' 36" S 71° 21' 30" W 1168 m	Poaceae	<i>Anthoxanthum juncifolium</i>	Root	10	44	6078	
Araucariaceae			<i>A. araucana</i>	Root	10	48	8323		
Poaceae			<i>Festuca pallescens</i>	Root	10	43	4772		
Rosaceae			<i>Potentilla chilensis</i>	Root	10	50	9396		
		Soil	9	34	2532				
Republic of South Africa	Fynbos 02/02/2010	JFx	33° 59' 17" S 18° 57' 20" E 319 m	Fabaceae	<i>Aspalathus cordata</i>	Root	4	17	7224
				Poaceae	<i>Cymnopogon nardus</i>	Root	4	19	1822
				Montiniaceae	<i>Montinia caryophyllacea</i>	Root	4	24	2571
				Polygalaceae	<i>Muraltia alopecuroides</i>	Root	4	13	926
			Soil	9	51	14996			
	JFy	33° 59' 35" S 18° 58' 27" E 379 m	Rosaceae	<i>Cliffortia cuneata</i>	Root	3	6	1072	
			Poaceae	<i>Cymnopogon nardus</i>	Root	3	14	1764	
			Asteraceae	<i>G. crocea</i>	Root	4	33	5703	
			Montiniaceae	<i>Montinia caryophyllacea</i>	Root	4	16	2732	
			Soil	8	62	7489			
	Clearcut of pine forest 02/02/2010	JCx	33° 58' 25" S 18° 56' 39" E 294 m	Asteraceae	<i>Conyza canadensis</i>	Root	1	5	482
				Asteraceae	<i>H. radicata</i>	Root	2	15	245
				Poaceae	<i>Tribolium uniola</i>	Root	1	7	96
						Soil	8	22	7594
	JCy	33° 58' 22" S 18° 56' 39" E 312 m	Asteraceae	<i>Conyza canadensis</i>	Root	3	9	493	
			Asteraceae	<i>H. radicata</i>	Root	3	15	264	
Scrophulariaceae			<i>Pseudoselago serrata</i>	Root	1	34	432		
Asteraceae			<i>Senecio rigidus</i>	Root	3	6	1558		
		Soil	7	20	5167				
Pine (<i>P. radiata</i>) plantation 02/02/2010	JPx	33° 58' 22" S 18° 56' 30" E 346 m	Poaceae	<i>Bromus diandrus</i>	Root	2	3	146	
			Asteraceae	<i>G. crocea</i>	Root	2	5	76	
			Asteraceae	<i>H. radicata</i>	Root	3	8	782	
					Soil	5	16	1328	
JPy	33° 58' 22" S 18° 56' 30" E 346 m	Poaceae	<i>Briza maxima</i>	Root	3	15	381		
		Asteraceae	<i>H. radicata</i>	Root	3	9	193		
		Soil	4	10	1123				

Davison et al. (2015), while all other samples are published here for the first time.

Statistical analyses

We used species accumulation curves (SAC) and rarefaction analyses to display the accumulated number of taxa in relation to sampling intensity (Gotelli and Colwell 2001). To calcu-

late the estimated number of species and its standard deviation for a given sampling intensity, we used subsampling without replacement ('random method' with 1000 permutations; Gotelli and Colwell 2001). The analyses were performed in the R statistical environment (R Development Core Team 2013) with the function *speccaccum* from the vegan package (Oksanen et al. 2013).

Three different indices were used to represent different components of diversity: taxon richness, rarefied taxon richness and

the inverse of Simpson's dominance index (Hurlbert 1971; Hill 1973; Heck, van Belle and Simberloff 1975). Because differences in observed numbers of species may be a consequence of variable sampling intensity, we rarefied VT richness by standardizing sequencing depth per sample by rarefaction to the median number of sequences per sample (de Carcer et al. 2011). The inverse Simpson index (the so-called Hill's H2) gives more weight to the abundance of common species than, for instance, the exponential Shannon index (Hill's H1). Its scale is also intuitively understandable: when the abundance of all species in a sample is equal, i.e. there is a maximum evenness, the value of the inverse Simpson index is equal to the species richness (cf. Heip, Herman and Soetaert 1998).

To study variation in the diversity of AM fungal VT among habitat types, we used linear mixed effect models (LME; Pinheiro et al. 2014). In each model, habitat type was used as a fixed factor. The random structure of models varied between the root and soil data: for analyses of root data, we used a nested random structure with each plant host nested in plot identity; for soil data, we used plot as a random factor.

We described AM fungal community composition using the relative number of VT reads in a sample as a measure of abundance (Moora et al. 2014). We used nonmetric multidimensional scaling (NMDS; Legendre and Legendre 1998) with Bray–Curtis dissimilarity to visualize patterns in AM fungal community composition. For each region, separate NMDS solutions were generated for root and soil samples.

In order to test for differences in AM fungal community composition and beta diversity between habitat types, we used Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001) and analysis of multivariate homogeneity of group dispersion (betadisper; Anderson 2006), respectively. PERMANOVA analyses of root samples were performed using the function *nested.npmanova* from the BiodiversityR package (Kindt and Coe 2005). Since root samples were collected from different plant species, we used habitat as a fixed factor and plant species identity as a random factor. PERMANOVA analyses of soil samples were performed applying the *adonis* function from the *vegan* package (Oksanen et al. 2013). Post-hoc pair-wise PERMANOVA analyses were used to test which habitats differed significantly from each other in the case of South African samples (where there were three different habitat types).

We applied multilevel pattern analysis to evaluate the presence of indicator species associated with particular habitat types. For each region, analyses were performed for root and soil samples separately. We applied the function *multipatt* from the *indicspecies* package (De Cáceres and Legendre 2009). The function calculates an indicator value of the association between a species and a particular habitat type by multiplying the relative abundance and frequency of the species in each particular habitat type.

To address phylogenetic community composition, we used a phylogenetic tree containing the type sequences of all VT in the MaarjAM database (Ópik et al. 2013, using an updated tree from Grilli et al. 2015). In order to represent phylogenetic community composition, we performed NMDS using a pairwise between-sample phylogenetic distance matrix. The matrix, which was weighted by a matrix of AM fungal VT relative abundances, was constructed using the function *comdist* from the *picante* package (Kembel et al. 2010). In order to test for differences in AM fungal VT phylogenetic community composition and beta diversity, we applied the PERMANOVA analyses as detailed above.

RESULTS

A total of 431 019 quality-filtered SSU rRNA gene sequences were recovered, of which 199 560 recorded a match in the AM fungal database MaarjAM. From 280 root and 90 samples subjected to the molecular analysis, 215 and 77 yielded AM fungal sequences (Table 1). The AM fungal sequences were assigned to a total of 124 VT: 11 Acaulosporaceae (8.9% of VT, 1.9% of reads), two Ambisporaceae (1.6%/0.5%), five Archaeosporaceae (4%/2.8%), four Claroideoglomeraceae (3.2%/11.9%), 5 Diversisporaceae (4%/0.2%), six Gigasporaceae (4.8%/3.1%), 90 Glomeraceae (72.6%/79.3%) and one Paraglomeraceae VT (0.8%/0.3%). Gigasporaceae were not detected in the Argentinean samples. The most abundant VT in South Africa were *Claroideoglossum* VT57 (25% of reads from South Africa) and *C. VT193* (7% of reads; related to *C. claroideum-etunicatum* species group), and in Argentina *Glomus* VT327 (29% of reads from Argentina) and *G. VT191* (27% of reads; no related morphospecies known) (Table S1, Supporting Information). Of the remaining reads, 69% could be matched against sequences in the INSD nucleotide collection (at sequence similarity $\geq 90\%$). These hits represented Metazoa (32%, mostly Arthropoda and Tardigrada), Viridiplantae (10%), unclassified eukaryotes (11%) and other Fungi (17%).

The total number of recorded AM fungal VT was higher in South Africa (108) than in Argentina (82) (Fig. 1). The two regions shared about 53% of VT. In South Africa, the different habitat types exhibited quite a high proportion of unique VT, especially when soil AM fungal communities were considered. Fynbos contained the most and pine plantation the fewest unique AM fungal VT. By contrast, the two habitat types addressed in Argentina exhibited large overlap of VT, both in the cases of root and soil AM fungal communities.

VT accumulation curves indicated that the overall number of VT was higher in South Africa than Argentina; and that additional sampling may have resulted in the detection of more additional VT in South Africa than Argentina (Fig. 2). We only recorded significant differences in VT richness per sample, rarefied VT richness and inverse Simpson diversity per sample between habitat types in South Africa (Figs 3 and 4; Table 2). In the case of root samples, both the richness per sample and rarefied richness were higher in the fynbos and clearcut than in pine plantation. In the case of soil samples, fynbos showed significantly higher richness per sample and rarefied richness than both clearcut and pine plantation. Species diversity (inverse of Simpson's dominance index) exhibited the same pattern, although post hoc analysis did not confirm the significance of the higher soil AM fungal diversity in fynbos. In Argentina, there were no differences in AM fungal richness per sample, rarefied richness or diversity per sample between natural and planted forest. When comparing the two regions, VT numbers per root or soil sample tended to be lower in South Africa (on average 4–11 VT per sample) than in Argentina (15–18 VT per sample; Fig. 3), indicating high turnover among samples in South Africa, as the total VT pool sizes showed the opposite trend.

VT composition of AM fungal communities in both roots and soil varied significantly between habitat types in South Africa (Fig. 5; Table 3). Post hoc pair-wise analyses revealed that root AM fungal community composition differed significantly between fynbos and pine plantation, and between fynbos and clearcut, but not between clearcut and pine plantation. When considering soil samples, we found significant differences between all three habitat types. In Argentina, there were no differences in the composition of native and planted forest AM fungal communities in roots or in soil (Table 3).

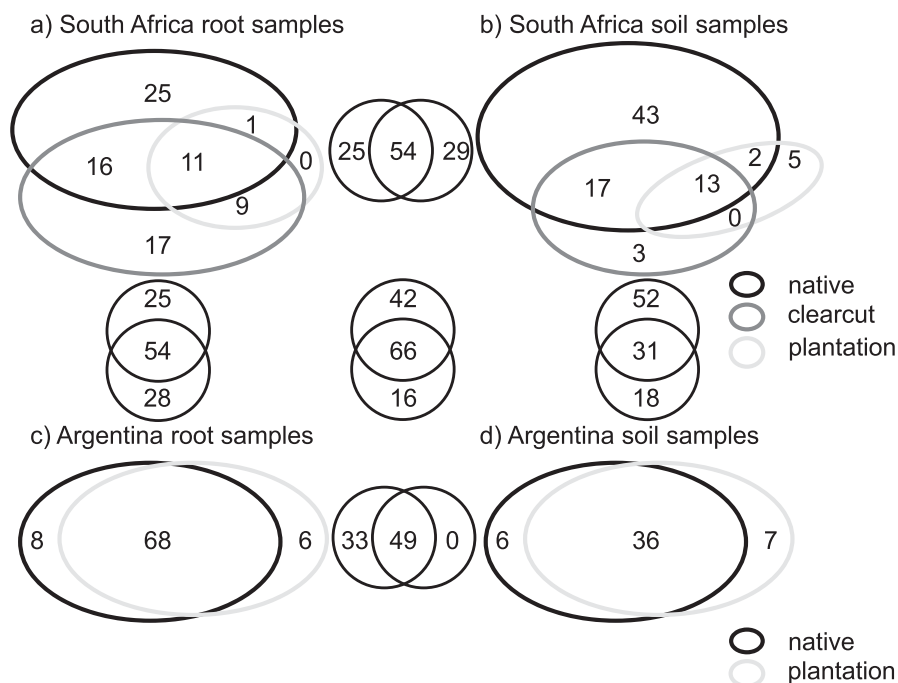


Figure 1. Shared and unique AM fungal molecular taxa (VT) across regions and habitats. For root and soil samples in each habitat type we show the shared and unique number of VT. The colors indicate different habitat types. In addition, the small circles indicate the shared and unique number of VT between: different regions (center), soil samples in different regions (middle right), root samples in different regions (middle left) and soil and root samples in the same region (center above and below).

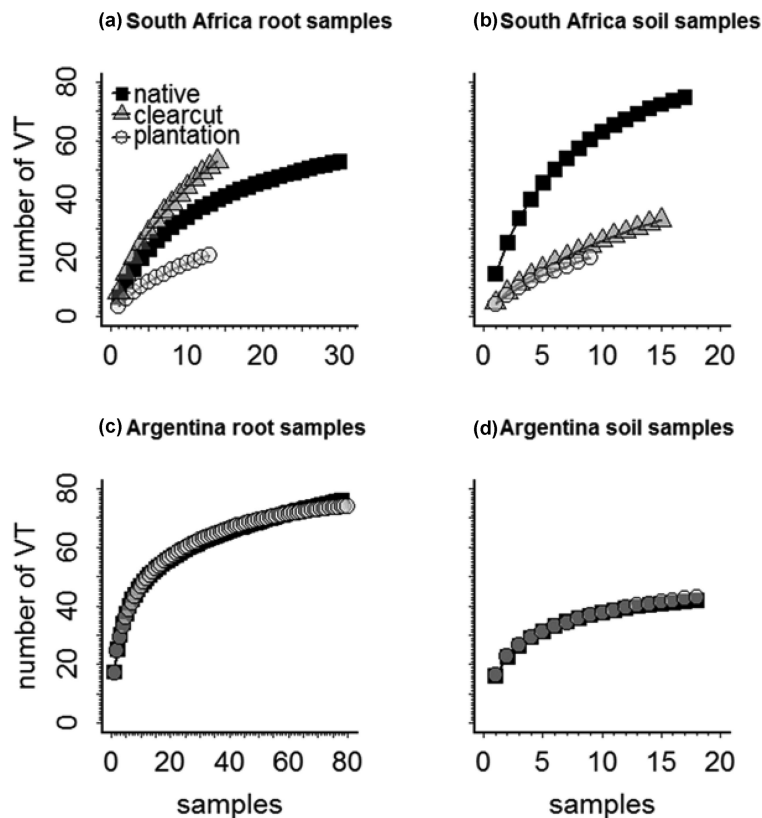


Figure 2. AM fungal molecular taxon (VT) accumulation curves across habitats and regions. Values show the cumulative number of AM fungal VT with increasing numbers of samples.

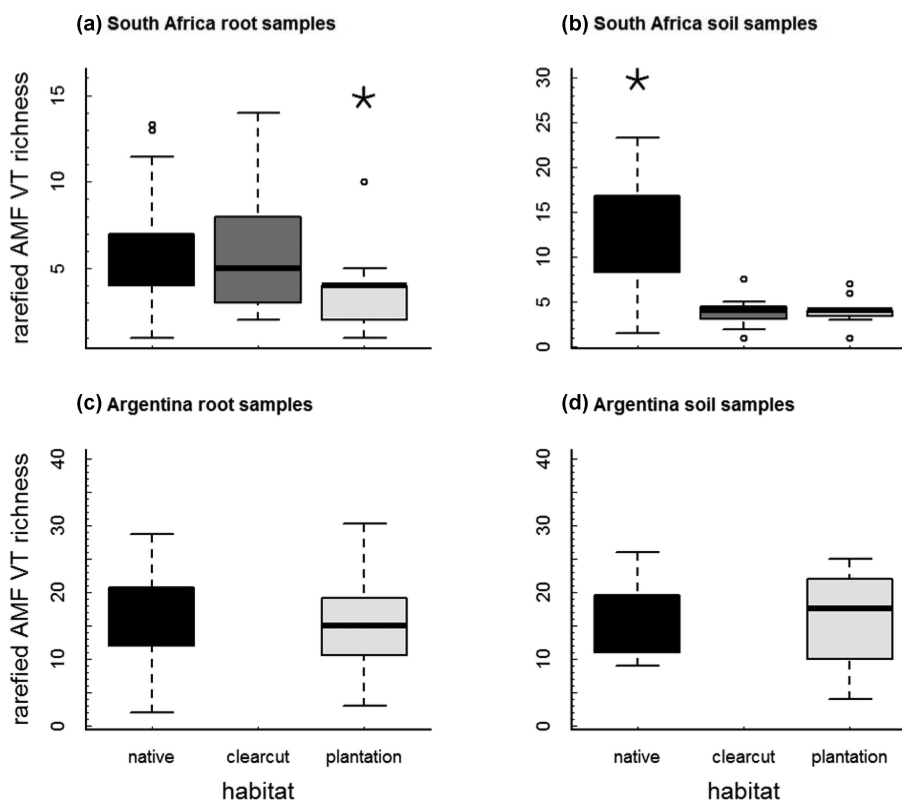


Figure 3. Richness of AM fungal molecular taxa (VT), rarefied to the median number of sequences per sample, across regions and habitats. Asterisks indicate significant differences between habitats (see Table 2). Colors indicate different habitat types (cf. Fig. 1). Median (bold line), interquartile range (box) and range (whiskers) are shown (outlying values are indicated by points).

Indicator species analyses confirmed that there were marked differences between the two regions (Table 4). In South Africa, several AM fungal VT were significantly associated with either fynbos or pine plantation, and to a lesser extent with clearcut. In Argentina, only one AM fungal VT in roots showed significant association with native forest and one VT with planted pine forest.

The phylogenetic composition of AM fungal communities in roots only differed significantly between habitat types in Argentina (Fig. 6; Table 3). There were no differences in the phylogenetic composition of soil AM fungal communities in Argentina. However, there were differences in South Africa, with post hoc analysis showing that native fynbos was different from clearcut and that pine plantation did not differ significantly from either native fynbos or clearcut.

We found few differences in the beta diversity (multivariate dispersion) of either taxonomic or phylogenetic AM fungal community composition (Table S2, Supporting Information). When considering taxonomic composition, no significant differences for AM fungal community composition in root and soil were found in either South Africa or Argentina. In the case of phylogenetic community composition, we found significant differences in root AM fungal communities from South Africa.

DISCUSSION

Our results support the hypothesis that the impact of alien EcM pines on local AM fungal communities differs significantly between regions with different biogeographic histories. In South Africa, where EcM fungi have been historically absent, planting of alien EcM pines has resulted in a decrease in local AM fun-

gal richness and in significant changes to AM fungal community composition. In Argentina, where EcM trees and EcM fungi are a natural component of ecosystems, introducing alien EcM pines did not result in changes to the richness or composition of local AM fungal communities. At the same time, the presence of alien pines resulted in slight changes to the phylogenetic structure of root AM fungal communities in both regions. In South Africa, we found that clearcut areas in EcM pine plantations exhibited richness and composition of AM fungal communities that were intermediate between the native fynbos and the non-native pine plantation, suggesting that natural regeneration of the former AM fungal community had begun to occur following pine removal and recovery of AM vegetation. These findings suggest that despite programs of pine afforestation lasting at least 20 years in the studied locations, recovery of original AM fungal communities remains possible.

The potential for changes to occur in mycorrhizal associations following introduction of alien plants has been discussed in several reviews (Richardson et al. 2000; Pringle et al. 2009; Inderjit and van der Putten 2010; Johnson et al. 2013), but information about actual shifts in the (molecular) diversity and composition of fungal communities remains scarce. Descriptions of AM fungal communities in the roots of alien plants indicate some differences to those in native plant roots (Moora et al. 2011; Lekberg et al. 2013; Bunn et al. 2014). Such differences may occur due to overall shifts in local AM fungal community composition due to plant invasion, but may also reflect host specific differences in fungal communities, irrespective of the alien status of the plant species. Barto et al. (2011) focused on the roots of native tree seedlings in sites invaded by a nonmycorrhizal exotic plant and recorded changes to AM fungal community composition, but not

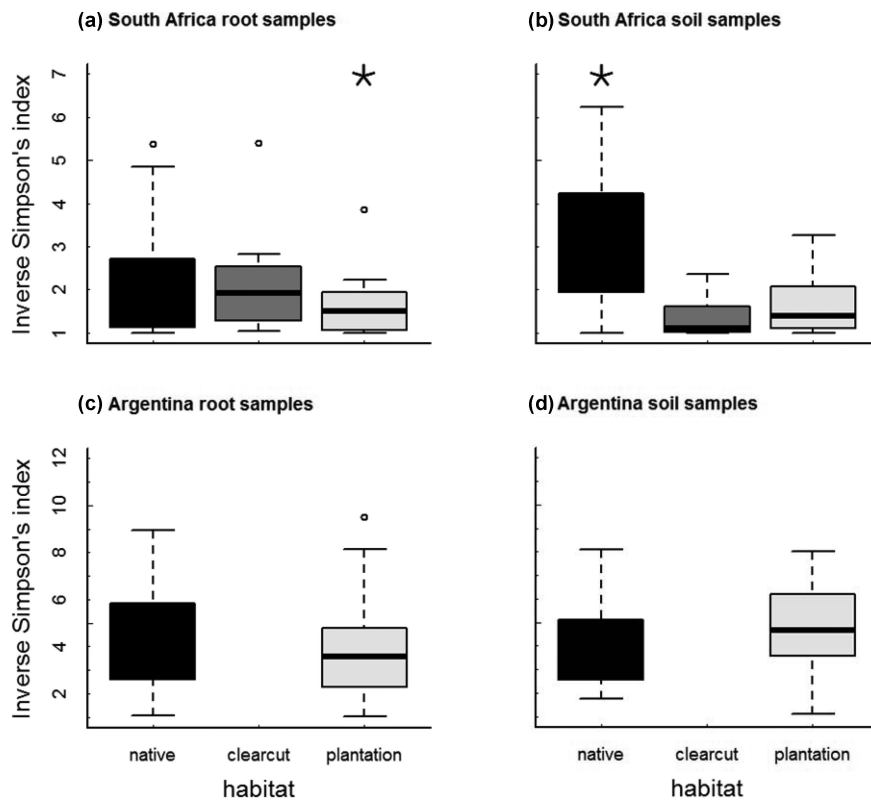


Figure 4. AM fungal taxon (VT) diversity in samples (inverse Simpson index) across regions and habitats. Asterisks indicates significant difference between habitats (see Table 2). Colors indicate different habitat types: black—native; grey—clearcut; light—pine plantation (cf. Fig. 1). Median (bold line), interquartile range (box) and range (whiskers) are shown (outlying values are indicated by points).

Table 2. Results of linear mixed effect models. AM fungal VT richness per sample (VTR), rarefied VT richness and inverse Simpson diversity (1/S) per sample for root and soil communities were regressed against habitat type—native (fynbos or araucaria forest), pine plantation, clearcut (in South Africa). Note that different random effect structures were used for models with root and soil richness as response variables: a nested structure (host within plot) was used for root models while a single-level structure (plot) was used for soil models. F statistics and their associated probabilities are shown. F statistics and their associated probabilities are shown and statistically significant values are indicated in bold.

		VT		Rarefied VTR		1/S	
		Habitat		Habitat		Habitat	
		F	P	F	P	F	P
Root	South Africa	16.32	0.001	15.32	0.001	6.37	0.04
	Argentina	0.97	0.35	1.10	0.32	1.52	0.25
Soil	South Africa	12.52	0.040	12.97	0.03	8.82	0.06
	Argentina	0.11	0.93	0.29	0.88	0.25	0.67

to diversity. Meanwhile, Bunn *et al.* (2014) recorded only small changes in soil and root AM fungal community composition in local plant communities under stronger invasion pressure.

In many parts of the world, alien EcM trees are planted into matrices of natural vegetation that is predominantly AM (Nunez and Dickie 2014), and some of the introduced tree species go on to become invasive. Furthermore, in regions where the natural biota does not contain EcM fungi, EcM trees used in forestry are accompanied by EcM fungal inoculum (Schwartz *et al.* 2006; Nunez and Dickie 2014). Introduction of EcM trees and fungi to ecosystems has significant potential to alter ecosystem processes (Hynson *et al.* 2013; Nunez and Dickie 2014), and this in turn may influence the performance of soil microbes, including local AM fungi. To the best of our knowledge, our study is the first to directly record changes to AM fungal communities in a

region where EcM plants have been introduced into natural AM vegetation. The data from South Africa confirm an important effect of novel introduced EcM trees and fungi on local AM fungal communities. However, in Patagonia, where EcM fungi are naturally present, the increased share of EcM plants brought about by the introduction of alien pines did not result in significant changes to local AM fungal communities. It is known from previous studies that EcM plants can exert a negative influence on AM fungi by causing alterations to the chemical properties of soil (Tyndall 2005; Cumming and Kelly 2007) and litter (Piotrowski, Morford and Rillig 2008; Becklin, Pallo and Galen 2012). A more complex view is presented by Dickie *et al.* (2014) who proposed that EcM fungi could inhibit organic matter decomposition by altering the substrate stoichiometry, thus, reducing mineralization rates and decreasing the availability of mineral nutrients to

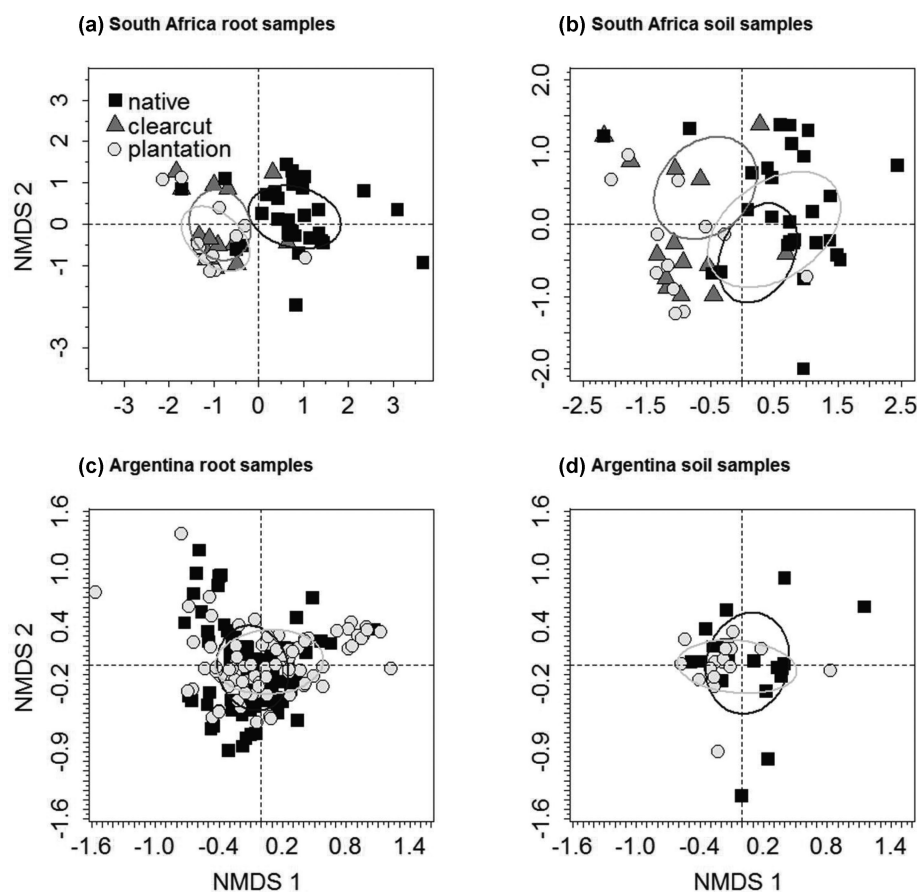


Figure 5. NMSD ordination showing variation in AM fungal taxonomic community composition across habitats and regions. The ordination was calculated using Bray-Curtis distance and sample relative abundance data. Colors indicate different habitat types: black—native; grey—clearcut; light—pine plantation (cf. Fig. 1). Dispersion ellipses represent one standard deviation of point scores around group centroids.

Table 3. PERMANOVA analyses performed to test the differences in taxonomic and phylogenetic AM fungal community composition among habitat types. The analyses were performed using AM fungal composition, either taxonomic or phylogenetic, as response matrix and habitat type as fixed factor (plant species identity as random factor in the case of root samples). The pseudo *F* statistic and its associated probability are shown. Post hoc PERMANOVA pair-wise tests were applied in the case of the data from South Africa to test for the differences between each habitat type. Letters represent groups based on the statistical differences between habitat types: native (fynbos), plantation (pine plantation) and clearcut. Statistically significant values are indicated in bold.

		Habitat		Native	Plantation	Clearcut
Taxonomy						
Root	South Africa	F	P	a	b	b
	Argentina	1.18	0.50			
Soil	South Africa	2.74	<0.01	a	b	c
	Argentina	0.88	0.52			
Phylogeny						
Root	South Africa	3.47	0.08			
	Argentina	1.75	0.01			
Soil	South Africa	2.08	0.05	a	ab	b
	Argentina	0.98	0.52			

AM plants. Wider ecosystem changes resulting from the presence of EcM vegetation, such as reduced AM host plant density, may also importantly influence AM fungal communities.

Our finding that changes to AM fungal diversity in the presence of introduced EcM pines were most pronounced in the region, where EcM plants have historically been absent appears likely to reflect mechanisms acting over biogeographic time

scales. Biotic and abiotic filters to AM fungal community composition imposed by introduced EcM pines in Argentina have presumably been present throughout the long-term history of coexistence between AM and EcM vegetation. While our data cannot provide information about the precise mechanisms that have occurred, it seems plausible that the historical presence of filters imposed by EcM vegetation has shaped local AM fungal

Table 4. Indicator species analyses. The table shows the number of AM fungal molecular taxa (VT) significantly associated with each habitat type, and their indicator species value (de Caceres and Legendre 2009) and associated probability. We show the VT associated with each habitat type: native or invaded habitat (considering either pine plantation, clearcut or both in South Africa) when considering root or soil data.

Family	Species	AMF VT	Indicator value	P-value
		South Africa		
		Root		
Glomeraceae	Glomus	Native VT360	0.730	0.001
Glomeraceae	Glomus	VT112	0.608	0.008
Gigasporaceae	Scutellospora, Dentiscutata colliculosa, dipapillosa, heterogama, reticulata	VT255	0.511	0.042
Glomeraceae	Glomus	VT370	0.483	0.024
Glomeraceae	Glomus	VT103	0.483	0.037
Glomeraceae	Glomus	Plantation VT166	0.597	0.005
Glomeraceae	Glomus	VT362	0.516	0.012
Claroideoglomeraceae	Claroideoglomus	VT57	0.754	0.002
Glomeraceae	Glomus	VT253	0.638	0.001
Glomeraceae	Glomus	VT399	0.609	0.002
Acaulosporaceae	Acaulospora laevis, scrobiculata	VT30	0.577	0.001
Glomeraceae	Glomus	VT185	0.471	0.026
		Soil		
Claroideoglomeraceae	Claroideoglomus, claroideum, etunicatum, lamellosum, luteum, viscosum, Entrophospora infrequens	Native VT193	0.728	0.002
Archaeosporaceae	Archaeospora	VT338	0.721	0.001
Ambisporaceae	Ambispora callosa, leptoticha	VT242	0.705	0.009
Glomeraceae	Glomus	VT360	0.686	0.001
Glomeraceae	Glomus	VT194	0.594	0.006
Glomeraceae	Glomus	VT154	0.594	0.007
Glomeraceae	Glomus	VT370	0.594	0.011
Glomeraceae	Glomus	VT191	0.542	0.020
Glomeraceae	Glomus	VT149	0.542	0.021
Gigasporaceae	Scutellospora, castanea, fulgida, gilmorei, gregaria, persica, weresubiae, Racocetra tropicana	VT41	0.529	0.038
Archaeosporaceae	Archaeospora trappei, schenckii	Plantation VT245	0.809	0.002
Acaulosporaceae	Acaulospora lacunosa, mellea	VT24	0.471	0.050
Acaulosporaceae	Acaulospora laevis, scrobiculata	VT30	0.744	0.031
		Argentina		
		Root		
Glomeraceae	Glomus	Native VT247	0.480	0.011
Archaeosporaceae	Archaeospora	Plantation VT338	0.310	0.015
		Soil		

taxon pools, by filtering out those AM fungal taxa unsuited to the conditions created by EcM vegetation. It is notable that the total number of AM fungal molecular taxa found in the naturally EcM ecosystems in Argentina was somewhat lower than in naturally EcM-free South Africa, although both were relatively taxon rich when compared to similar available data from other sites (Öpik et al. 2013). However, it is also possible that AM fungal lineages cooccurring with EcM vegetation in Argentina adapted to effectively exploit the soil nutrient or other conditions influenced by EcM plants, while South African lineages—representing some of the same VT present in Argentina—did not.

It should be noted however that ecological conditions may have varied in the two regions in ways not directly related to the introduced EcM pines. While introducing alien pines in *Araucaria* forest does not bring about major changes in vegetation structure, planting of pines in fynbos shrubland considerably alters vegetation structure. Light conditions in the understorey of emerging forest undergo changes that may influ-

ence local AM fungal communities (Öpik et al. 2009). Although we do not have detailed information about plant community changes in the study sites, it is perhaps notable that we were able to sample most of the same dominant plant species in the *Araucaria* forest and pine plantation in Argentina, but only one sampled dominant plant species was shared between different South African habitats. Although the effect of host species identity on AM fungal community composition appears to be relatively weak (Davison et al. 2015), the more profound change to plant community composition brought about by planting pines in South Africa, compared to Argentina, might still contribute to different responses of AM fungal communities in the two continents.

We also found that AM communities in both regions experienced small shifts in phylogenetic composition in response to the presence of the alien pine. Because the phylogenetic relatedness of AM taxa may to a certain degree reflect functional similarity (Chagnon et al. 2013), this could represent the

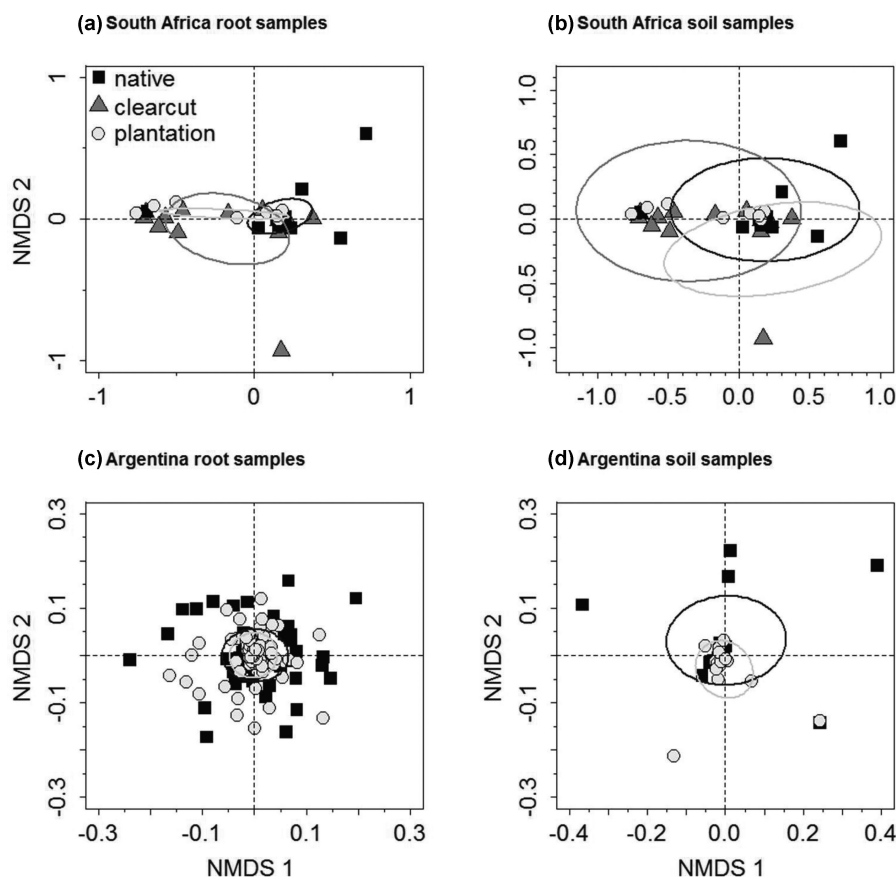


Figure 6. NMDS ordination showing variation in AM fungal phylogenetic community composition across habitats and regions. The ordination was calculated using relative-abundance-weighted phylogenetic distances between samples. Colors indicate different habitat types: black—native; gray—clearcut; light—pine plantation (cf. Fig. 1). Dispersion ellipses represent on standard deviation of point scores around group centroids.

proliferation of lineages with functional attributes that enable them to coexist with the alien pine. It should be noted that, as with many biogeographic-scale investigations, our study had limited opportunity for replication, and particular characteristics of the sites or introduced pine species may have influenced our results in ways unconnected to the biogeographic context.

Our data from South Africa suggest that changes to AM fungal communities brought about by the introduction of EcM plants and fungi are not irreversible, at least following an introduction that persists for about 20 years. We recorded a proliferation of AM fungal diversity in formerly pine-planted areas of fynbos. Following clearcutting of pines, vegetation containing a greater share of AM plant species starts to develop and this appears to facilitate the development of a diverse AM fungal community (Korb, Johnson and Covington 2003; Overby et al. 2015). There is evidence that AM fungal spores may maintain their viability in the absence of host plants for several years (Varga et al. 2015). Furthermore, dispersal of AM fungi to a virgin habitat from the neighborhood within less than one year has been reported (Dodd et al. 2002). Together with the soil seed bank (Heelemann et al. 2013), an AM fungal propagule bank would create favorable conditions for attempts to regenerate and restore valuable native vegetation such as fynbos, where over half of plant species characteristically form AM (Allsopp and Stock 1993). Our findings provide hope that appropriate management of alien pines can allow AM fungal communities to recover toward a state associated with undisturbed vegetation.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

ACKNOWLEDGEMENTS

The authors would like to thank CapeNature for providing research permit AAA005-00159-0028.

FUNDING

This research was funded by grants from the Estonian Science Foundation (9050, 9157); Estonian Research Council (20-28); the European Regional Development Fund (Centre of Excellence EcolChange); and the Ministry of Economy and Competitiveness (Contrato Formacion Postdoctoral MINECO—FPDI 2013-16600 to AG).

Conflict of interest. None declared.

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