

# Species composition of arbuscular mycorrhizal fungi differ in semi-natural and intensively managed pastures in an isolated oceanic island (Terceira, Azores)

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**Abstract** Nothing is published about the arbuscular mycorrhizal fungi (AMF) of the Azores archipelago, either with regard to individual species, or at the community level. This study, based on identification through spore morphological characteristics, compares the AMF community structure of semi-natural and intensively managed pastures. Thirty-nine glomeromycotan fungal spore types were detected in soil, with species in the genera *Acaulospora*, *Ambispora*, *Archaeospora*, *Claroideoglossum*, *Entrophospora*, *Gigaspora*, *Paraglossum*, *Sclerocystis*, *Scutellospora sensu* Morton and Msiska (Mycorrhiza 20 483–496, 2010) and *Rhizophagus*. The two most representative groupings were the glomoid spore types and *Acaulospora* with 13 and 10

species respectively, followed by *Scutellospora* with 3. The glomeromycotan fungal richness was similar for both intensive and semi-natural pastures, with 28 spore types in the former and 23 in the latter but their composition differed. Semi-natural pastures were dominated by species from *Acaulospora* and *Scutellospora*, particularly *S. calospora* and *A. cf. myriocarpa*, while for intensively farmed pastures, species with glomoid spores, and members of the two genera *Claroideoglossum* and *Paraglossum* were found most frequently and abundantly. Spore densities of the most commonly found groupings — *Acaulospora*, *Claroideoglossum*, *Scutellospora* and the glomoid spores were correlated with soil chemical properties, suggesting that soil characteristics influence the AMF communities. These results indicate that intensity of pasture management may not influence AMF richness but is probably an important factor influencing their composition and abundance.

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management · Pastures

## 1 Introduction

Arbuscular mycorrhizal fungi (AMF) are symbiotic associations between plants and obligate symbiotic fungi of the phylum *Glomeromycota* thought to have originated 400–500 million years ago (Schübler et al. 2001). In this intimate association the fungi provide their hosts with minerals nutrients (particularly inorganic phosphate) and water (Querejeta et al. 2007), and increase resistance to biotic and abiotic stresses, including pathogens (Pozo and Azcon-Aguilar 2007), water limitation and environmental pollutants (Hildebrandt et al. 2007). In turn, the fungi obtain carbon in

organic compounds from their photoautotrophic partners (Smith and Read 2008).

These beneficial effects of AMF are important in natural ecosystems, although it has been suggested that they may be less functionally important in high-input agriculture (Barea and Jeffries 1995; Galvez et al. 2001). Agricultural practices such as intensity of cultivation, nature and quantity of applied fertilisers and plant protection strategies have considerable impacts on soil and soil organisms (Miller et al. 1995; Boddington and Dodd 2000). The community structure of AMF is no exception (Sieverding 1989; Douds and Millner 1999; Oehl et al. 2003; Li et al. 2007; Liu et al. 2012), but it has proved difficult to identify any consistent effects attributable to different management practices such as tillage, fertiliser input, pesticide use and crop rotation (Gosling et al. 2006; Gai et al. 2006). Populations of AMF can be affected by disturbance or change of host plant use, and Jansa et al. (2002; 2003) reported that tillage affected significantly the community structure of AMF, decreasing the sporulation of some members of the *Diversisporales*. Also Oehl et al. (2004) found that differences between conventional *versus* organic farming were reflected in both species richness and composition. Although they detected no change in abundance of species of *Glomus* (in the broad sense that the genus name was used before Schübler and Walker 2010: see below) between farming systems, the same pattern was not observed in relation to spore abundances of *Acaulospora* and *Scutellospora* species, which were more abundant in organic systems. These findings suggest that conventional management practices diminish local differences in community composition, resulting in homogenization and domination by AMF generalist species.

AMF respond to a range of environmental factors such as pH and nutrient status (Helgason and Fitter 2009; Dumbrell et al. 2010). It has been showed in several studies that pH it is an important factor influencing the AMF species composition (Wang et al. 1985; Ezawa et al. 2001; Oehl et al. 2010). Some AMF species (e.g., some former '*Glomus*') are said to prefer alkaline and neutral soil, while others (e.g., *Acaulospora*) are thought to sporulate more abundantly in acid soils (Gai and Liu 2003; Oehl et al. 2010). Differences in AMF composition between conventional and organic systems can also be explained by the nutrients applied which lead to different levels of soil phosphorus (P) (Eason et al. 1999; Oehl et al. 2004). Higher available soil-P can reduce AMF formation, either through a direct effect on external hyphal growth or indirectly associated with host P status (Sanders 1975). The genus '*Glomus*' due to its large ecological plasticity is generally dominant in intensively managed sites (Oehl et al. 2003, 2004), being for this reason classified as a P-tolerant genus (Johnson 1993; Bhadalung et al. 2005).

Land use changes are important factors impacting biodiversity in island ecosystems. The native forests of the Azores archipelago had been mostly destroyed since human

occupation and converted into agricultural land (Martins 1993; Borges et al. 2008). During the second half of the 20th century, after failure of different monoculture crops such as wheat (*Triticum* spp.), woad (*Isatis tinctoria*) and orange (*Citrus* spp.), milk production has grown in importance (Martins 1993; Borges and Brown 1999), and intensively managed pastures now dominate the landscape in the archipelago, covering 67.7 % of the arable land. There are also semi-natural pastures comprising 11.2 % of the total area (Garcia and Furtado 1991), most of which are located at high altitude in recent infertile acid volcanic soils. The intensively managed pastures are dominated by introduced grasses and legumes, whereas native legumes such as *Lotus uliginosus*, grasses, rushes, sedges and ferns are more frequent in the less intensively managed semi-natural localities (Borges and Brown 1999). Both kinds of pasture are dominated by *Holcus lanatus*, a non-indigenous plant with a frequency more than 50 % (Dias 1996; Silva and Smith 2006; Kueffer et al. 2010). In Terceira Island it is very common, being the third most frequent exotic plant (Silva and Smith 2006) in all kinds of soil.

Although AMF are generally considered of great ecological importance, nothing is published about the diversity and composition of communities in the Azores. Traditional studies on AMF diversity are based mainly on spore morphology (Walker 1992). However, taxonomic identification of AMF spores collected directly from the field is quite difficult because they are often unidentifiable due to degradation or parasitism by other organism, and thus do not necessarily reflect the AMF populations in soil (Clapp et al. 1995). Additionally, fungal spore diversity differs seasonally, with some fungi sporulating in late spring and others at the end of summer. However, an advantage is that spore numbers are indicators over a longer time period, i.e. over months, which allow us to describe AMF community structures based on spore morphology (Douds and Millner 1999; Oehl et al. 2003), with the exception of putative non-sporulating AMF species. However, the extraction of spores from soil is a rapid and non-expensive method, and it may reveal taxa not easily detected by molecular methods (e.g. Gollotte et al. 2004; Gamper et al. 2009; Krüger et al. 2009). The use of successive trap cultures and subsequent extraction and study of spores takes much longer but also reveals significantly greater diversity than a 'one-off' spore extraction (Oehl et al. 2004).

We assessed the qualitative and quantitative differences of the AMF community, based on spore morphological characterisation in semi-natural and intensively managed pastures in Terceira Island, the third largest island of the Azorean archipelago. We predict that both species richness and abundance of AMF will be lower in intensively managed pastures, and species composition should differ between semi-natural and intensively managed pastures.

## 2 Materials and methods

### 2.1 Study sites and sampling procedure

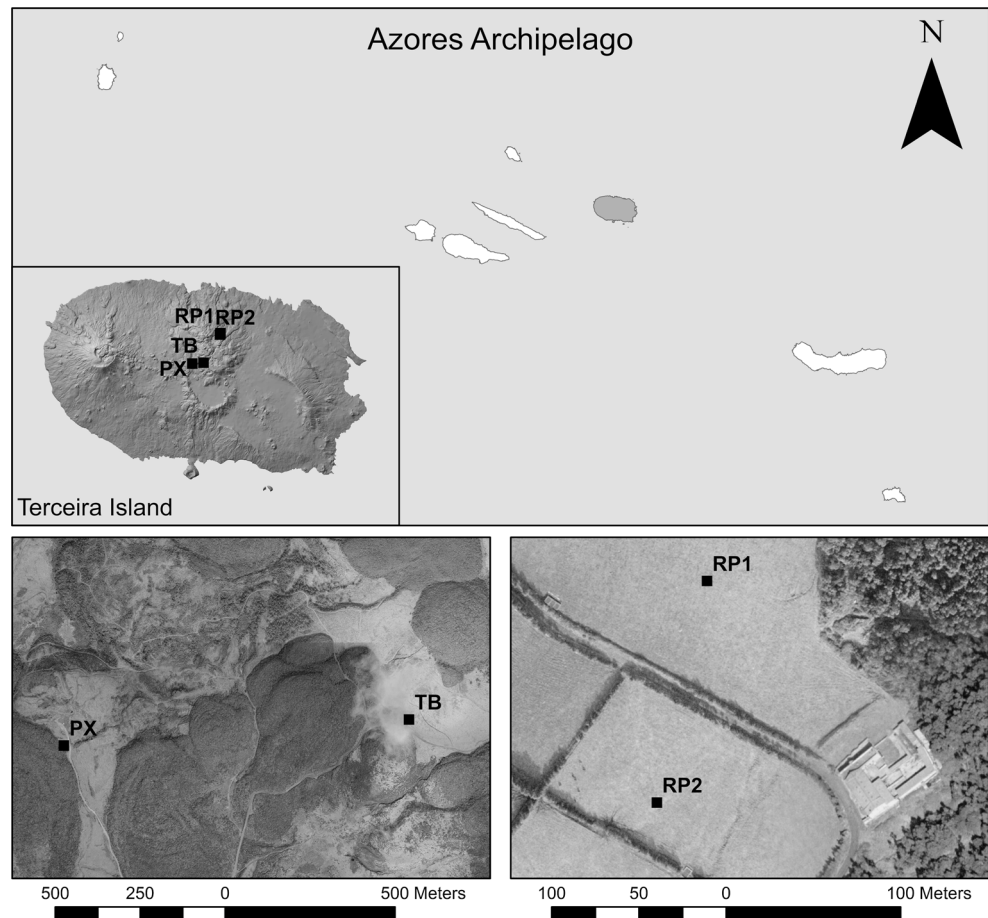
This study was conducted in the Terceira Island, the third island of the Azorean archipelago in terms of size (402 km<sup>2</sup>). The Azores archipelago have a temperate oceanic climate characterised by high levels of relative atmospheric humidity that could reach 95 % at high altitude native forests and ensures slight thermal variations throughout the year (Azevedo et al. 1999). The average temperature is 17.5 °C in low altitudes, while the maximum temperature is in August and minimum in February (Azevedo 1996). The pluviometric regime reaches its peak in January-February and minimum in July (Azevedo 1996): Angra do Heroísmo (47 m): 969 mm year (140 mm in January and 40 mm in July) and Serra de S. Bárbara (1.023 m): 3.000 mm year (Borges 1997).

The sampling areas were cattle-grazed upland pastures of two different types: semi-natural pastures with low grazing intensity and frequency (managed for more than 50 years, with a relatively high diversity of grasses and forbs; see Borges and Brown (2001) and intensively managed pastures with high grazing intensity and frequency (managed for more

than 30 years, characterised also by a depauperate vascular flora of five or fewer dominant species).

The semi-natural pastures, Pico Galhardo (PX) (38° 41' 51.71" N 27° 13' 25.34" W) with a area of 4.8 ha, and Terra Brava (TB) (38° 41' 59.74" N Longitude 27° 12' 41.37" W) with a area of 12.4 ha (Fig. 1), are included in the Natura 2000, a site of communitarian interest (SIC- Serra de Santa Barbara e Pico Alto), and are part of the protected area Terceira Island Park. They are at high-altitude, and therefore located in the called "cloud-zone forest" (Sjögren 1990), where the rainfall (vertical and horizontal) is high, leaving the air saturated with moisture and the soil waterlogged. In these conditions, anoxia phenomena occur in the soil, decreasing the mineralisation of soil organic matter (Borges 1997). They are dominated by the perennial grasses *Holcus lanatus* and *Agrostis castellana*, have a high floristic diversity (Dias 1996; Borges 1997), often including other grasses such as *Anthoxanthum odoratum*, *Lolium multiflorum*, *Holcus rigidus* and *Poa trivialis* and non-forage species, including *Lotus uliginosus*, *Rumex acetosella* ssp. *angiocarpus*, *Potentilla anglica*, *Hydrocotyle vulgaris*, *Plantago lanceolata*, *Lobelia urens*, *Cerastium fontanum*, *Conyza bonariensis*, *Anagallis arvensis*, *Hypochoeris radicata*, *Ranunculus repens*, *Pteridium*

**Fig. 1** Location of the semi-natural (PX and TB) and intensively managed (RP1 and RP2) pastures in Terceira island



*aquilinum* and *Juncus effusus* (Dias 1996; Borges 1997). The traditional silvopastoral system is practiced in these pastures where bullfight cattle are given free range. Fertilization is not allowed and the number of cattle is limited. The intensively managed pastures, Agualva 1 (RP1) at 38° 45' 27.18" N 27° 11' 41.55" W, with an area of 6.4 ha, and Agualva 2 (RP2) 38° 45' 24.44" N 27° 11' 42.24" W, with an area of 1.2 ha, (Fig. 1). These pastures resulted of the conversion of native forest to wood production and, finally, to permanent pastures. The pastures show different topographic conditions resulting of the conversion process, which limit the management practices, and change the intensity of grazing (Borges and Brown 1999). They are surrounded by exotic eucalyptus plantations. Though dominated by *H. lanatus* and *Lolium perenne* they may also have high populations of *Trifolium repens* (Borges 1997; Dias 1996) *P. lanceolata*, *Cyperus esculentus*, *Mentha suaveolens*, *Cerastium fontanum* and *Rumex conglomeratus* (Dias 1996; Borges 1997). They are characterised by closed fields in large dimensions with wooded fencerows of *Cryptomeria japonica*, (Borges 1997). The grazing system is rotative characterised by heifers and beef cattle with a stocking rate average of 2.5 cows/ha during the late spring and early autumn (Borges 1997). Every year is applied Nitrogen (NH<sub>4</sub>NO<sub>3</sub> with 27 % N, 81 kg ha<sup>-1</sup>) to both pastures after each period of grazing with approximately 5 weeks between the next grazing. Every 5 years the pastures are tilled and re-sow with annual grasses such as *Lolium perenne*.

## 2.2 Sample collection

A total of 40 soil samples with associated roots were randomly collected in the target areas in August 2007, of which twenty were in semi-natural pastures (PX and TB) and the remaining twenty in the intensively managed pastures (RP1 and RP2). Approximately 2 kg of soil was collected from the rooting zone of the dominant plant species, *H. lanatus*, to a depth of 20 cm. The soil samples were air-dried, passed through a 2-mm sieve and stored at 4 °C before analysis.

## 2.3 Soil analyses

The soils of semi-natural and intensive pastures are sandy loams (Pinheiro 1990). The soil analyses (Table 1) were

performed on samples collected in August 2007. Ten samples from each site were pooled and analysed at the University of Azores Soil Laboratory (CITA-A). Potassium (K), calcium (Ca) and magnesium (Mg) were extracted with sodium acetate (1/5) at pH 7, and determined using a Varian ICP atomic emission spectrophotometer. Soil pH was measured from a soil and water paste (1:2.5 v/v), and available phosphorus (P) (Olsen and Sommers 1982) by atomic absorption spectrometry after extraction with a 0.5 M NaHCO<sub>3</sub> solution at pH 8.5. Total soil nitrogen (N) (Kjehldahl) content (Allen 1989) and organic matter (OM) (by dry-ashing) were also measured.

## 2.4 Establishment of trap cultures

Open pot trap cultures (Gilmore 1968) were established with 1-week-old *Zea mays* seedlings in fresh soil from each site mixed with autoclaved sand and volcanic soil "bagacina" (2:1:1 v/v/v). Ten pots containing 1.5 kg of the mixture were prepared from each of 10 field soil samples per site. Host plant seeds were surface sterilised by immersion in alcohol (96 %) for 30 s, and 4 % bleach for 2 min, followed by two rinses in sterile distilled water, sown in pots on sterilised sand soil, and germinated in the greenhouse. Four seedlings were transplanted to each pot, and maintained in a greenhouse for 5 months. All pots were watered every 2 days with distilled water, and no nutrient solution was added.

## 2.5 Spore extraction and morphological identification

This work was a preliminary study of AMF diversity and composition based on spores extracted from trap cultures, because identification from field-collected samples is hampered by such factors as parasitism, degradation through age and environmental alteration (e.g., discoloration) and low spore numbers. Discrepancies between data from trap cultures and field samples are likely to be the result of the conditions in the pot cultures, which may favour certain AMF taxa (Brundrett et al. 1999; Oehl et al. 2003, 2004; Hijri et al. 2006; Wang et al. 2008). Oehl et al. (2004) reported that three of 35 species recorded at a field site failed to produce spores from such traps. Also, Hijri et al. (2006) noted that *Paraglomus* was never detected in trap cultures, although it occurred frequently in the field site experiment. However, the

**Table 1** Chemical soil parameters at field sites differing in land use in August 2007. Semi-natural pastures (PX – Galhardo; TB – Terra Brava); intensively managed pastures RP1 – Agualva 1; RP2 – Agualva 2. The soil analysis for each site was derived from a pooled sample ( $n=10$ )

Land use	Field site	pH	P (Olsen) (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	N (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	OM (%)
Semi-natural	PX	5.7	7	143	0.55	93	84	24
	TB	5.7	11	190	0.40	89	73	21
Intensive	RP1	5.7	4	102	0.11	54	17	14
	RP2	5.7	10	80	0.26	45	13	16

opposite was observed in relation to *Archaeospora trappei* which only be detected in trap cultures (Hijri et al. 2006). Consequently, trap culture may provide a different picture of AMF communities than analysis of field-collected roots, which could contribute to a lack of consistent differences across the two managements practices investigated. However, trap cultures may give a better indication of what was actually alive in the soil, whereas spores from soil might represent dead leftovers from species that were present in the past, but have died out.

Glomeromycotan spores were extracted from 50 g of air-dry soil of each sample from field soil and trap cultures by wet sieving and sucrose centrifugation (Walker et al. 1982) and were stored at 4 °C in autoclaved water pending examination. Different spore types were initially separated under a stereomicroscope, and then examined through a compound microscope in a 1:1 mixture of polyvinyl alcohol lacto-glycerol (PVLG) and Melzer's reagent. They were then classified into known species, or spore types that could not be placed in a current species, based on colour, size, surface ornamentation, hyphal attachment, reaction to Melzer's reagent, and wall structure. Counts were made for of the total number of spores of each type.

There is considerable confusion and controversy with regard to the taxonomy of the *Glomeromycota*. There is a revision based largely on molecular evidence (Schüßler and Walker 2010; Krüger et al. 2012), and another erecting a large number of genera (Oehl et al. 2011a), particularly for the genus *Glomus* in a broad sense, based largely on the appearance of the subtending hypha of the spore and the nature of the occlusion of spore contents. These two views are largely incompatible, and we have chosen to follow the former. Consequently, we use the genus '*Glomus*' (which we therefore continue to place in inverted commas) in a broad sense, including the species that are listed in Schüßler and Walker (2010) as 'Species of uncertain position in *Glomus sensu lato*', and we similarly follow the narrow definitions of *Rhizophagus*, and *Sclerocystis* as circumscribed in that publication, and the germination shield bearing genera (termed 'the *Scutellospora* group' herein) in the *Gigasporaceae* as presented in Redecker et al. (2013).

## 2.6 Data analyses

Spore density, richness, and occurrence were based on characterisation of spores extracted from the trap cultures, because most of them extracted directly from field soil were in low number and parasitised by other organism, which could hinder accurate identification, thus all results after this point are relate to trap cultures.

Spore density (number per 50 g dried soil), frequency of occurrence (number of samples containing particular taxon) and species richness (number of taxa found in a particular land

use type) were calculated. We conducted a two-dimensional ordination of the four sites using nonlinear multidimensional scaling (MDS) in the software Community Analysis Package v. 4.0 (CAP 4) (Seaby et al. 2004). This method has some advantages over correspondence analysis (CA) and Principal component analysis (PCA) because it can be performed with any similarity measure and is independent of judgment by the researcher. Bray-Curtis distances were computed to model dissimilarities. A log-linear saturated model was constructed to examine the relationships among the land use types.

Pearson correlation coefficients were calculated for chemical properties of each soil type and the total AMF spore density or the AMF spore density of the more common groups (*Acaulospora*, *Claroideoglomus*, '*Glomus*' and the *Scutellospora* group).

The sampling followed a hierarchical nested design, with field sites nested within land use type. Variation of species richness between land uses and field sites within each land use was analysed by Nested ANOVA (Minitab version 13.31, 2000). Variation of total AMF spore density and of the commonest genera (*Acaulospora*, *Claroideoglomus*, '*Glomus*' and the *Scutellospora* group) was similarly analysed. All data were tested for normality to fulfil the assumptions of Nested ANOVA.

## 3 Results

### 3.1 AMF spore types

Thirty-nine AM spore types from among eleven genera were detected in the soil traps from the two land use types studied (Table 1). However, only six of these were detected by extraction directly from soil: *Gigaspora* sp., *Acaulospora laevis*, *A. cf koskei*, *Sclerocystis rubiformis*, *Scutellospora calospora* and *Cetraspora pellucida* (Table 2).

Nineteen species could not confidently be named to species, the majority of them belonging to the genera '*Glomus*' (13) and *Paraglomus* (2) followed by *Acaulospora*, *Ambispora*, *Gigaspora* and *Racocetra* each with just one species not identified to the species (Table 2). The two most representative genera were '*Glomus*' and *Acaulospora* with 14 and 10 species respectively, followed by the genera *Paraglomus* and the *Scut.* group, each with 3 species (Table 2).

### 3.2 AMF species richness and composition

Similar numbers of species were found from soil traps from both land uses types. The semi-natural and intensive pastures

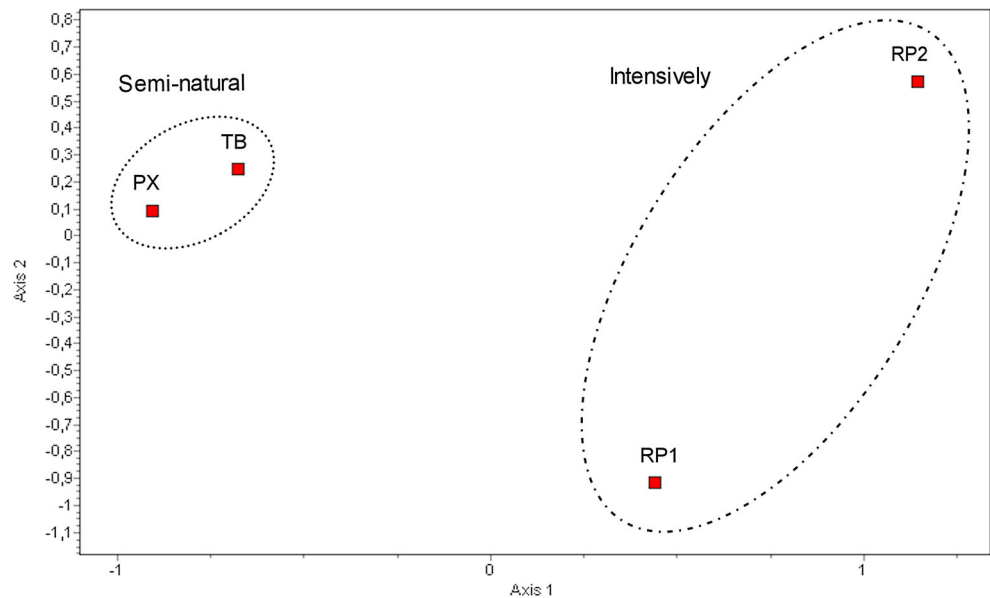
**Table 2** Species and unidentified morpho-taxa of glomeromycotan fungi extracted from semi-natural (PX and TB) and intensively managed (RP1 and RP2) pastures. Spore density based on ten subsamples of 50 g air dried substrate from soil trap pot culture. The AMF species is listed by spores density in the soil trap from semi-natural pastures, allowing a comparison with differential species density in intensively managed pastures

Management type	Spore density (spores per 500 g air dried substrate)					
	Semi-natural			Intensive		
	PX	TB	Total density	RP1	RP2	Total density
Pasture identity						
Species recorded						
<i>Acaulospora</i> cf. <i>myriocarpa</i>	75	26	101	12	4	16
<i>Scutellospora calospora</i>	62	29	91	24	4	28
<i>Cetraspora pellucida</i>	27	24	51	5	1	6
<i>Acaulospora laevis</i>	21	27	48	1	3	4
<i>Paraglomus</i> sp. 1	0	35	35	54	39	93
<i>Acaulospora</i> sp.	9	16	25	0	0	0
<i>Racocetra</i> sp.	9	14	23	1	1	2
<i>Acaulospora</i> cf. <i>paulinae</i>	8	8	16	15	44	59
<i>Ambispora</i> sp.	11	4	15	0	0	0
<i>Acaulospora excavata</i>	0	15	0	0	0	0
<i>Acaulospora thomii</i>	13	0	13	0	0	0
<i>Acaulospora delicata</i>	12	0	12	0	0	0
<i>Acaulospora elegans</i>	12	0	12	0	0	0
<i>Archaeospora schenckii</i>	10	0	10	0	0	0
Glomoid spore type 4	0	10	10	0	0	0
<i>Acaulospora tuberculata</i>	0	8	8	0	0	0
<i>Gigaspora</i> sp.	8	0	8	0	0	0
<i>Sclerocystis rubiformis</i>	3	5	8	3	4	7
Glomoid spore type 3	0	7	7	0	0	0
<i>Acaulospora</i> cf. <i>koskei</i>	6	0	6	5	1	6
<i>Rhizophagus clarus</i>	2	2	4	14	14	28
<i>Rhizophagus</i> cf. <i>intraradices/irregularis</i>	4	0	4	0	0	0
Glomoid spore type 1	3	0	3	9	0	0
Glomoid spore type 2	3	0	3	0	9	9
<i>Claroideoglomus etunicatum</i>	0	0	0	51	27	78
<i>Paraglomus brasilianum</i>	0	0	0	6	9	15
Glomoid spore type 5	0	0	0	14	0	14
Glomoid spore type 9	0	0	0	0	11	11
Glomoid spore type 6	0	0	0	9	0	9
Glomoid spore type 7	0	0	0	9	0	9
Glomoid spore type 11	0	0	0	0	9	9
<i>Paraglomus</i> sp. 2	0	0	0	0	9	9
Glomoid spore type 10	0	0	0	0	8	8
<i>Claroideoglomus lamellosum</i>	0	0	0	0	8	8
<i>Entrophospora infrequens</i>	0	0	0	0	6	6
Glomoid spore type 8	0	0	0	6	0	6
Glomoid spore type 12	0	0	0	0	6	6
Glomoid spore type 13	0	0	0	0	6	6
<i>Glomus globiferum</i>	0	0	0	0	3	3

yielded 23 and 28 glomeromycotan taxa or spore types respectively, and no significant differences were found in species richness between land uses (Nested Anova:  $F=1.39$ , d.f.=1,2,  $p=0.25$ ) or between field sites within each land use (Nested Anova:  $F=0.29$ , d.f.=1,2,  $p=0.75$ ).

In contrast, AMF species composition differed between land uses. Along with the log-linear analysis, the Bray-Curtis-based MDS analysis highlighted differences in community composition for the different tillage systems (Fig. 2). The first axis, clearly separated the semi-natural locations with

**Fig. 2** Bray-Curtis-based multidimensional scaling (MDS) analysis emphasizing changes in the patterns of AM fungal community composition between in semi-natural (TB and PX) and intensively managed (RP1 and RP2) pastures. Key: samples of the same land use type are circled

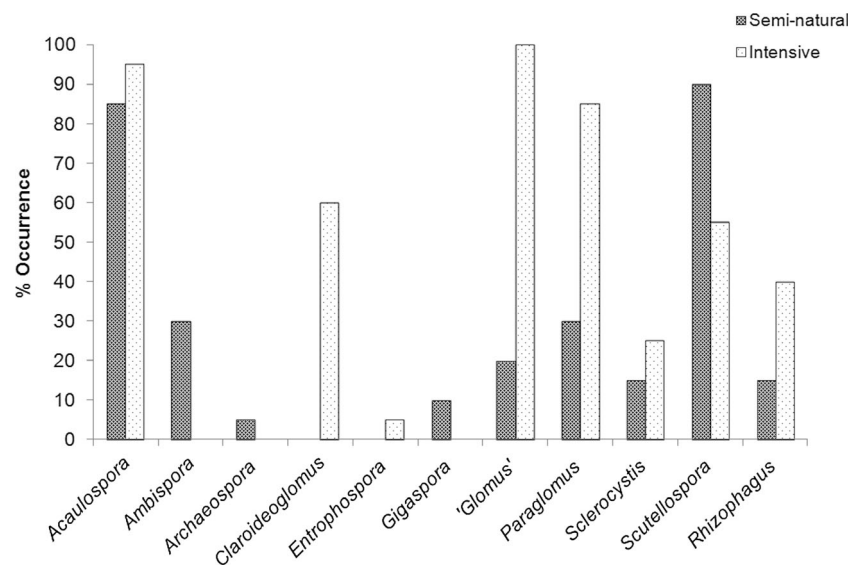


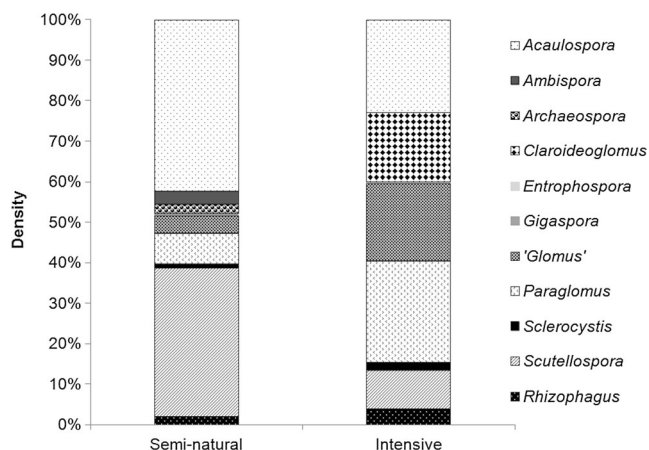
lower levels of land use (PX; TB), from the intensively managed sites (RP1; RP2), while the second axis show some degree of heterogeneity between the two intensively managed sites (Fig. 2).

Approximately 30 % of AMF taxa were common to both land uses (*A. cf. koskei*, *A. laevis*, *A. cf. myriocarpa* (considered by some authors to be in the genus *Archaeospora* (Oehl et al. 2011b) or *Ambispora* (Blaszkowski 2012), *Acaulospora* cf. *paulinae*, glomoid spore types 1 and 2, *Paraglomus* sp. 1, *S. calospora*, *Cetraspora pellucida*, *Racocetra* sp. *Rhizophagus clarus* and *Sclerocystis rubiformis*), demonstrating a ‘generalist’ behaviour (Table 2). Some taxa occurred exclusively in just one land-use type or in just one field site. Most species of *Acaulospora*, and the only representatives of *Ambispora* and *Gigaspora* were confined to the semi-natural

pasture, while intensive pasture soil was dominated by *Claroideoglomus* and ‘*Glomus*’ species (Table 2). Members of *Acaulospora* and the Scut. group occurred most frequently (Fig. 3) and were most abundant (Fig. 4) in semi-natural sites, *S. calospora* and *A. cf. myriocarpa* being dominant. In contrast, spores from the ‘*Glomus*’ grouping occurred most frequently in all samples from the intensive pastures (Fig. 3) and were most abundant, representing more than 20 % of the total AMF spores density (Fig. 4). Among the various taxa of this group, glomoid spore type 4 was the most common. Species of *Paraglomus* were also more frequent and abundant in intensive field sites than in semi-natural ones (Figs. 3 and 4), and *Acaulospora* spp. were present in almost all samples of intensive pasture (Figs. 3 and 4), with *A. cf. paulinae*, being the dominant species of this genus in RP2. There were also

**Fig. 3** Frequency of occurrence of each AMF genus present in the trap soil from semi-natural (PX and TB) and intensively managed (RP1 and RP2) pastures





**Fig. 4** Proportion of each AMF genus present in the soil trap from semi-natural (PX and TB) and intensively managed (RP1 and RP2) pastures

differences between sites within each land use. In the semi-natural pastures we observed that *A. delicata*, *A. thomii*, *Archaeospora schenckii*, *Gigaspora* sp. and *R. cf. intraradices/irregularis* only occurred in PX, whereas *A. excavata*, *A. tuberculata* and glomoid spore types 3 and 4 were detected only in TB (Table 2). In the intensive pastures, we also noted that some species like *A. elegans*, and glomoid spore types 5, 6, 7, 8 and 9 occurred only in RP1 (Table 2). Nine taxa as *Glomus globiferum*, glomoid spore types 1, 9, 10, 11, and 12, *Claroideoglomus lamellosum*, *Entrophospora infrequens* and *Paraglomus* sp. 2 were detected only in RP2 (Table 2).

### 3.3 AMF spore density

The total AMF spore densities did not differ among the two studied land uses (Nested Anova:  $F=0.17$ , d.f.=1, 2,  $p \leq 0.683$ ), or between field sites within each land use (Nested Anova:  $F=0.64$ , d.f.=2,36,  $p \leq 0.531$ ). Nevertheless, significant differences were found between land uses for the spore densities of *Acaulospora*, *Claroideoglomus*, 'Glomus' and the Scut. group (Table 3). The spore density of *Acaulospora* spp. was higher in the semi-natural pastures than in intensive ones, being the dominant genus in both semi-natural field sites,

**Table 3** F and p values from Nested ANOVA checking the effect of land use and field sites within land use in the AMF spore densities of the genera *Acaulospora*, *Claroideoglomus*, 'Glomus' and *Scutellospora*

AM Fungus	Land use		Field site within land use	
	F	p	F	p
<i>Acaulospora</i>	6.90	0.013	2.17	0.13
<i>Claroideoglomus</i>	11.04	0.002	2.11	0.14
'Glomus'	0.84	0.008	0.79	0.46
<i>Scutellospora</i>	9.06	0.005	1.23	0.30

especially in PX with a relative abundance of 46 % (Fig. 4). The Scut. group spore density was also significantly influenced by management intensity, contributing approximately 40 % (Fig. 4) to that of the semi-natural pastures. In contrast, about 40 % of the AMF spores from the intensive pasture were from the genus *Claroideoglomus* and the 'Glomus' grouping. The glomoid spore density also varied significantly between the two land uses. Spores belonging to *Claroideoglomus* were detected only in intensively managed pastures.

### 3.4 Soil nutrient parameters

Total AMF spore density was not significantly correlated with any soil nutrients, but correlations were shown for AMF spore densities amongst the four groups sporulating at the highest densities (*Acaulospora*, *Claroideoglomus*, 'Glomus' and the Scut. group) (Table 4). The density of *Acaulospora* spores was positively correlated with N ( $r=0.467$ ), Mg ( $r=0.408$ ), OM ( $r=0.457$ ) and Ca ( $r=0.379$ ). Negative and significant correlations occurred between *Claroideoglomus* spores density and all soil nutrients analysed. The same result was also observed in relation to 'Glomus' and the Scut. group spore density except for P, which was not significantly correlated with spore density.

## 4 Discussion

### 4.1 AMF species diversity

Pasture management can affect the structure of AMF communities causing a decline in AMF diversity and producing a shift in the AMF community composition (Boddington and Dodd 2000; Jansa et al. 2003). In the current study we found that, based on spore morphology, there were no differences in AMF diversity in semi-natural and intensive pastures. A similar result was obtained in relation to AMF spore density, which although it was slightly higher in semi-natural systems was not statistically significant. Lack of consistent differences across the two managements practices investigated are opposite to previous reports of lower diversity (Oehl et al. 2004; Gai et al. 2009; Verbruggen et al. 2010) and abundance of AMF spores (Eason et al. 1999; Oehl et al. 2003; Castillo et al. 2006a) in disturbed soils than in less disrupted environments.

Our results were, however, consistent with some other reports. Jansa et al. (2002) found that spore abundance was not significantly affected by soil tillage, and Sjöberg et al. (2004) did not find significant differences in spore densities between semi-natural grassland and ploughed fields. Schalamuk et al. (2006) identified spores of 24 species of AMF in non-tilled and tilled wheat soils in Argentina suggesting that lack of disruption to the hyphal network in the no-tillage plots favoured members of the family *Glomeraceae*,



**Table 4** Correlation coefficients of total AMF spore density and AMF spore densities of *Acaulospora*, *Claroideoglossum*, ‘*Glomus*’ and *Scutellospora* and soil nutrient levels

Spore density					
	Total	<i>Acaulospora</i>	<i>Claroideoglossum</i>	‘ <i>Glomus</i> ’	<i>Scutellospora</i>
N	0.087	0.467**	−0.505**	−0.376*	0.407**
P	−0.125	0.079	−0.392*	0.016	−0.037
K	0.010	0.211	−0.364*	−0.340*	0.342*
Mg	0.090	0.408**	−0.447**	−0.435**	0.461**
Ca	0.090	0.379*	−0.414**	−0.438**	0.463**
OM	0.094	0.457**	−0.491**	−0.410**	0.441**

though current phylogenetic classification (Krüger et al. 2012) shows that those species belong to several families in the order *Glomerales*.

In Terceira, the influence of human activities on the simplification of ecosystems is quite dramatic with very few endemic arthropods occurring in intensive pastures (Borges and Brown 1999; Cardoso et al. 2009). In fact, it is known that the input of fertilisers and pesticides and several other management practices (e.g., grazing intensity) are important in defining the quality of the pasturelands for AMF (e.g. Gange et al. 1993; Dhillon and Gardsjord 2004). However, the input of fertilisers to Azorean pastureland is lower than average inputs to pastures on the European mainland (Garcia and Furtado 1991), which means that at Terceira, both semi-natural and intensively managed pastures are reasonably benign habitats for AMF (Borges and Brown 1999). Moreover, topographical differences could create different communities within the site, some more inaccessible to grazing and others more intensively grazed, leading to some heterogeneity among pastures of the same land use type (Borges and Brown 1999).

#### 4.2 AMF spores composition

Distinct differences were found in species composition between the two types of land use in our study. Species from the ‘*Glomus*’ grouping were dominant in intensively managed pastures, perhaps indicating adaptability in adjusting patterns of sporulation to environmental conditions (see also Castillo et al. 2006a; Gai et al. 2009; van der Gast et al. 2011), while species from *Acaulospora* and *Scut.* group were dominant in semi-natural pastures. Such differences in species composition could be related to differences in propagative units among the glomeromycotan families (Jansa et al. 2003; Castillo et al. 2006a). Some members of *Glomeraceae* may have a highly infective extra-radical mycelium that can directly colonise roots (Hart and Reader 2002). Species of *Rhizophagus* and *Funnelformis* are known to self-anastomose thus being able to re-establish an interconnected network after mechanical disturbance (Daniell et al. 2001). It is thought that, on the

contrary, members of the *Gigasporaceae* are only capable of propagation via spore dispersal or infection from an intact mycelium and thus would colonise plant roots more slowly than members of *Glomeraceae*. Thus, under tillage the yearly disruption of the extraradical hyphae of these fungi would reduce their capacity of infection (Jansa et al. 2003; Börstler et al. 2006).

Spore dormancy and specific environmental conditions for spore germination probably slow the rate at which regenerating AMF can colonise plant roots (Hart and Reader 2002). For this reason, prevalence of species with glomoid spores in agricultural soil with repetitive severe physical disturbance, such as ploughing is widely reported (Jansa et al. 2002; Mathimaran et al. 2007; Castillo et al. 2006b; Lee and Eom 2009; Wang et al. 2008).

It is noteworthy that spores of *Acaulospora* spp. were often found in the intensively managed land use areas and together with those from ‘*Glomus*’ formed the highest proportion of spores in intensive pastures. Some members of *Glomeraceae* and *Acaulosporaceae* families respond to stress by producing more spores, allowing them to persist and dominate disturbed landscapes for longer (Castillo et al. 2006a; Jefwa et al. 2009).

It is also important to highlight the highest abundance and occurrence of *Paraglomus* species in intensive pastures. This result is consistent with a previous report by Lee and Eom (2009) which showed that *P. occultum* was only detected in the conventional farming while organic farming was dominated by *Acaulospora* species. However, a different result, based on molecular phylogenetic methods, was described by Gosling et al. (2014), who showed that members of the *Paraglomerales* were more common in soils under organic management than in sites managed conventionally in England. These authors also showed that distribution of *Paraglomus* spp. was not related to soil physical characteristics.

#### 4.3 Soil nutrient availability

The effect of land use on AMF communities is probably strongly linked to the changes in soil fertility due to

management practices (Titus and Leps 2000; Ezawa et al. 2001). In our study, the density of the four most abundant group - *Acaulospora*, *Claroideoglossum*, 'Glomus' and the Scut. group - present in the both semi-natural and intensively managed pastures, was correlated with almost all measured soil nutrients. Khanam et al. (2006) also found a positive correlation between soil nutrients levels (N, K, OM) and spore numbers in agricultural crops in Bangladesh. Nevertheless, the opposite has been observed in relation to total AMF spore density, which supported the generalisation that inorganic fertilisers may have a deleterious effect on many glomeromycotan species (Jefwa et al. 2006).

Among soil nutrients, phosphorus is considered to reduce the composition and diversity of AMF communities as well as spore and mycelium densities in both temperate and tropical systems (Agwa and Al-Sodany 2003; Oehl et al. 2004; Jansa et al. 2005; Khanam et al. 2006). Kahiluoto et al. (2001) demonstrated reduced AM colonisation of roots and AMF spore density in soil with increasing P fertilisation for several crops on two soils with low and intermediate concentrations of available P. However, in our study no statistically significant correlations were obtained between AMF spore density and this soil nutrient, as found also by Mathimaran et al. (2007) and Rodriguez-Echeverria et al. (2007). It is interesting to note that the P level in the two land use types under study was low and below the level considered prejudicial to AMF. Under P-limited conditions, the investment of a host plant in AMF is expected to increase to maintain the uptake of-limiting nutrients (Johnson et al. 2003). As predicted by the functional equilibrium model, in low-P soil, relative allocation to arbuscules, coils and extraradical hyphae is generally increased by N enrichment (Johnson et al. 2003). Consequently, in these Azorean pastures, we can observe an increase in AMF productivity, species richness and diversity with N, being the most responsive AMF taxa *Acaulospora*, the Scut. group and *Gigaspora*. In fact the lower C/N ratio in semi-natural pastures (27.8 vs. 54.6 in intensive pastures) could be explained by a higher mycorrhizal activity and higher cover abundance of legumes in these pastures (Borges and Brown 2001), which promoted the mineralisation process of OM resulting in an increase of the soil N (Atul-Nayyar et al. 2009). The opposite occurs in P-rich soils with N fertilisation application, observing a reduction of AMF community due to the loss of less common AMF species and the increase on abundance of 'Glomus' species (Egerton-Warburton et al. 2007).

#### 4.4 Concluding remarks

This is the first published report of glomeromycotan species from the Azores. We conclude that whether pastures were

intensively managed or not had no significant effect on AMF spore diversity and total density. However clear differences in AMF species composition were found between land use types. Species of 'Glomus' were dominant in intensive pastures, while *Acaulospora* and the Scut. group predominated in semi-natural pastures. This might be explained through different strategies of colonisation adopted by AMF and by soil nutrient levels. Members of *Glomeraceae* have a highly infective extra-radical mycelium that could allow immediate colonisation of plant roots, while members of *Gigasporaceae* are only capable of propagation via spore dispersal, colonising plant roots more slowly than members of *Glomeraceae*. Moreover, many of the species with glomoid spores form anastomoses between mycelia, and such species will have the ability to re-establish an interconnected network after mechanical disturbance. Therefore, management intensity affected the relative proportion of species from the *Glomeraceae* and *Gigasporaceae*.

The spore densities of the four most representative taxonomic groups, 'Glomus', *Acaulospora*, *Claroideoglossum* and the Scut. group, were positively correlated with soil nutrients. Thus, the improvement of soil fertility, especially the availability of K, N, Ca and Mg, appear to have provided favourable environments for mycorrhizal formation and function in these systems. However, a correlation between spore density and soil P content was not found. The low level of soil P content and its implication on absorption of other soil nutrients, particularly N, might also explain some change in the AMF spore composition.

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