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Original Article

Properties related to communities of arbuscular mycorrhizal fungi along an altitudinal gradient in a Brazilian cloud forest

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ABSTRACT

This was the first study conducted on the diversity and abundance of arbuscular mycorrhizal fungi (AMF) species and quantification of glomalin-related soil protein (GRSP) fractions, as well as on their relationship with environmental and soil chemical and physical properties along an elevation gradient above 1000 m in a Brazilian Atlantic cloud forest. AMF diversity was assessed by examining spores extracted from soil samples collected near the roots of the 18 most common plant species in permanent plots established in the field at altitudes of 1500, 1700, 1900, and 2100 meters above sea level. Fifteen AMF species were found, most of them belonging to the families Acaulosporaceae, Glomeraceae, and Gigasporaceae. Compositions of the AMF community varied among the altitudes; *Acaulospora* was the predominant genus, with six species. The total Bradford-reactive soil protein (BRSP) and the easily extractable BRSP (EE-BRSP) soil glomalin were the highest at the altitude of 2100 m (5.7 and 3.8 mg.g soil^{-1}, respectively). Altitude and environment and soil characteristics affected the composition and diversity (Shannon index) of the AMF communities. However, the effect of altitude on AMF diversity can be estimated, indirectly, through its effect on plant diversity.

Keywords: Glomeromycota, species richness, glomalin, tropical montane forest, upper montane forest, tropical montane ecosystem

Introduction

The Brazilian Atlantic Forest is one of the most important biodiversity "hotspots" (Myers *et al.* 2000). The vegetation types present in the Atlantic Forest include cloud forests, which are found at high elevations and are covered by clouds most of the time (Webster 1995). The clouds or the fog that envelop the vegetation supplement rainfall and, in some cases, are the main source of water for this ecosystem (Stadtmüller 1987; Bruijnzeel 2002). Under these humid conditions, the amount of water directly intercepted by the vegetation in the cloud forests can be up to 15-20% of

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the amount of direct rain (Bubb *et al.* 2004). These forests not only are of hydrological importance, particularly in the protection and maintenance of watershed headwaters, but also shelter range-restricted species and are still considered one of the rarest and most threatened ecosystems in the world, which makes them priority sites for conservation (Bruijnzeel *et al.* 2011).

Cloud forests are particularly threatened by climate changes, because these changes affect temperature, rainfall, and cloud formation in mountain areas (Bubb *et al.* 2004). Due to the narrow environmental tolerance of these ecosystems, human actions that cause climate change will potentially become the greatest threat to these forests in the near future (Ponce-Reyes *et al.* 2012). Despite the importance of cloud forests, there are few reports in the literature regarding their ecology, resulting in only fragmentary knowledge of these ecosystems (Churchill *et al.* 1995). Studies identifying plant species, phenology, yield, and seed development are still incipient (Homeier & Breckle 2002). In Brazil, research on cloud forests focuses particularly on the South and Southeast regions, because these ecosystems are more common in these regions (Koehler *et al.* 2002; Falkenberg 2003; Oliveira-Filho 2009).

Altitudinal gradients lead to pronounced changes that directly affect the composition and structure of vegetation, and the lower temperatures at high altitudes also decrease the rate of biomass decomposition, causing greater accumulation of organic matter in the soil (Scheer & Mocochinski 2009; Charan *et al.* 2013). Due to these changes that occur as the altitude increases, it is expected that the soil microbiota associated with plants, such as arbuscular mycorrhizal fungi (AMF), also undergo changes, as observed by Lugo *et al.* (2008) in an altitudinal gradient in a South American puna grassland.

Arbuscular mycorrhizal fungi (AMF – phylum Glomeromycota) are important components of the soil microbiota that contribute to the diversification and stability of natural ecosystems (van der Heijden *et al.* 1998). AMF may promote the aggregation, stability, and retention of C in the soil, which occurs through different mechanisms, one being the production of an insoluble and hydrophobic glycoprotein called glomalin. When deposited in the soil by fungal hyphae, glomalin works as a cementing agent and carbon reservoir in the soil (Purin & Rillig 2007). Therefore, the presence of AMF may be essential for the sustainability of the ecosystems in both plant development and for the maintenance of biological diversity.

AMF are obligate mutualistic symbionts that colonize the roots of more than 80% of plant families and establish an association known as arbuscular mycorrhiza (Schübler *et al.* 2001). The majority of the three hundred and fifty species already known worldwide (Wijayawardene *et al.* 2022) occur in tropical regions; there are at least 128 species in South America, with a relatively large number recorded in Brazil (Stürmer *et al.* 2018). However, this AMF diversity data is generally restricted to certain Brazilian vegetation types or ecosystems, and little is known, for instance, about the mycorrhizal status of the vegetation that makes up the tropical montane cloud forests in Brazil and in much of the world (Kottke 2002).

Association of mycorrhiza with plants has been reported in cloud forests, and it has been suggested that it is an important strategy for assisting plants to overcome extreme environmental conditions (Smith & Read 2008), such as those that occur in high-altitude ecosystems. Association of plants and AMF has been reported in the high altitudes of the Peruvian Andes (up to 5391 m) (Schmidt *et al.* 2008), in the Bolivian Andes (heights from 3700 to 4000 m) (Urcelay *et al.* 2011), and in the Patagonian Altoandina in Argentina (830-2005 m) (Velázquez *et al.* 2016).

Species richness of AMF has also been reported in high-altitude environments, such as along an altitudinal gradient in the South American puna grassland (Lugo *et al.* 2008) and in the subalpine foreland of the Morteratsch glacier, located at approx. 1900–2100 m altitude in Switzerland (Oehl *et al.* 2011). New AMF species, such as *Pacispora robigina*, *Otospora bareae*, *Entrophospora nevadensis*, *Acaulospora alpina*, *A. nivalis*, *Septoglomus altomontanum*, *Acaulospora pustulata*, *A. tortuosa*, and *A. viridis*, have been described in high altitude sites (*e.g.*, Oehl & Sieverding 2004; Palenzuela *et al.* 2013). Although most papers also evaluated soil properties, most of them did not evaluate relationships between soil properties and AMF diversity. In addition, usually only a few properties were evaluated by most of these papers.

Seeking to better understand the occurrence and diversity of AMF associated with the rich and endemic flora of cloud forests, the objective of this study was to assess the effect of the altitude gradient and the environmental and soil characteristics, peculiar to each altitude, on the composition of the AMF community (diversity and abundance of AMF spores) and on the levels of glomalin produced by these fungi associated with the rhizosphere of dominant plant species within each altitude of a Brazilian tropical montane cloud forest.

Materials and Methods

Study area and sampling to obtain soil and microclimate data

The study area is in the municipality of Itamonte, state of Minas Gerais, in the Alto-Montana Private Reserve of Natural Heritage (Reserva Particular do Patrimônio Natural - RPPN) of Serra Fina, at the following geographical coordinates: 22°21'55" S and 44°48'32" W. The area is part of the Atlantic Forest domain, a cloud forest in the dense high montane rain forest formation (above 1500 m altitude).

The climate in the municipality has Cwb type (Köppen climate classification) characteristics; it is a mesothermal climate with dry winters and mild rainy summers. The mean temperature of the hottest month is lower than 17.3 °C, and the coldest month is warmer than 12.7 °C (Sá Júnior *et al.* 2012). According to the WorldClim database (Hijmans *et al.* 2003), the mean annual rainfall for the study area is 2050 mm.

To determine the occurrence of AMF and to evaluate glomalin levels, six composite soil samples were collected using an auger at a depth of 0-20 cm in six permanent plots (plots of 40 m × 10 m) that were established in the field at each of the altitudes under study: 1500, 1700, 1900, and 2100 m, for a total of 24 samples. The composite samples were formed by 18 single subsamples/plot collected near the roots of the dominant plant species present at each altitude (Table 1). The subsamples were placed in plastic bags, homogenized, and stored at 4 °C until processing (chemical and physical analyses of the soil, assessment of AMF diversity, and quantification of glomalin). Samples were collected in April 2011.

The microclimate data were collected from four WatchDog Model 2900 ET automatic weather stations set up at each altitude (1500, 1700, 1900, and 2100 m). The collected data represent the year 2011. Data regarding humidity (mean, maximum, and minimum), temperature (mean, maximum, and minimum), extreme values of maximum and minimum temperatures, and mean and maximum wind speeds were collected. These data are shown in Table 2. To assess whether there were microclimate variations along the elevation gradient at each elevation, WatchDog model 2900ET weather stations were also set up, which performed measurements over 2013 and 2014 (Mariano *et al.* 2020) (Fig. 1).

Table 1. Identification of plant species around which soil samples were collected near the roots to study the diversity of arbuscular mycorrhizal fungi along an altitudinal gradient in a Brazilian cloud forest.

Altitude (m)	Family	Species
1500	Myrsinaceae	*Myrsine umbellata* Mart.
1500	Myrtaceae	*Myrcia splendens* (Sw.) DC.
1500	Fabaceae	*Dalbergia villosa* (Benth.) Benth.
1500	Salicaceae	*Xylosma prockia* (Turcz.) Turcz.
1500	Vochysiaceae	*Vochysia tucanorum* Mart.
1500	Proteaceae	*Roupala montana* Aubl.
1700	Myrtaceae	*Myrcia splendens* (Sw.) DC.
1700	Annonaceae	*Guatteria australis* A.St.-Hil.
1700	Rubiaceae	*Rudgea jasminoides* (Cham.) Müll.Arg.
1700	Rubiaceae	*Psychotria vellosiana* Benth.
1900	Myrtaceae	*Myrceugenia miersiana* (Gardner) D.Legrand & Kausel
1900	Myrtaceae	*Pimenta pseudocaryophyllus* (Gomes) Landrum
1900	Rosaceae	*Prunus myrtifolia* (L.) Urb.
1900	Proteaceae	*Roupala rhombifolia* Mart. ex Meisn
2100	Myrtaceae	*Myrceugenia rufescens* (DC.) D.Legrand & Kausel
2100	Clethraceae	*Clethra scabra* Pers.
2100	Euphorbiaceae	*Croton piptocalyx* Müll.Arg.
2100	Proteaceae	*Roupala rhombifolia* Mart. ex Meisn
2100	Solanaceae	*Solanum bullatum* Vell.
2100	Rosaceae	*Prunus myrtifolia* (L.) Urb.

Table 2. Microclimate data (2011) along an altitudinal gradient in a Brazilian cloud forest.

Parameter	Altitude (m)			
	1500	1700	1900	2100
Relative humidity (%)	79.7	83.7	81.6	83.5
Minimum humidity (extreme value) (%)	7.7	8.5	8.8	6.4
Mean maximum humidity (%)	90.6	93.4	91.3	94.0
Mean minimum humidity (%)	62.7	68.5	68.0	67.0
Mean temperature (°C)	15.6	14.2	13.1	11.8
Maximum temperature (extreme value) (°C)	28.4	25.6	24.1	23.8
Minimum temperature (extreme value) (°C)	2.6	0.0	0.3	-1.7
Mean maximum temperature (°C)	20.0	18.2	16.6	16.0
Mean minimum temperature (°C)	12.8	11.1	10.1	8.4
Mean wind speed (km h^{-1})	0.4	0.3	0.4	0.5
Maximum wind speed (km h^{-1})	12.0	9.0	11.0	11.0

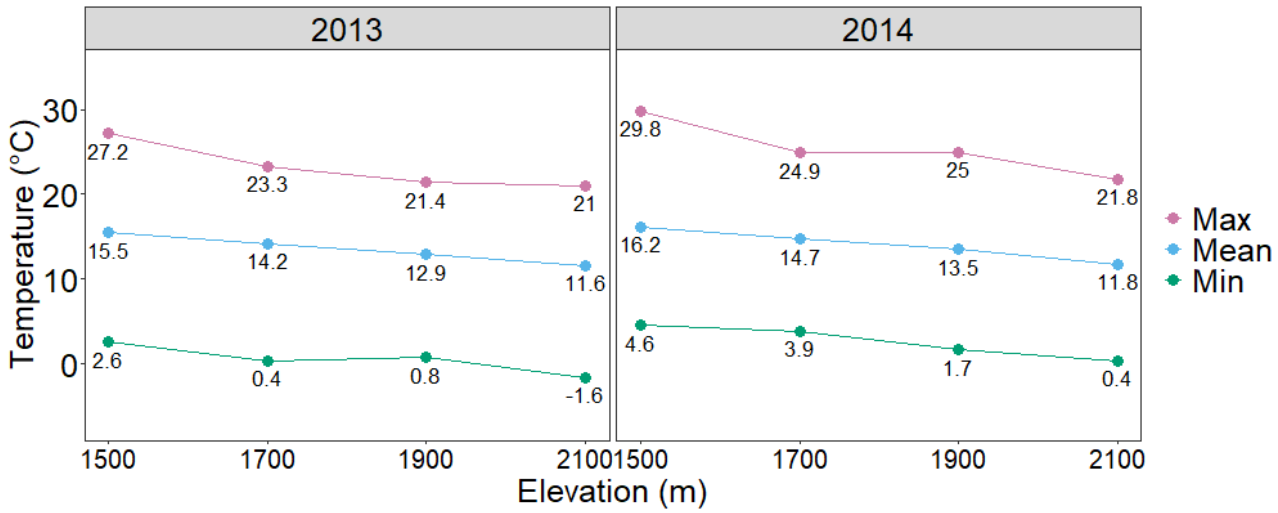

Figure 1. Average, maximum and minimum temperature values (°C) at each elevation level in the two years of observation collected through meteorological stations in the tropical montane forest in the Atlantic Forest region (Souza *et al.* 2023).

Chemical and physical soil properties

A sub-sample was extracted from each composite sample. The sub-samples were air dried and passed through a 2-mm mesh sieve. Chemical and physical (texture) analyses were performed on the samples in the Soil Fertility and Plant Nutrition Laboratory at the Federal University of Lavras. The following chemical properties were analyzed: pH in H_2O (1:2.5 m/v); Al, Ca, and Mg [extracted by 1 mol KCl L^{-1} (1:10) and determined by atomic absorption spectrometry (Ca and Mg) and titration with NaOH (Al)]; available P, K, Zn, Fe, Mn, and Cu [extracted by Mehlich-1 solution and determined by photometry (K), colorimetry (P), and atomic absorption spectrophotometry (micronutrients)]; remaining phosphorus (P-rem) [calculated after the addition of 60 ppm of P-KH_2PO_4 and determined in P-solution by colorimetry]; S [extracted by dicalcium phosphate (0.01 M) and analyzed by turbidimetry/colorimetry]; potential acidity (H+Al) extracted by SMP; B (extracted with hot water and determined by colorimetry); and organic matter [wet oxidation with 4N $Na_2Cr_2O_7$ + 10N H_2SO_4 and determined by colorimetry]. The organic carbon content (Corg) in the soil samples was determined by the dry combustion method on an Elementar brand Vario Cube TOC analyzer. Approximately 15 mg of the sample was incinerated in the combustion chamber at 950 °C, and the CO^2 released from the soil sample was detected by an NDIR sensor. A soil sample certified by Elementar (ref. 35.00-0158) was used

to verify the results. The results obtained from the sorption complex allowed for the following calculations: exchangeable bases (SB), effective (t) and potential (T) cation exchange capacities, base saturation (V%), and Al saturation (m%). The soil textures were analyzed by the Bouyoucos method.

AMF extraction, identification, and classification

To study the occurrence of AMF in these soils, AMF spores were extracted from each composite soil sample using the wet sieving technique (Gerdemann & Nicolson 1963), followed by sucrose density gradient centrifugation. Under a dissecting microscope, the spores were separated by morphotype and were counted. Permanent slides were prepared with the spores using PVLG (Polyvinyl-Lacto-Glycerol) and PVLG mixed with Melzer's reagent. The AMF species were identified based on the size, color, and shape of the spores under a dissecting microscope; by an analysis of the sub-cellular structures of the spores under a light microscope; by comparison with the manual of Schenck and Perez (1998); and by description of the species in the pages of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu) and the Dept. of Plant Pathology, University of Agriculture, in Szczecin, Poland (http://www.agro.ar.szczecin.pl/~jblaszkowski/). Furthermore, species were identified and matched with morphotypes, with integration of the most recent updates (Błaszkowski *et al.* 2022; Wijayawardene *et al.* 2022).

The frequency of occurrence (FO) of each species was calculated using the equation Fi = Ji/K, where Fi is the species and i is the frequency; Ji is the number of samples in which i species occurred; and K is the total number of soil samples. The approximation of the FO, proposed by Zhang *et al.* (2004), was used to classify the AMF species present at each altitude as dominant (FO > 50%), very common (30% < FO ≤ 50%), common (10% < FO ≤ 30%), and rare (FO ≤ 10%).

The following indices were used to evaluate and compare the differences in community composition at each altitude: diversity (Shannon-Weaver), richness (Margalef), and equitability (J - Shannon-Wiener)

Total glomalin content (BRSP) and easily extractable glomalin content (EE-BRSP)

The total Bradford-reactive soil protein (BRSP) and easily extractable Bradford-reactive soil protein (EE-BRSP) levels were evaluated following the method proposed by Wright and Upadhyaya (1998), and the extraction of glomalin and glomalin-related soil proteins (GRSP) was performed according to the method of Bradford (1976). To extract EE-BRSP, 1 g of soil was added to 8 mL of sodium citrate (20 mM, pH 7.0), followed by autoclaving (121 °C / 30 min) and centrifugation (3,300 rpm for 15 minutes). The total glomalin (BRSP) was extracted by adding 8 mL of sodium citrate (50 mM, pH 8.0), followed by autoclaving (121 ºC / 1 hour) and centrifugation (3,300 for 30 min.) in a repeated manner until the supernatant was not reddish-brown in color. The supernatants resulting from extraction cycles were collected and stored at 4 °C for later quantification using the Coomassie Brilliant Blue G-250 reagent and bovine serum albumin (BSA) for the standard curve, with readings on a spectrophotometer at 595 nm. The values were expressed as $mg.g^{-1}$ of soil.

Statistical analysis

The data regarding soil properties, species richness, and spore abundance were statistically analyzed, and the means for each altitude were grouped by the Scott-Knott test at 5% probability (Scott & Knott 1974). The values found for the Shannon-Weaver index were compared pairwise by the Scott-Knott test. The following indices were used to evaluate and compare the differences in community composition at each altitude: diversity (Shannon-Weaver), richness (Margalef), and equitability (Pielou), using the DIVES software (Rodrigues 2005).

Detrended Correspondence Analysis (DCA) was applied to the data of the AMF community, and four response variables were obtained: number of spores, species richness, axis 1, and axis 2. As the axes in a DCA are orthogonal, it is possible to analyze them independently and use them as a synthesis of the relations of composition of AMF species. From the altitude, soil, and tree vegetation data, we obtained 8 response variables: altitude (m), number of individual trees, basal area of the tree community (m^2), axes 1 and 2 of a DCA performed with the abundance of tree species in the sampling units, and axes 1 and 2 of a Principal Component Analysis (PCA) with all the edaphic variables collected. Pearson correlation analyses were used on these explanatory variables to identify redundancy in explanation, from which overall models with non-correlated variables were set up. Generalized Linear Models (GLM) were used for each overall model of each response variable in accordance with the explanatory variables, and from them, submodels were obtained through the dredge function, which were selected considering ΔAICc ≤ 2 (Burnham *et al.* 2011). The Multi-Model Inference (Burnham *et al.* 2011) through the "model.avg" function of the "MuMIn" package (Bartón 2009) was used on the set of submodels selected from each overall model of each response variable to capture the uncertainty of the effects of all the predictors on the response variables. Based on the result of the submodels, we posited the criterion of ΔAICc ≤ 2 to then calculate the mean values of the coefficients and obtain the values of significance of the explanatory variables in each one of the four response variables. The variables for number of spores and species richness of AMFs were worked on within the distribution of Poisson residues, ensuring the presupposition of non-existence of overdispersion, whereas

the variables for DCA 1 and DCA 2 were worked on within the Gaussian family, meeting the criterion of normality of residues (Shapiro-Wilk test). None of the overall models had spatial self-correlation. All analyses were performed on the R v. 3.5.1 program (2018).

Results

The soils at all altitudes generally had low pH values (Table 3). The 1500 m altitude had the highest soil pH (5.3), and the altitudes of 1700 and 2100 m had the most acidic soils (pH 4.4). The other chemical and physical soil properties also varied among altitudes ($p < 0.05$). Soil organic matter, organic carbon, P, and N were greater at higher altitudes (1900 and 2100 m). The 1500 m altitude exhibited the greatest difference from the other altitudes ($p < 0.05$), with the highest levels of K, Ca, Mg, sum of bases, effective cation exchange capacity (t), remaining phosphorus, Zn, and Mn. There were no differences among altitudes for Fe, Cu, and B ($p > 0.05$) (Table 3).

A total of 15 AMF morphotypes were recovered along the altitudinal gradient, and 12 of them were identified to the species level (Tables 4 and 5). Only the species *Acaulospora scrobiculata* and *Glomus* sp. occurred at all altitudes. Most species (73%) belonged to the families Acaulosporaceae (*Acaulospora*), Gigasporaceae (*Gigaspora* and *Scutellospora*), and Glomeraceae (*Glomus* and *Rhizoglomus*). The genera *Glomus*, *Rhizoglomus*, *Entrophospora*, *Paraglomus*, *Dentiscutata*, and *Cetraspora* were represented by only one species each (Table 4).

The largest number of species that occurred only once were observed at the altitude of 1500 m, namely *Acaulospora delicata*, *A. foveata*, *A. mellea*, *Cetraspora pellucida*, and *Rhizoglomus clarum*. The species *Gigaspora margarita* and *Entrophospora etunicata* were also exclusive to certain altitudes (1700 and 2100 m, respectively). Six AMF species were found at 1700 and 2100 m, and five species at 1900 m (Table 4). The mean spore densities (50 mL of soil) at altitudes were 27.3 at 1500 m, 29.5 at 1700 m, 68.2 at 1900 m, and 45.2 at 2100 m. The species with the highest total abundance of spores (per 50 mL soil) was *Glomus* sp. (377), followed by *Entrophospora etunicata* (147), *Acaulospora spinosa* (132), *Gisgaspora* sp. (129), and *Paraglomus occultum* (119). Despite differences in the number of species and spore densities among altitudes, there was no effect ($p > 0.05$) of the altitudinal gradient on these properties of AMF communities (Table 4).

Table 3. Chemical and physical attributes of soils under a cloud forest along an altitudinal gradient.

Soil Attribute	Unit	Altitude (m)				Coefficient of Variation (%)
		1500	1700	1900	2100	
pH (H_2O)	—	5.3a	4.4c	4.7b	4.4c	4.7
P	mg dm^{-3}	1.7b	1.3b	3.2a	3.9a	37.2
K	mg dm^{-3}	159a	81.2b	61.8b	94.3b	28.1
Ca	cmol dm^{-3}	2.4a	0.2b	0.2b	0.5b	102.9
Mg	cmol dm^{-3}	1.0a	0.1b	0.1b	0.3b	56.7
Al	cmol dm^{-3}	1.4d	3.6b	2.5c	4.8a	25.9
H+Al	cmol dm^{-3}	7.6b	14.8b	14.1b	22.7a	16.9
SB	cmol dm^{-3}	3.8a	0.5b	0.5b	0.9b	70.8
t	cmol dm^{-3}	4.9a	4.1b	3.0b	5.8a	23.9
T	cmol dm^{-3}	11.4c	15.4b	14.6b	23.7a	14.6
V	%	33.2a	3.4b	3.3b	4.1b	75.4
m	%	26.1b	87.6a	82.2a	83.5a	13.9
OM	dag kg^{-1}	4.8b	5.6b	11.3a	12.2a	47.5
Corg	g kg^{-1}	3.8c	4.8c	10.6b	14.2a	27.9
P-rem	mg L^{-1}	9.9a	3.8b	2.7c	2.2c	20.2
Zn	mg dm^{-3}	3.8a	1.2b	1.9b	2.4b	45.4
Fe	mg dm^{-3}	42.9a	92.9a	72.4a	64.2a	38.9
Mn	mg dm^{-3}	73.8a	24.1b	22.1b	213b	37.8
Cu	mg dm^{-3}	0.2a	0.1a	0.1a	0.1a	42.0
B	mg dm^{-3}	0.4a	0.3a	0.2a	0.3a	39.6
S	mg dm^{-3}	7.9c	13.5c	19.8b	31.2a	27.3
N	g kg^{-1}	3.0c	3.7c	7.3b	10.1a	30.1
Clay	dag kg^{-1}	53.5a	55.4a	32.8b	30.2b	26.2
Sand	dag kg^{-1}	14.3b	22.8b	29.2a	36.8a	33.0
Silt	dag kg^{-1}	32.2a	21.8b	38.0a	33.0a	27.3

SB = sum of bases; t = effective cation exchange capacity; T = cation exchange capacity at pH 7; V = base saturation; m = aluminum saturation; OM = organic matter content; P-rem = remaining phosphorus. Numbers within a line followed by the same letter are not statistically significant ($p < 0.05$) according to the Scott-Knott test.

Table 4. Occurrence, number of spores, and diversity indices for arbuscular mycorrhizal fungi communities along an altitudinal gradient in a cloud forest in Brazil.

AMF Family/Species	Number of spores (50 mL of soil)[1]				Total spore density per AMF species
	Altitude (m)				
	1500	1700	1900	2100	
Acaulosporaceae					
Acaulospora delicata C. Walker, C.M. Pfeiff. & Bloss	16				16
Acaulospora foveata Trappe & Janos	12				12
Acaulospora mellea Spain & N.C. Schenck	4				4
Acaulospora morrowiae Spain & N.C. Schenck	12	19	22		53
Acaulospora scrobiculata Trappe	1	9	22	35	67
Acaulospora spinosa C. Walker & Trappe	5		71	56	132
Glomeraceae					
Glomus sp.	118	104	113	42	377
Rhizoglomus clarum (TH Nicolson & NC Schenck) Sieverd., G.A. Silva & Oehl	28				28
Entrophosporaceae					
Entrophospora etunicata (W.N. Becker and Gerd.) Błaszk., Niezgoda, B.T. Goto & Magurno				147	147
Paraglomeraceae					
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	104			15	119
Gigasporaceae					
Gigaspora margarita (W.N. Becker & I.R. Hall)		15			15
Gigaspora sp.			113	16	129
Scutellospora sp.	11	21		6	38
Dentiscutataceae					
Dentiscutata heterogama (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl	6	9			15
Racocetraceae					
Cetraspora pellucida (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd	11				11
Mean Density of spores[2]	54,6	29,5	56,8	52,8	
Total Number of Species[1]	12 a	6 a	5 a	6 a	
Total Spores per altitude[1]	328 a	177 a	341 a	317 a	
Shannon Index (H)[3]	0.76 a	0.57 b	0.61 b	0.64 b	
Margalef's index[3]	4.37 a	2.22 b	1.57 b	2.00 b	
Pielou's equitability[3]	0.71 a	0.73 a	0.87 a	0.82 a	

[1]Sum of six plots. [2]Sum of all species density/ six plots. [3]Mean of six plots. Numbers within a line followed by the same letter are not statistically significant ($p < 0.05$) according to the Scott-Knott test.

Ten species of AMF – *Acaulospora morrowiae, A. spinosa, A. scrobiculata, Gigaspora margarita, Entrophospora etunicata, Glomus sp., Gigaspora* sp., *Paraglomus occultum, Dentiscutata heterogama*, and *Scutellospora* sp. – were detected in over 30% of the soil samples from at least one altitude (Fig. 2). Among these species, *Glomus* sp. stood out as the only species with a frequency of occurrence greater than or equal to 30% at all altitudes. At 1500 and 1900 m, this species had the greatest frequency of occurrence recorded in our study (67%). We found that seven AMF species – *Acaulospora delicata, A. foveata, A. mellea, Rhizoglomus clarum, Gigaspora margarita, Gigaspora* sp., *and Cetrasposa pellucida* – were classified as rare (FO ≤ 10%); and only *Glomus* sp. was classified as dominant (FO > 50%).

In general, 1163 spores distributed among 15 morphotypes of AMFs were found, with the number of spores per species ranging from 377 (*Glomus* sp.) to 4 (*Acaulospora mellea*). In relation to the tree community, 1889 individual trees were found from 161 species, which represent a basal area of 36.69 m^2/ha and a density of 2053.6 individual/ha. The sample units in the altitudinal levels varied in relation to their edaphic characteristics (Fig. 3), with a main differentiation between the 1500 m level of lowest fertility positioned to the right in the diagram, and the other levels of greater fertility and greater acidity by aluminum positioned to the left. Within the levels of 1700, 1900, and 2100 m, the units were organized in accordance with the variables of texture, organic matter, and organic carbon content (Corg), with a gradient at axis 2 of the diagram that goes from the 1700 m level, with a higher amount of clay, to the levels of 1900 and 2100 m, with greater amounts of sand, silt, organic matter, and Corg. Thus, the altitude represented a synthesis of edaphic variations, though in a non-linear manner in relation to the gradient.

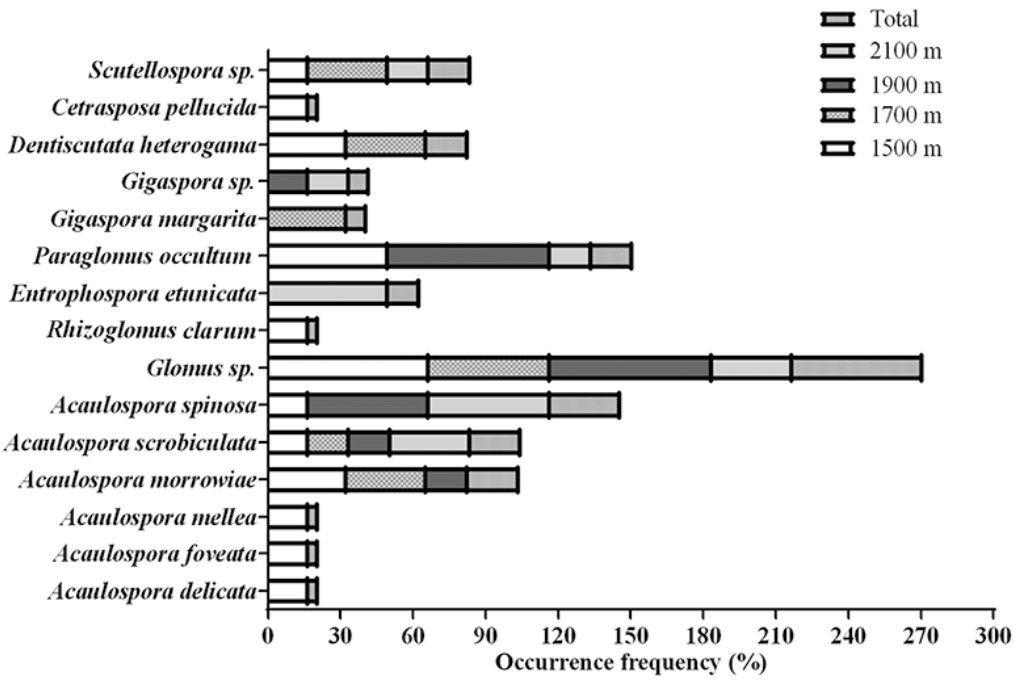

Figure 2. Frequency of occurrence (%) of arbuscular mycorrhizal fungi along an altitudinal gradient of a Brazilian tropical cloud forest.

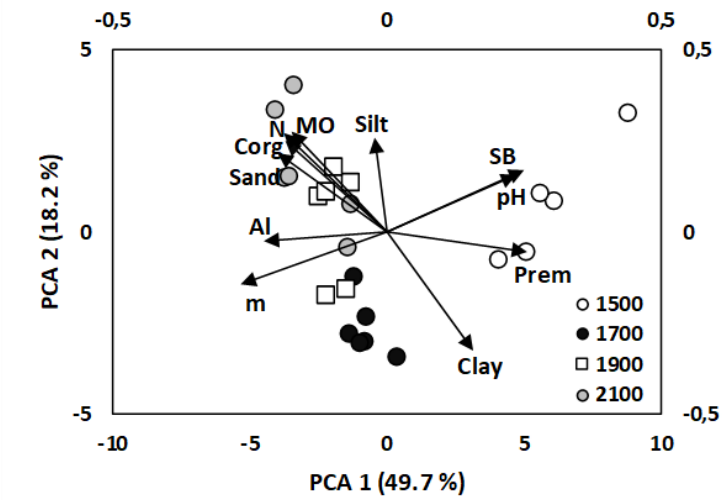

Figure 3. Principal Component Analysis (PCA) from edaphic variables of the plots along the altitudinal gradient studied. N: Nitrogen; OM: organic matter; Corg: organic carbon; Al: exchangeable aluminum; m: aluminum concentration; P-rem: remaining phosphorus; SB: sum of bases.

Total (BRSP) and easily extractable (EE-BRSP) glomalin levels differed significantly among altitudes (Table 6). The highest levels of both glycoprotein fractions were recorded at altitudes of 1900 m (4.7 and 3.6 mg.g soil^{-1} for BRSP and EE-BRSP, respectively) and 2100 m (5.7 and 3.8 mg.g soil^{-1} for BRSP and EE-BRSP, respectively), which differed statistically from the remaining altitudes. The altitudes of 1500 and 1700 m did not differ in terms of BRSP content; however, there were significant differences between these altitudes for the EE-BRSP content (2.3 mg.g soil^{-1} at 1500 m and 2.8 mg.g soil^{-1} at 1700 m).

The relationship between the response variables of AMFs and the explanatory edaphic variables is shown in Table 7. The number of spores was explained in a significant manner by the edaphic characteristics of the two axes and by the altitude, with positive relations for all of them; whereas the richness of microorganism species was not affected by any of the variables considered. As altitude is not a

linear synthesis of the edaphic characteristics, and from preliminary analyses, altitude was not strongly correlated with the PCA axes, the variables are probably associated with different explanations. This is shown by the incongruence of the result upon stating that locations of higher values in axis 1 of the PCA (1500 m level) would have a greater number of AMF spores, as well as upon stating that locations of higher altitude value (2100 m level) would have a larger number of spores. Therefore, the tendency is for the number of spores to have a non-linear response across the altitudinal gradient, affected by the combination of edaphic effects and of additional altitudinal effects (Fig. 4).

The pattern of composition of AMF species was only explained in a significant manner by axis 2 of the DCA, in accordance with the composition of the plant species, axis 1 of the DCA – positive relationship, and by axis 2 of the PCA, with a negative relationship (Table 1). Thus, the main pattern of differentiation of composition that is synthesized in axis 1 of the DCA cannot be explained by any of the variables evaluated, including by the altitude. The variables associated with texture (OM, Corg, and N), synthesized by axis 2 of the PCA, and the main differentiation of the vegetation, synthesized in the axis of the DCA of the vegetation, are explanatory of the secondary differentiation of the similarity relationships (Fig. 5). As shown in the DCA of AMFs, the sampling units at the different levels were not organized in a linear manner over composition relationships, with no pattern found; they were possibly associated with combinations among substitution of species of tree vegetation, edaphic characteristics, and other factors not measured with high explanatory power.

Table 5. Pairwise comparisons of Shannon's diversity index among all altitudes. Values represent the t-statistic.

Altitude (m)	1500 m	1700 m	1900 m	2100 m
1500	—			
1700	4.8613*	—		
1900	5.4572*	1.2224	—	
2100	3.9964*	1.9105	1.2518	—

* Significant at $p < 0.05$.

Table 6. Levels of total and easily extractable glomalin in soils under a cloud forest along an altitudinal gradient

Altitude (m)	Total Glomalin (mg.g soil^{-1})	Easily Extractable Glomalin (mg.g soil^{-1})
1500	2.8 b	2.3 c
1700	3.2 b	2.8 b
1900	4.7 a	3.6 a
2100	5.7 a	3.8 a
CV(%)	20.9	12.5

Numbers within a column followed by the same letter are not statistically significant (P<0.05)

Table 7. *Multimodel inference* results on generalized linear model (GLM) analysis, representing the relationship between the response variables of AMFs (columns) and the explanatory variables (rows). Non-significant explanations are shown in white, significant positive explanations in black, and significant negative explanations in gray, considering 0.05 as the level of significance. N spores: number of mycorrhizal (mycor.) spores; S mic: species richness of mycor.; DCA mycor. 1 and DCA mycor. 2: scores of axis 1 and 2 in a Detrended Correspondence Analysis with mycor. data; N veg: number of trees; S veg: species of trees; Basal area: basal area (m^2) of trees; DCA veg 1 and DCA veg 2: scores of axis 1 and 2 in a Detrended Correspondence Analysis with vegetation data; Soil PCA 1 and Soil PCA 2: scores of axis 1 and 2 of Principal Component Analysis with edaphic variables; Altitude: elevation (m).

	N spores	S mycor.	DCA mycor. 1	DCA mycor. 2
N veg				
S veg				
Basal area				
DCA veg 1				
DCA veg 2				
Soil PCA 1				
Soil PCA 2				
Altitude				

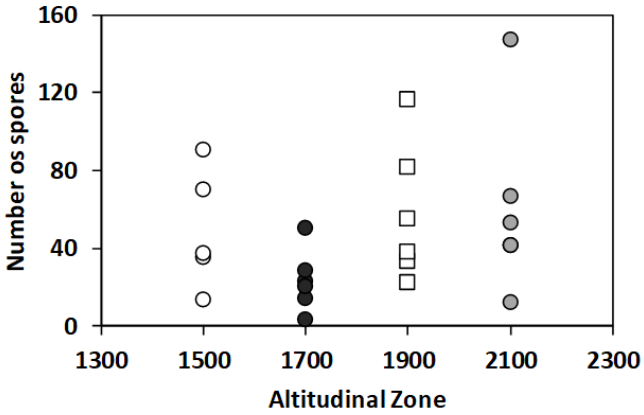

Figure 4. Number of spores/50 mL soil in the sampling points along an altitudinal gradient in a cloud forest.

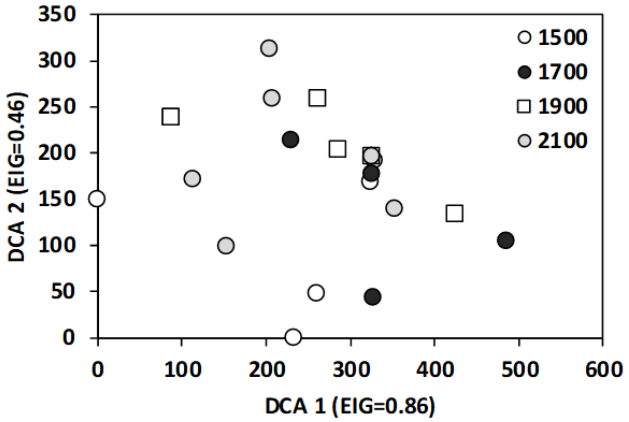

Figure 5. Correspondence analysis (DCA) for community composition and diversity of arbuscular mycorrhizal fungi (AMF) along an altitudinal gradient.

Discussion

We characterized the composition of the AMF community and determined spore diversity and abundance among field-collected spores at different sites along an altitudinal gradient [1500, 1700, 1900, and 2100 meters above sea level (m.a.s.l.)] in a Brazilian Atlantic cloud forest. Qualitative differences in the composition of the AMF community and spore abundance were important components to demonstrate the effect of altitude and its particular edaphic characteristics, as well as the plant species present. Our data are based on field-collected spores and thus represent only a certain moment by documenting the species sporulating at the time of sampling, but we emphasize the importance of the present study because it is the first record of the occurrence and diversity of AMF associated with plant species in a Brazilian tropical montane cloud forest at high altitude (1500-2100 m.a.s.l.).

Studies in Brazil have identified AMF on mountains, as seen in de Carvalho *et al.* (2012), Bonfim *et al.* (2015) and Vieira *et al.* (2019). However, the first two studies investigated AMF species diversity along an altitudinal gradient whose elevation (80-1000 m.a.s.l.) was much lower than in our study, and the third one was carried out in an altitudinal gradient (1451 to 1958 m) in a northeastern region of Brazil where the predominant vegetation types were rupestrian field shrubland and Cerrado (Brazilian tropical savanna).

The total of 15 species and 9 genera within 7 families detected in the present study is in agreement with some of the previous investigations of high-altitude environments, whether by the number of species or the number of genera found. Lugo *et al.* (2008) recovered ten AMF species and 4 genera over a transect of altitudes ranging from 3,320 to 3,870 m in an altitudinal gradient in the South American puna grassland. Gai *et al.* (2009) detected 23 AMF species representing 10 genera in the Tibetan plateau, up to the 3,500 to 5,200 m altitude range. Liu *et al.* (2011) recorded a total of 21 AMF phylotypes in 6 genera from other high-altitude alpine environments. Oehl *et al.* (2011) recovered 28 AMF species distributed in 8 genera across seven sites at altitudes ranging from 1,922 to 2,012 m.a.s.l. in the Swiss eastern-central Alps. Velázquez *et al.* (2016) detected 27 AMF species in 10 genera in high altitude sites (830-2005 m.a.s.l.) of the Patagonian Altoandina region in Nahuel Huapi National Park (Argentina).

Reports on AMF diversity in montane tropical forests are still scarce in the literature, but we highlight two studies conducted in southern Ecuador – Kottke *et al.* (2008) and Haug *et al.* (2010). The first evaluated only the occurrence of AMF, but did not report the species diversity of these fungi. Haug *et al.* (2010) used a molecular phylogenetic analysis and reported a high diversity of AMF whose DNA sequences were distributed between two groups of *Glomus* [Group A (69) and Group B (3)] and among the families Acaulosporaceae (16), Archaeosporales (11), and Gigasporaceae (3).

Comparisons of studies in high-altitude environments must be done with caution and after considering the different plant communities, soil types, and characteristics of the climate that can affect the composition of the AMF communities (Velázquez *et al.* 2016). Qualitative differences in the composition of the AMF community were an important component for demonstrating the effect of altitudes in our study. First, among the three most abundant species at each altitude, only one (*Glomus* sp.) was common to all altitudes. *Glomus* sp. was the most frequent species at 1500, 1700, and 1900 m, and the third most common at 2100 m. *Paraglomus occultum* was the second most frequent species at 1500 m but was not among the most frequent at 1700 and 2100 m. *Entrophospora etunicata* was the most frequent species at 2100 m but was not recorded at the other altitudes. Second, the most abundant AMF species differed among the various altitudes, and only *Glomus* sp. was shared

as one of the most abundant species, as previously explained. Third, the number of *Acaulospora* species (six) was vastly superior to the number of species in other genera at 1500 m. However, the number of species from this genus decreased by at least half at the other altitudes: two species (1700 m), three species (1900 m), and two species (2100 m). Moreover, the species *Acaulospora delicata, A. foveata*, and *A. mellea* occurred exclusively at 1500 m. Similarly, *Gigaspora margarita* occurred exclusively at 1700 m, and *C. etunicatum* only occurred at 2100 m.

Studies based on field-spore recoveries in high-altitude environments indicate that members of Glomeraceae are dominant (Haug *et al.* 2010; Oehl *et al.* 2011; de Carvalho *et al.* 2012; Vieira *et al.* 2019). In this study, Acaulosporaceae was the dominant family in terms of species richness, representing 40% of all the species recovered. The highest species diversity was found for the genus *Acaulospora*, which has frequently been found in humid forests (Lugo *et al.* 2008). Although the genus *Acaulospora* is strongly linked to tropical ecosystems (Allen *et al.* 1995), this genus was observed to be prominent in high mountainous and alpine regions of the Swiss Alps (Oehl *et al.* 2006) and in high altitude sites of the Patagonian Altoandina (Velázquez *et al.* 2016), suggesting the versatility of the genus *Acaulospora* in adapting to different environmental conditions. Other authors in studies on high-altitude environments reported other AMF species found in our study: *Acaulospora mellea, A. morrowiae, A. spinosa, Entrophospora etunicata, Rhizoglomus clarum, Paraglomus occultum*, and *Cetrasposa pellucida* (Börstler *et al.* 2006; Lugo *et al.* 2008; Haug *et al.* 2010; Liu *et al.* 2011).

Our data on Shannon diversity indices suggested that soil pH was a strong predictor of AMF diversity, corroborating studies by Huang *et al.* (2019) and Xu *et al.* (2016), though they were carried out in ecosystems different from ours. Just as in Zhu *et al.* (2020), the diversity of AMF and pH were positively related, since the highest pH value was recorded at an altitude of 1,500 m, where a greater diversity of AMF was also observed. Furthermore, higher levels of K were found at an altitude of 1,500, corroborating classic studies (Sieverding 1991; Munyanziza *et al.* 1997) that demonstrated the beneficial effect of K on AMF proliferation. Studies on AMF distribution over altitude gradients in particular have shown that spore abundance and AMF species richness decrease with increasing altitude (Gai *et al.* 2009), or they have shown maximum spore abundance at an intermediate altitude, as reported by Li *et al.* (2014) and Coutinho *et al.* (2015). In the present study, altitude, environment and soil characteristics affected the composition and diversity (Shannon index) of the AMF communities. However, the effect of altitude on AMF diversity can be estimated, indirectly, through its effect on plant diversity. Furthermore, we need to consider the possibility of random/stochastic effects and processes that tend to have a considerable effect on a small scale and under non-measurable microclimate conditions. Thus, we agree with Velázquez *et al.* (2016) when they suggest that the variation in spore abundance and species richness over altitude gradients is, in fact, site-dependent and that variable conditions among those common to mountain environments – e. g., low temperatures, rainfall, moisture regimes, and frost – affect the abundance and richness of AMFs in different ways.

Thus, our results add to those of other authors who demonstrate that there is no pattern for AMF diversity and abundance on elevation gradients. Velázquez *et al.* (2016) reported no effect of the altitudinal gradient on spore abundance and species richness and no relationship of the latter with chemical and physical soil properties. Vieira *et al.* (2019) found no major differences in AMF communities associated with different mountain habitats, but they observed that the diversity of AMF species was related to the heterogeneity of habitats (*e.g.*, plant communities) and that soil texture was most related to the structure of these fungal communities.

Among the ways used to study AMF communities, the detection of glomalin-related soil protein (GRSP) fractions has been gaining attention (Purin & Klauberg Filho 2011). In Brazil, studies on GRSP are recent, and this study is the first to address the levels of BRSP and EE-BRSP in soils from montane forests along an altitudinal gradient. We found that the levels of total (BRSP) and easily extractable (EE-BRSP) glomalin varied among altitudes, and the levels of both glomalin fractions were greater at higher elevations, where greater concentrations of C and N were also recorded. Thus, our results corroborate those obtained by Rillig *et al.* (2003), who also found a positive correlation between glomalin levels and levels of C and N in the soil; this finding led the authors to suggest the use of glomalin as a marker for impacts caused by land use. Until now, the highest levels of glomalin recorded in Brazilian soils were 5.12 and 1.23 mg.g soil^{-1} (BRSP and EE-BRSP, respectively) in a native field (Purin *et al.* 2006) and 4.7 and 1.2 mg.g soil^{-1} (BRSP and EE-BRSP, respectively) in a preserved Caatinga ecoregion (Mergulhão, 2006). In our study, we found levels exceeding those at an altitude of 2100 m (5.7 mg.g soil^{-1} for BRSP and 3.8 mg.g soil^{-1} for EE-BRSP), which were most likely related to the high levels of C.

The habitats examined in this study showed differences in soil texture (clay, sand, and silt content), nutrient and organic matter content, and pH, among other characteristics. We observed that differentiation between the 1500 m level and the other levels was mainly due to the lower acidity and the higher sum of bases and of remaining P in the soil. This may explain the trend observed for greater diversity of AMF species (Shannon index) at 1500 m, since the availability of P in the soil can alter the effectiveness of some AMF species through its effect on root colonization (Nogueira & Cardoso 2007). However, the better nutritional conditions of the soil found at higher altitudes may have contributed to the predominance of plant species with lower mycorrhizal dependency, and therefore affected AMF species diversity.

The microclimate data for the forest under study revealed a decrease in temperature along the altitudinal gradient, which can result in partial freezing of some anatomical structures of the vegetation, and this may cause stress to plant communities. The low temperatures recorded for the higher altitudes may have contributed to accumulation of organic matter in the soil, which was greater at higher altitudes (1900 and 2100 m). This is due to decreased rates of biomass decomposition (Charan *et al.* 2013). According to Bruijnzeel (2002), this soil characteristic indicates high potential for carbon sequestration and water retention. Soils with high organic matter levels tend to be more fertile and promote better plant development, consequently decreasing the dependence of plant communities on mycorrhizal fungi.

Conclusions

Our results indicated that altitude gradient and the environmental and soil characteristics, peculiar to each altitude, affected the composition of the AMF community, the Shannon index, and the levels of glomalin produced by these fungi that are associated with the rhizosphere of dominant plant species within each altitude of a Brazilian tropical montane cloud forest. However, the effect of altitude on AMF diversity can be analyzed, though indirectly, that is, through its effect on plant diversity. Acaulosporaceae was the dominant family in terms of richness, representing 40% of the total AMF diversity found at all altitudes. Only the *Glomus* sp. morphotype was common to all altitudes, and it also had the highest spore abundance, demonstrating the high resilience of this species in occupying different niches. The highest concentrations of glomalin (BRSP and EE-BRSP) occurred where the highest concentrations of C and N were recorded (2100 m). Soil texture (clay, sand, and silt contents), nutrient and organic matter content, pH, and other characteristics varied among the altitudes. The main soil characteristics that differentiated the 1500 m level from the other levels was the lower acidity and the higher sum of bases and of remaining P in the soil.

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References

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil 170: 47-62.

Bartón K. 2009. MuMIn: Multi-Model Inference. R package version 1.42.1. https://cran.r-project.org/package=MuMIn.

Błaszkowski J, Sanchez-Garcia M, Niezgoda P *et al.* 2022. A new order, Entrophosporales, and three new Entrophospora species in Glomeromycota. Frontiers in Microbiology 13: 4615.

Bonfim JA, Vasconcellos RLF, Gumiere T, Mescolotti DLC, Oehl F, Cardoso EJBN. 2015. Diversity of arbuscular mycorrhizal fungi in a brazilian atlantic forest toposequence. Microbial ecology 71: 164-177.

Börstler B, Renker C, Kahmen A, Buscot F. 2006. Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. Biology and Fertility of Soils 42: 286-298.

Bradford DMM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248.

Bruijnzeel LA, Scatena FN, Hamilton L. 2011. Tropical Montane Cloud Forests: Science for Conservation and Management. Cambridge, Cambridge University Press.

Bruijnzeel LA. 2002. Hydrology of tropical montane cloud forests: a reassessment. In: Gladwell JS (ed.), Proceedings of the Second International Colloquium on Hydrology and Water Management in the Humid Tropics. Paris, IHP-UNESCO. p. 353-383.

Bubb P, May I, Miles L, Sayer J. 2004. Cloud Forest Agenda. Cambridge, United Nations Environment Programme-World Conservation Monitoring Centre.

Burnham KP, Anderson DR, Huyvaert KP. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65: 23–35. doi: 10.1007/s00265-010-1029-6

Charan G, Bharti VK, Jadhav SE *et al.* 2013. Altitudinal variations in soil physico-chemical properties at cold desert high altitude. Journal of Soil Science and Plant Nutrition 13: 267-277.

Churchill SP, Griffin III D, Lewis M. 1995. Moss diversity of the tropical Andes. In: Churchill SP, Balslev H, Forero E, Luteyn JL (eds.). Biodiversity and conservation of Neotropical Montane Forests. New York, The New York Botanical Garden. p. 335-346.

Coutinho ES, Fernandes GW, Berbara RLL, Valério HM, Goto BT. 2015. Variation of arbuscular mycorrhizal fungal communities along an altitudinal gradient in rupestrian grasslands in Brazil. Mycorrhyza 25: 627-638.

de Carvalho F, de Souza FA, Carrenho R, Moreira FMS, Jesus EC, Fernandes GW. 2012. The mosaic of habitats in the high-altitude Brazilian rupestrian fields is a hotspot for arbuscular mycorrhizal fungi. Applied Soil Ecology 52: 9-19.

Falkenberg DB. 2003. Matinhas nebulares e vegetação rupícola dos Aparados da Serra Geral (SC/RS), sul do Brasil. PhD Thesis, Universidade Estadual de Campinas, Campinas.

Gai JP, Christie P, Cai XB *et al.* 2009. Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau. Ecological Research 24: 1345-1350.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal endogene species extracted from soil by wet sieving and decanting. Transaction British Mycological Society 46: 235-244.

Haug I, Wubet T, Weiß M, Aguirre N, Weber M, Günter S, Kottke I. 2010. Species-rich but distinct arbuscular mycorrhizal communities in reforestation plots on degraded pastures and in neighboring pristine tropical mountain rain forest. Tropical Ecology 51: 125-148.

Hijmans RJ, Condori B, Carillo R, Kropff MJ. 2003. A quantitative and constraint-specific method to assess the potential impact of new agricultural technology: The case of frost resistant potato for the Altiplano (Peru and Bolivia). Agricultural Systems 76: 895-911.

Homeier J, Breckle SW. 2002. Waldstruktur und Baumdiversität im montanen Regenwald der Estación Científica San Francisco in Südecuador. Beihefte der Tüuxengesellschaft 14: 109-118.

Huang Z, Zhao F, Wang M, Qi K, Wu J, Zhang S. 2019. Soil chemical properties and geographical distance exerted effects on arbuscular mycorrhizal fungal community composition in pear orchards in Jiangsu Province, China. Applied Soil Ecology 142: 18-24.

Koehler A, Galvão F, Longhi SJ. 2002. Floresta Ombrófila Densa Altomontana: Aspectos florísticos e estruturais de diferentes trechos da serra do mar. Ciência Florestal 12: 27-39.

Kottke I, Haug I, Setaro S *et al*. 2008. Guilds of mycorrhizal fungi and their relation to trees, ericads, orchids and liverworts in a neo-tropical mountain rain forest. Basic and Applied Ecology 9: 13-23.

Kottke I. 2002. Mycorrhizae – rhizosphere determinants of plant communities. In: Waisel Y, Eshel A, Kafkafi U (eds.). Plant roots: The hidden half. New York, Marcel Dekker Inc. p. 919-932.

Li X, Gai J, Cai X *et al.* 2014. Molecular diversity of arbuscular mycorrhizal fungi associated with two co-occurring perennial plant species on a Tibetan altitudinal gradient. Mycorrhiza 24: 95-107.

Liu Y, He J, Shi G, An L, Öpik M, Feng H. 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. FEMS Microbiology Ecology 78: 355-365.

Lugo MA, Ferrero M, Menoyo E, Estévez MC, Siñeriz F, Anton A. 2008. Arbuscular Mycorrhizal fungi and rhizospheric bacteria diversity along an altitudinal gradient in South American Puna Grassland. Microbial Ecology 55: 705-713.

Mariano RF, Rezende VL, Mendes CN, Santos RMD, Souza CRD, Moura ASD, Fontes MAL. 2020. Phylogenetic beta diversity in an upper montane Atlantic Forest along an altitudinal gradient. Plant Ecology 221: 671-682.

Mergulhão ACES. 2006. Aspectos ecológicos e moleculares de fungos micorrízicos arbusculares. PhD Thesis, Universidade Federal de Pernambuco, Recife.

Munyanziza E, Kehri HK, Bagyaraj DJ. 1997. Agricultural intensification, soil biodiversity and agro-ecosystem function in the tropics: the role of mycorrhiza in crops and trees. Applied Soil Ecology 6: 3-16.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Nogueira MA, Cardoso EJBN. 2007. Phosphorus availability changes the internal and external endomycorrhizal colonization and affects symbiotic effectiveness. Scientia Agricola 64: 295-300.

Oehl F, Schneider D, Sieverding E, Burga CA. 2011. Succession of arbuscular mycorrhizal communities in the foreland of the retreating Morteratsch glacier in the Central Alps. Pedobiologia 54: 321-331.

Oehl F, Sieverding E. 2004. Pacispora, a new Vesicular arbuscular mycorrhizal fungal genus in the glomeromycetes. Journal of Applied Botany 78:72-82.

Oehl F, Sýkorová Z, Redecker D, Wiemken A, Sieverding E. 2006. Acaulospora alpina, a new arbuscular mycorrhizal fungal species characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98: 286-294.

Oliveira-Filho AT. 2009. Classificação das fitofisionomias da América do Sul cisandina tropical e subtropical: Proposta de um novo sistema – prático e flexível – ou uma injeção a mais de caos? Rodriguésia 60: 237-258.

Palenzuela J, Azcón-Aguilar C, Barea JM, Silva GA, Oehl F. 2013. Acaulospora pustulata and Acaulospora tortuosa, two new species in the Glomeromycota from Sierra Nevada National Park (southern Spain). Nova Hedwigia 97: 305-319.

Ponce-Reyes R, Reynoso-Rosales VH, Watson JEM, VanDerWal J, Fuller RA, Pressey RL, Possingham HP. 2012. Vulnerability of cloud forest reserves in Mexico to climate change. Nature Climate Change 2: 448-452.

Purin S, Klauberg Filho O, Stürmer SL. 2006. Mycorrhizae activity and diversity in conventional and organic apple orchards from Brazil. Soil Biology and Biochemistry 38: 1831-1839.

Purin S, Klauberg Filho O. 2011. Glomalina: nova abordagem para entendermos a biologia dos fungos micorrízicos arbusculares. In: Siqueira JO, de Souza F, Cardoso EJBN, Tsai SM (eds.). Micorrizas: 30 anos de pesquisa no Brasil. Lavras, UFLA. p. 503-524.

Purin S, Rillig MC. 2007. The arbuscular mycorrhizal fungal protein glomalin: Limitations, progress and a new hypothesis for its function. Pedobiologia 51: 123-130.

Rillig MC, Ramsey PW, Morris S, Paul EA. 2003. Glomalin, an arbuscular mycorrhizal fungal soil protein, responds to land use change. Plant and Soil 253: 293-299.

Rodrigues WC. 2005. DivEs – Diversidade de espécies. Versão 2.0 Software e Guia do Usuário.

Sá Júnior A, Gonsaga LC, Silva FF, Alves MC. 2012. Application of the Köppen classification for climatic zoning in the state of Minas Gerais, Brazil. Theoretical and Applied Climatology 108: 1-7. doi: 10.1007/s00704-011-0507-8

Scheer MB, Mocochinski AY. 2009. Florística vascular da Floresta Ombrófila Densa Altomontana de quatro serras no Paraná. Biota Neotropica 9: 51-70.

Schenck NC, Perez Y. 1998. A manual of identification of vesicular-arbuscular mycorrhizal fungi. Gainesville, University of Florida.

Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW. 2008. Mycorrhizal and Dark-Septate Fungi in Plant Roots Above 4270 Meters Elevation in the Andes and Rocky Mountains. Arctic, Antarctic, and Alpine Research 40: 576-583.

Schübler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycological Research 102: 1413-1421.

Scott AJ, Knott MA. 1974. Cluster analysis method for grouping means in the analysis of variance. Biometrics 30: 507-512.

Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Eschborn, Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Federal Republic of Germany.

Smith SE, Read DJ. 2008. The symbiontes forming arbuscular mycorrhizas. In: Smith SE, Read DJ (eds.). Mycorrhizal Symbiosis. San Diego, Academic Press. p. 13-41.

Stadtmüller T. 1987. Cloud Forests in the Humid Tropics: A bibliographic review. Costa Rica, United Nations University, Tokyo and CATIE, Turrialba.

Stürmer SL, Bever JD, Morton JB. 2018. Biogeography of arbuscular mycorrhizal fungi (Glomeromycota): A phylogenetic perspective on species distribution patterns. Mycorrhiza 28: 587- 603.

Urcelay C, Acho J, Joffre R. 2011. Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation. Mycorrhiza 21: 323-330.

van der Heijden MGA, Klironomos JN, Ursic M *et al*. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396: 69-72.

Velázquez MS, Stürmer SL, Bruzone C, Fontenla S, Barrera M, Cabello M. 2016. Occurrence of arbuscular mycorrhizal fungi in high altitude sites of the Patagonian Altoandina region in Nahuel Huapi National Park (Argentina). Acta Botanica Brasilica 30: 521-531.

Vieira LC, Silva DKA, Silva IR, Gonçalves CM, Assis DMA, Oelh F, Silva G. 2019. Ecological aspects of arbuscular mycorrhizal fungal communities in different habitat types of a Brazilian mountainous area. Ecological Research 34: 182-192.

Webster GL. 1995. The panorama of Neotropical Cloud Forests. In: Churchill SP, Balslev H, Forero E, Luteyn JL (eds.). Biodiversity and conservation of neotropical montane forests: Proceedings of neotropical montane forest biodiversity and conservation symposium. New York, The New York Botanical Garden. p. 53-57.

Wijayawardene NN, Hyde KD, Dai DQ *et al*. 2022. Outline of Fungi and fungus-like taxa-2021. Mycosphere 13: 53-453.

Wright SF, Upadhyaya A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198: 97-107.

Xu T, Veresoglou SD, Chen Y *et al*. 2016. Plant community, geographic distance and abiotic factors play different roles in predicting AMF biogeography at the regional scale in northern China. Environmental Microbiology Reports 8: 1048-1057.

Zhang Y, Gui LD, Liu RJ. 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. Plant and Soil 261: 257-263.

Zhu X, Yang W, Song F, Li X. 2020. Diversity and composition of arbuscular mycorrhizal fungal communities in the cropland black soils of China. Global Ecology and Conservation 36: e00964.