Arbuscular mycorrhizal fungal communities in soils under three phytophysiognomies of the Brazilian Atlantic Forest

Lorrane Marques Duarte, Simone Cristina Braga Bertini, Sidney Luiz Stürmer, Marcio Rodrigues Lambais, and Lucas Carvalho Basilio Azevedo

Received: June 30, 2018
Accepted: September 5, 2018

ABSTRACT
Arbuscular mycorrhizal fungi (AMF) play an important role in plant community productivity and structure, and so studying the factors that affect the diversity and structure of this fungal community is important for understanding their ecology in tropical forests. We investigated AMF spore communities and root colonization under three forest phytophysiognomies (Restinga Forest, REF; Lowland Ombrophilous Dense Forest, LLF; and Montane Ombrophilous Dense Forest, MTF). Spore abundance was lowest in LLF and highest in REF, with no statistical differences relative to MTF. Spore diversity indices and root colonization rates were not statistically different among the phytophysiognomies. However, principal components analysis revealed that AMF community structure differed according to forest phytophysiognomy. Hierarchical partitioning analysis indicated that most of the AMF community variables were better explained by phytophysiognomy than by chemical and physical attributes of the soil. In addition to the plant community, clay content, pH, Boron, P, S and CEC best explained some of the AMF community variables. Thus, we conclude that while several factors determine AMF community structure in the Atlantic Forest, phytophysiognomy is the most significant.

Keywords: arbuscular mycorrhiza, Glomeromycotina, hierarchical partitioning, plant community, soil attributes, soil microbiology, tropical rain forest

Introduction
The Atlantic Forest of Brazil is a hot spot of biological diversity with high levels of endemism. However, this biome has been threatened by historical deforestation for agriculture and urban development (Mittermeier et al. 1998; Myers et al. 2000). It has been estimated that only 11% to 16% of the original area (1.29 × 10^6 km^2) remains (Morellato & Haddad 2000; Ribeiro et al. 2009), and consequent losses in biodiversity and ecosystem function are unquestionable (Myers et al. 2000).

Losses in biodiversity are mainly observed in plants and animals (Myers et al. 2000). Conversely, little is known about the effects of anthropic activities on microbial diversity in this biome. Recent findings on the bacterial communities of the phyllosphere, dermosphere and rhizospheric soil in the Atlantic Forest show high levels of bacterial diversity associated with specific tree species and highly similar bacterial communities within individual plants of the same taxon, suggesting that each plant species has its own microbiome (Lambais et al. 2014).

Among various plant-microbe associations, Arbuscular mycorrhiza (AM) play an important role in the shaping
Arbuscular mycorrhizal fungal communities in soils under three phytophysiognomies of the Brazilian Atlantic Forest

Diagramação e XML SciELO Publishing Schema: www.editoraletra1.com.br


and functioning of an ecosystem (Heijden et al. 1998; Rillig & Mummey 2006; Smith & Read 2008). Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycotina) can develop symbiotic relationships with most known plant species (Smith & Read 2008; Spatafora et al. 2016). The most prominent consequence of this relationship is enhanced phosphate uptake from the soil solution and transfer to plants. AM may also increase plant productivity and diversity, and drive plant community structure (Heijden et al. 1998; Klironomos et al. 2011; Garcia de León et al. 2016). In addition, the external mycelia of AMF may increase soil aggregation and carbon stock in the soil (Rillig & Mummey 2006).

Several interdependent environmental variables, including edaphic and climatic factors, influence AMF community structure (Antunes et al. 2011; Davison et al. 2011; León et al., 2016). The structure of the plant community may also affect the AMF community. Certain plant species may create species-specific soil attributes, microclimates and associations with AMF species (Johnson et al. 1992; Mummey & Rillig 2006). Thus, vegetation and soil types can be considered as drivers of AMF community structure (Oehl et al. 2010; Pagano et al. 2013; Mathimaran et al. 2005).

Studies on the tropical rain forest have focused on how different management practices and forest disturbance intensities (León et al. 2018; Pereira et al. 2018), altitudinal and ecological restoration gradients (Bonfim et al. 2016; Silva et al. 2015b), and early stages of plant succession (Stürmer et al. 2006; Zangaro et al. 2013) affect AMF community structure. The occurrence of AMF species in the Atlantic Forest has also been compiled by Jobim et al (2018). However, the effects of phytophysiognomy type on diversity of mycorrhizal fungi needs to be better clarified. Different phytophysiognomies are common in the biome and vary with altitude. Differences in plant community structure (Assis et al. 2011; Campos et al. 2011; Padgurschi et al. 2011) suggest that AMF communities may also vary by phytophysiognomy type. Therefore, quantifying the extent to which phytophysiognomy and specific soil physical and chemical variables determine AMF variables could enhance our understanding of community structure in the Atlantic Forest.

We evaluated AMF community structure, diversity, and root colonization within permanent plots in three preserved phytophysiognomies of the Atlantic Forest (Restinga Forest, Lowland Ombrophilous Dense Forest and Montane Ombrophilous Dense Forest). We examined whether AMF sporulation, community structure, diversity, and root colonization were affected by phytophysiognomy, and the physical and chemical properties of the soil. Given that edaphic climatic conditions and plant community could influence the composition of the AMF community, we hypothesized that AMF species composition is more strongly influenced by phytophysiognomy than by the physical and chemical properties of the soil.

Materials and methods

Study area

Soil and root samples were collected from permanent plots (100 m x 100 m) in three phytophysiognomies of the Atlantic Forest (established using the Biota-Program from the São Paulo State Research Foundation) located in the Serra do Mar State Park, São Paulo, Brazil (Joly et al. 2012) (Fig. 1). The permanent plots were set up within a

Figure 1. Study areas in Brazil were permanent plots of Restinga Forest (10 m elevation), Lowland Ombrophilous Dense Forest (75m elevation) and Montane Ombrophilous Dense Forest (1000 m elevation) within the Serra do Mar State Park in the Atlantic Forest.
Restinga forest (REF), (elevation 9.5 to 10.5 m, 23°21’22"S 44°51’03"W), a Lowland Ombrophilous Dense Forest (LLF) (elevation 64 to 89 m, 23°20’05"S 44°49’55"W), and a Montane Ombrophilous Dense Forest (MTF) (elevation 1010 to 1040 m, 23°20’36"S 45°04’22"W). Henceforth, the Lowland Ombrophilous Dense Forest will be referred to as the “Lowland forest” and the Montane Ombrophilous Dense Forest as the “Montane forest”.

The Restinga forest hadTypic Quartzipsamment sandy soil that was seasonally waterlogged, acidic (pH 3.9 to 5.0) and had high Al saturation (approximately 57% of the cation exchange capacity (CEC)). Assis et al (2011) identified 84 plant species from 32 families in the Restinga forest plot. Myrtaceae, Arecaceae and Euphorbiaceae were the most abundant plant families and accounted for 57% of the individuals (Joly et al. 2012).

The Lowland forest featured strongly undulating relief with clayey Typic Dystrochrept soil that was acidic (pH 4.1 to 4.7), had high Al saturation (averaging 55% of CEC), and was stony with outcrops of granite/gneiss. The Lowland forest plot contained 142 plant species within 41 families (Campos et al. 2011). The most abundant species were Euterpe edulis Mart., Mollinedia schottiana (Spreng.) Perkins, Bathysa mendonicae K. Schum., Coussarea accedens Mull. Arg. and Rustia formosa (Cham. & Schltdl.) Klotzsch. while Myrtaceae, Rubiaceae and Fabaceae were the most abundant plant families.

The Montane forest was on strongly undulating relief, with clayey Typic Dystrochrept soil that was acidic (pH 4.1 to 4.3) with high Al saturation (averaging 72% of CEC). Padgurschi et al. (2011) identified 149 plant species from 40 families in the Montane forest plot. The most abundant species were Euterpe edulis Mart., Licania hoehnei Pilg., Calyptranthes lucida Mart., Ocotea catharinensis Mez. and Mollinedia argyrogyra Perkins while Arecaceae, Myrtaceae, and Monimiaceae were the most abundant families.

The climate in the Restinga and Lowland forests is Tropical/Subtropical Humid (Af/Cfa, Köppen classification) without a dry season. The average annual rainfall is over 2200 mm, while the average annual temperature is 22 °C (Joly et al. 2012; Setzer 1966; Embrapa 2009) and the average minimum temperature is 18 °C (measured from Sep 2006 to Nov 2007, Siegloch 2010). The climate of the Montane forest is Humid subtropical (Cwa, Köppen classification) with an average annual precipitation of 2500 mm, average annual temperature of 21 °C and average minimum temperature of 15 °C (measured between Sep 2006 and Nov 2007, Siegloch 2010).

**Soil and root sampling**

Soil and roots were collected at the end of the dry season (October, 2011), and randomly sampled (six replicates) within each of the permanent plots in the Restinga, Lowland and Montane forests. Specifically, three subsamples (spaced equidistantly ~100 cm) were collected from each random point using a soil probe (20 cm depth). These subsamples were then pooled to form a single sample per point. Therefore, a total of 18 samples were collected (three phytosociognomies x six replicates). The soil and root samples were placed in plastic bags and stored at 4 °C until processing.

**Chemical and physical analysis of the soil**

Soil pH and concentrations of H+Al, Al, Ca, Mg, P, organic carbon (OC), K, S, B, Cu, Fe, Mn and Zn were determined according to Silva (2009). CEC, base saturation and Ca, Mg, K and Al saturation of CEC were calculated as: CEC = (Ca+Mg+K+Al+H); base saturation = (Ca+Mg+K/CEC)*100; Ca, Mg and K saturation = [(Ca or Mg or K)/CEC]*100; Al saturation = [Al/(Ca+Mg+K+Al)]*100. Soil texture was determined accordingly to Gee & Or (2002).

**Arbuscular mycorrhizal fungi spores: counts, richness and diversity estimations**

AMF spores were extracted from 50 g of soil by wet sieving (Gerdemann & Nicolson 1963) and centrifugation in water and in a 70% sucrose solution. The spores were rinsed in tap water and collected with a 38 µm mesh sieve. The spores were then mounted on glass slides using polyvinyl-lacto-glycerol (PVLG) and PVLG with Melzer’s reagent (1:1, v/v) for identification and then separated by morphotype. AMF spores were identified at the genus/species level according to Schenck & Pérez (1990) and using morphological descriptions from the INVAM website (http://invam.cav.wvu.edu) and Glomeromycotina species list website (http://www.lrz.de/~schuessler/amphylot/).

The frequency of occurrence of each AMF species was calculated as the percentage of samples in which a species was observed. Species richness was estimated as the number of species observed in each treatment using the non-parametric estimators ACE (Chao & Lee 1992) and CHAO-1 (Chao 1984) determined using the SPADE program (Chao & Shen 2010). The Shannon diversity index and the reciprocal of the Simpson index were calculated using AMF spore abundance. The Pielou index of equitability was determined as described by Magurran (1988).

**Root colonization**

Roots were randomly separated from the soil samples using tweezers (1 g from each soil sample), rinsed in tap water to eliminate soil debris, immersed for 15 hours in 10% KOH solution at room temperature and then clarified in a water bath at 60 °C for 10 min. After discarding the KOH solution, the roots were washed with tap water, immersed in HCl 1% solution and then stained (5% Parker blue pen ink, 5% acetic acid and 10% lactic-glycerol) (Vierheilig et al. 2012).
Arbuscular mycorrhizal fungal communities in soils under three phytophysiognomies of the Brazilian Atlantic Forest

1998) for three min at 90 °C. Mycorrhizal root colonization was measured under a dissecting microscope using the grid-line intersect method (Giovannetti & Mosse 1980).

Statistical analysis

Variables were tested for homogeneity of variance (Bartlett test) and normality (Shapiro-Francia and Lilliefors (Kolmogorov–Smirnov)). Spore counts were transformed to \((x + 0.5)^{0.5}\) and root colonization data were normalized using \(\text{arcsin}(x)^{0.5}\). ANOVA was used when the assumptions for the parametric tests were met, while the Kruskal–Wallis test \((p < 0.05)\) was employed when the normality and homoscedasticity assumptions were not met. ANOVA was conducted using the PROC GLM procedure in SAS 9.2 (Littell et al. 2006) and the Kruskal–Wallis test was performed using R software (RStudio, Boston, MA). The means from the normal data were compared using the Tukey Kramer test \((p < 0.05)\) (SAS version 9.2; SAS Institute, Cary, NC).

Principal component analysis (PCA) was used to visualize relationships among AMF species, biological variables (diversity indexes, spore counts and root colonization), and associations with soil physical and chemical properties, and to determine whether the samples could be differentiated by phytophysiognomy (Lepš & Šmilauer 2003). PCA was performed with a focus on inter-species correlations (Lepš & Šmilauer 2003) and using Canoco software version 4.5 (Biometris, Netherlands).

Hierarchical Partitioning analysis (HP) was performed as in Bertini et al. (2014) (“hier.part” package version 1.04, Walsh & Mac Nally 2015, and implemented using the R software, R Development Core Team 2017) to estimate the percent of total variation in spore number, root colonization, AMF species and diversity variables explained by a given environmental variable (phytophysiognomy, physical and chemical soil properties) (Chevan & Sutherland 1991; Mac Nally 2000; Mac Nally & Walsh 2004). Thus, the environmental variables or predictor variables consisted of categories (phytophysiognomy), physical soil properties and chemical soil properties. Multicollinearity between predictor variables was determined using the ‘car’ package of R software. Variables with Variance Inflation Factors (VIFs) > 5 and Tolerance < 0.2 were considered collinear and not used to construct the models (Montgomery & Peck 1992). All independent effect/joint effect ratios of predictor variable were higher than 1.0, which indicates low collinearity among predictor variables (Mac Nally 2000; Pont et al. 2005; Arenas et al. 2006). R-squared was used as the goodness-of-fit measure. Generalized linear models (GLMs) were used for response variables that did not meet the assumptions of the linear regression model (i.e. goodness-of-fit argument “Rsqu” and family argument of glm “gaussian”) (Millington et al. 2007; Walsh & Mac Nally 2015). The statistical significance of the independent contributions of each environmental variable was accepted at the upper 95% confidence limit \((Z \text{ score} \geq 1.65)\) and determined using a randomization approach with 100 interactions (Mac Nally 2000).

Results

Soil physical and chemical attributes

All soil samples were acidic \((\text{pH from 4.17 to 4.50})\) and had high Al saturation \((54.6 \% \text{ to } 73.1 \% \text{ of the CEC})\) (Tab. 1). Some soil properties were affected by different phytophysiognomies. Ten of the nineteen soil physicochemical attributes \((\text{pH}, \text{ Al}, \text{ CEC}, \% \text{ K}, \% \text{ Al}, \text{ Zn}, \text{ B}, \text{ sand}, \text{ silt and clay content})\) differed by phytophysiognomy type. Soil pH, Zn level and sand content were significantly greater in the Restinga forest than in the Montane forest. Conversely, Al %, Al content, clay and silt content were greater in the Montane forest than in the Restinga forest. CEC and K% were greater in the Lowland forest than in the Montane forest.

Table 1. Soil chemical and physical attributes in three phytophysiognomies (Restinga Forest – REF, Lowland Forest – LLF, and Montane Forest – MTF) in the Atlantic Forest.

<table>
<thead>
<tr>
<th>Soil Attributes</th>
<th>REF</th>
<th>LLF</th>
<th>MTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.50 ±0.3 A</td>
<td>4.37 ±0.2 AB</td>
<td>4.17 ±0.09 B</td>
</tr>
<tr>
<td>Al% (mmol kg⁻¹)</td>
<td>2.07 ±0.5 B</td>
<td>2.29 ±0.6 B</td>
<td>3.40 ±0.7 A</td>
</tr>
<tr>
<td>H⁺+Al⁺ (mmol kg⁻¹)</td>
<td>5.13 ±0.1 A</td>
<td>5.00 ±0.3 A</td>
<td>4.94 ±0.06 A</td>
</tr>
<tr>
<td>CEC</td>
<td>5.60 ±0.5 A</td>
<td>5.50 ±0.3 A</td>
<td>5.00 ±0.5 A</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>82.5 ±4.5 A</td>
<td>79.6 ±7.0 A</td>
<td>80.6 ±7.0 A</td>
</tr>
<tr>
<td>S (mg kg⁻¹)</td>
<td>69.11 ±18 A</td>
<td>70.9 ±7.3 A</td>
<td>63.6 ±11.7 A</td>
</tr>
<tr>
<td>Organic C (g kg⁻¹)</td>
<td>42.6 ±2.9 A</td>
<td>43.3 ±2.1 A</td>
<td>40.7 ±1.4 A</td>
</tr>
<tr>
<td>*Ca saturation (%)</td>
<td>13.1 ±2.9 A</td>
<td>14.4 ±3.7 A</td>
<td>11.4 ±1.9 A</td>
</tr>
<tr>
<td>*Mg saturation (%)</td>
<td>7.66 ±4.5 A</td>
<td>8.24 ±2.8 A</td>
<td>5.30 ±1.0 A</td>
</tr>
<tr>
<td>K saturation (%)</td>
<td>2.70 ±0.3 A</td>
<td>3.38 ±0.4 A</td>
<td>2.71 ±0.5 B</td>
</tr>
<tr>
<td>Al saturation (%)</td>
<td>57.1 ±0.9 B</td>
<td>54.6 ±13 B</td>
<td>75.1 ±5.5 A</td>
</tr>
<tr>
<td>Zn (mg kg⁻¹)</td>
<td>2.85 ±0.7 A</td>
<td>2.06 ±0.6 B</td>
<td>1.81 ±0.3 B</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>2.88 ±1.0 A</td>
<td>5.60 ±6.0 A</td>
<td>4.58 ±2.9 A</td>
</tr>
<tr>
<td>Cu (mg kg⁻¹)</td>
<td>0.71 ±0.2 A</td>
<td>0.61 ±0.3 A</td>
<td>0.56 ±0.3 A</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹)</td>
<td>392 ±174 A</td>
<td>385 ±134 A</td>
<td>269 ±71 A</td>
</tr>
<tr>
<td>B (mg kg⁻¹)</td>
<td>70.8 ±10 AB</td>
<td>64.3 ±6.9 B</td>
<td>76.0 ±3.0 A</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>91.8 ±21 A</td>
<td>75.6 ±49 A</td>
<td>67.6 ±62 C</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>5.5 ±21 C</td>
<td>17.6 ±28 B</td>
<td>22.3 ±37 A</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>2.61 ±16 B</td>
<td>6.63 ±30 A</td>
<td>1.0 ±32 A</td>
</tr>
</tbody>
</table>

Values are average ± standard deviation \((n = 6)\). Upper case letters compare the three phytophysiognomies. To normal data was used ANOVA and Tukey-Kramer test \((p < 0.05)\). * Kruskal-Wallis was performed to non-normal data \((p < 0.05)\). Values followed by different letters are significantly different and indicated by bold numbers. H⁺+Al⁺ (Potential acidity), CEC (Cation exchange capacity).

Arbuscular mycorrhizal fungi in soil and roots

Thirteen AMF species were identified from spores recovered in the field and assigned to Glomeraceae \((38 \%)\),
Acaulosporaceae (46%) and Gigasporaceae (16%) (Fig. 2). The species with the highest relative abundance (Fig. 2) were also the most frequent species recovered: *Glomus* sp. 1 (243 spores; 74% frequency), *Glomus* sp. 3 (179 spores; 68% frequency) and *Acaulospora mellea* (27 spores; 47% frequency) (Fig. 3).

The average spore number was significantly lower in the Lowland forest than in the Restinga and Montane forests (Tab. 2). Root colonization was approximately 50%; but as with the other diversity indices, did not differ statistically by phytophysiognomy (Tab. 2).

Table 2. Number of spores per 50 g of soil, root colonization (%), average observed richness, estimated species number and diversity index of the AMF community in three phytophysiognomies (Restinga Forest REF, Lowland Forest LLF, and Montane Forest MTF) in the Atlantic forest.

<table>
<thead>
<tr>
<th>Biological attributes</th>
<th>Spores 50g⁻¹ soil</th>
<th>Root colonization (%)</th>
<th>S&lt;sub&gt;AMP&lt;/sub&gt;</th>
<th>Shannon&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Evenness&lt;sup&gt;d&lt;/sup&gt;</th>
<th>ACE-1</th>
<th>CHAO-1&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>30.5 (±24.1) A</td>
<td>53 (±0.1) A</td>
<td>2.66 (±0.8) A</td>
<td>0.73 (±0.05) A</td>
<td>0.82 (±0.1) A</td>
<td>2.18 (±1.8) A</td>
<td>2.66 (±0.8) A</td>
</tr>
<tr>
<td>LLF</td>
<td>4.17 (±3.6) B</td>
<td>53 (±0.13) A</td>
<td>1.83 (±1.2) A</td>
<td>0.45 (±0.5) A</td>
<td>0.47 (±0.5) A</td>
<td>1.16 (±1.4) A</td>
<td>3.16 (±1.2) A</td>
</tr>
<tr>
<td>MTF</td>
<td>14.6 (±6.8) A</td>
<td>50 (±0.11) A</td>
<td>2.89 (±1.0) A</td>
<td>0.64 (±0.3) A</td>
<td>0.59 (±0.2) A</td>
<td>3.05 (±1.9) A</td>
<td>2.89 (±1.0) A</td>
</tr>
</tbody>
</table>

Values are average ± standard deviation (n = 6). * - Species richness of AMF spores; <sup>b</sup> - Shannon index by maximum likelihood estimator; <sup>d</sup> - Reciprocal of the Simpson index; <sup>c</sup> - Pielou’s Evenness; other letters - Estimator of species richness. Upper case letters compare the three phytophysiognomies. Values followed by different letters are significantly different according to the Tukey-Kramer test (p < 0.05) and indicated by bold numbers.

Influence of phytophysiognomy and soil properties on the AMF community

Principal component analysis (PCA) of the data set showed consistent differences among the phytophysiognomy types relative to biological, physical and chemical properties of the soil (Fig. 4). The Montane forest was separated from the Lowland forest along the first axis (Fig. 4A), which explained 22.7% of data variability. The Restinga forest was separated from Montane forest along the second axis, explaining 15.6% of data variability.

*Glomus* sp4, *Glomus* sp3, *Glomus* sp 2, *Glomus* sp1, *Acaulospora foveata*, *Acaulospora mellea*, root colonization, total spore number, Pielou evenness, Shannon, ACE-1, CHAO-1, S<sub>AMP</sub>, 1/D, P, Sand, pH, Zn, S and OC were positively associated with the Restinga forest, and negatively associated with the Lowland and Montane forests (Fig. 4A, B).

The Lowland forest was positively associated with *Scutellospora* sp1, *Scutellospora calospora*, *Acaulospora* sp3, *Acaulospora* sp1, *Rhizophagus fasciculatus*, K, K%, Cu and Mn, whereas the Montane forest was positively associated with *A. tuberculata*, *Acaulospora* sp2, Fe, B, Al, Al%, silt and clay content. Clay was positively associated with *Scutellospora* sp1 and *Scutellospora calospora*, and negatively associated with *Glomus* sp3. The diversity indices (ACE-1 and S<sub>AMP</sub>) and *Acaulospora mellea* were positively associated with P and B. CEC was positively associated with pH, Mg, Ca, K, OC, S and Zn, and negatively associated with evenness.

Contribution of individual soil properties and phytophysiognomy to AMF community determination

Regarding physical and chemical variables, only OC, pH, P, B, S, Fe, CEC and clay content showed no collinearity between variables and were kept for HP analysis. HP was performed to estimate the contribution of individual soil properties and phytophysiognomy on explaining the total variation of each biological (AMF) variable.

Phytophysiognomy most explained total spore number and the occurrence of five of the thirteen AMF species (*S. calospora*, *Acaulospora* sp3, *Glomus* sp1, *Glomus* sp3 and *Glomus* sp4) (Tab. 3). Clay content most explained the occurrence of *Scutellospora* sp1, but also explained

![Figure 2](image-url)
Arbuscular mycorrhizal fungal communities in soils under three phytophysiognomies of the Brazilian Atlantic Forest

Figure 3. Frequency of occurrence of AMF species in soil samples from three phytophysiognomies: Restinga Forest (REF), Lowland Ombrophilous Dense Forest (LLF) and Montane Ombrophilous Dense Forest (MTF) within the Serra do Mar State Park in the Atlantic Forest.

S. calospora, and Glomus sp3. The occurrence of A. mellea was mostly influenced by P, while CEC most explained Pielou evenness. S_{AMF} and ACE-1 were explained mostly by B, while A. foveata was best explained by S content. In summary, seven of the nine explanatory variables significantly explained at least one AMF variable (Tab. 3): phytophysiognomy explained six response variables, clay content explained three, B content and pH explained two, while S, P, and CEC explained one AMF variable each.

Discussion

Influences of soil, climate and plant community on AMF communities are well-documented (Johnson et al. 1992; König et al. 2010; Oehl et al. 2010; Antunes et al. 2011). In this paper, we tested the hypothesis that phytophysiognomy is a stronger determinant of AMF community composition than soil attributes in areas of the Atlantic Forest. Our analysis indicated that multiple factors affect AMF variables;
Table 3. Percent distribution of the independent effect (% I) of each environmental variable (phytophysiognomy, physical and chemical soil attributes) on the variability of the arbuscular mycorrhizal fungal (AMF) community.

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Phytophysiognomy</th>
<th>S</th>
<th>Fe</th>
<th>B</th>
<th>pH</th>
<th>P</th>
<th>Org-C</th>
<th>CEC</th>
<th>Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore number</td>
<td><strong>43.26</strong></td>
<td>2.28</td>
<td>7.71</td>
<td>11.34</td>
<td>10.64</td>
<td>0.91</td>
<td>2.49</td>
<td>6.76</td>
<td>14.60</td>
</tr>
<tr>
<td>Root colonization</td>
<td>14.01</td>
<td>7.34</td>
<td>2.12</td>
<td>1.46</td>
<td><strong>41.09</strong></td>
<td>15.86</td>
<td>8.79</td>
<td>2.97</td>
<td>6.36</td>
</tr>
<tr>
<td>S_Arbuscular</td>
<td>19.41</td>
<td>0.82</td>
<td>4.84</td>
<td><strong>39.11</strong></td>
<td>10.05</td>
<td>2.06</td>
<td>4.93</td>
<td>12.44</td>
<td>6.33</td>
</tr>
<tr>
<td>Shannon</td>
<td>12.93</td>
<td>6.37</td>
<td>4.53</td>
<td>35.21</td>
<td>2.56</td>
<td>2.72</td>
<td>5.98</td>
<td>16.92</td>
<td>12.77</td>
</tr>
<tr>
<td>L/D</td>
<td>9.15</td>
<td>11.92</td>
<td>2.59</td>
<td>30.08</td>
<td>1.55</td>
<td>5.66</td>
<td>10.42</td>
<td>22.21</td>
<td>6.42</td>
</tr>
<tr>
<td>Evenness</td>
<td>18.34</td>
<td>7.78</td>
<td>5.83</td>
<td>11.21</td>
<td>3.97</td>
<td>2.23</td>
<td>9.51</td>
<td><strong>25.80</strong></td>
<td>15.34</td>
</tr>
<tr>
<td>ACE-1</td>
<td>19.44</td>
<td>0.97</td>
<td>1.55</td>
<td><strong>47.19</strong></td>
<td>4.29</td>
<td>5.05</td>
<td>2.84</td>
<td>7.32</td>
<td>11.35</td>
</tr>
<tr>
<td>CHAO-1</td>
<td>23.11</td>
<td>16.21</td>
<td>8.42</td>
<td>0.97</td>
<td>11.04</td>
<td>2.32</td>
<td>15.96</td>
<td>10.61</td>
<td>11.37</td>
</tr>
<tr>
<td>AMF species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. fasciculatus</td>
<td>15.16</td>
<td>8.46</td>
<td>10.32</td>
<td>9.64</td>
<td>8.57</td>
<td>10.49</td>
<td>12.17</td>
<td>11.63</td>
<td>13.55</td>
</tr>
<tr>
<td>A. foveata</td>
<td>6.23</td>
<td></td>
<td></td>
<td></td>
<td><strong>41.18</strong></td>
<td>3.78</td>
<td>1.40</td>
<td><strong>38.13</strong></td>
<td>0.73</td>
</tr>
<tr>
<td>A. mellea</td>
<td>16.91</td>
<td>20.62</td>
<td>1.44</td>
<td>14.38</td>
<td>2.43</td>
<td><strong>32.54</strong></td>
<td>1.42</td>
<td>3.56</td>
<td>6.70</td>
</tr>
<tr>
<td>A. tuberculata</td>
<td>30.56</td>
<td>2.18</td>
<td>2.26</td>
<td>6.14</td>
<td>19.17</td>
<td>4.62</td>
<td>10.57</td>
<td>14.23</td>
<td>10.27</td>
</tr>
<tr>
<td>S. calospora</td>
<td><strong>15.16</strong></td>
<td>8.46</td>
<td>10.32</td>
<td>9.64</td>
<td>8.57</td>
<td>10.49</td>
<td>12.17</td>
<td>11.63</td>
<td><strong>13.55</strong></td>
</tr>
<tr>
<td>Scutellospora sp1</td>
<td>31.32</td>
<td>1.67</td>
<td>4.49</td>
<td>0.98</td>
<td>1.69</td>
<td>2.28</td>
<td>10.92</td>
<td>13.57</td>
<td><strong>33.08</strong></td>
</tr>
<tr>
<td>Acaulospora sp1</td>
<td>15.29</td>
<td>8.54</td>
<td>20.89</td>
<td>2.01</td>
<td>16.79</td>
<td>15.49</td>
<td>11.74</td>
<td>5.88</td>
<td>3.38</td>
</tr>
<tr>
<td>Acaulospora sp2</td>
<td>19.93</td>
<td>2.58</td>
<td>7.64</td>
<td>8.75</td>
<td>3.51</td>
<td>2.29</td>
<td>6.86</td>
<td>42.39</td>
<td>6.05</td>
</tr>
<tr>
<td>Acaulospora sp3</td>
<td><strong>40.55</strong></td>
<td>1.36</td>
<td>0.64</td>
<td>8.12</td>
<td>14.14</td>
<td>3.81</td>
<td>3.25</td>
<td>17.93</td>
<td>10.20</td>
</tr>
<tr>
<td>Glomus sp1</td>
<td><strong>49.88</strong></td>
<td>1.29</td>
<td>8.51</td>
<td>11.94</td>
<td>11.69</td>
<td>1.12</td>
<td>3.35</td>
<td>1.58</td>
<td>10.64</td>
</tr>
<tr>
<td>Glomus sp2</td>
<td>11.24</td>
<td>7.04</td>
<td>3.37</td>
<td>24.53</td>
<td>2.06</td>
<td>28.82</td>
<td>2.94</td>
<td>11.29</td>
<td>8.71</td>
</tr>
<tr>
<td>Glomus sp3</td>
<td><strong>45.61</strong></td>
<td>6.64</td>
<td>8.13</td>
<td>7.94</td>
<td>3.87</td>
<td>0.56</td>
<td><strong>1.09</strong></td>
<td>0.98</td>
<td><strong>25.17</strong></td>
</tr>
<tr>
<td>Glomus sp4</td>
<td><strong>50.81</strong></td>
<td>8.58</td>
<td>10.79</td>
<td>5.28</td>
<td>9.10</td>
<td>4.67</td>
<td>1.19</td>
<td>1.42</td>
<td>8.16</td>
</tr>
</tbody>
</table>

Bold numbers indicate the environmental attributes that most explained a given response variable. aVariability of individual species of AMF was based on spores abundance. S (Sulfur), Iron (Fe), Boro (B), P (Phosphorus), Org-C (Organic carbon), Cation exchange capacity (CEC), Clay content (Clay).

however, phytophysionomy, here represented by three distinct plant communities, was the explanatory variable that most shaped the AMF communities.

**Arbuscular mycorrhizal fungi in the soil**

Thirteen AMF morphospecies were found in the current study. Of these, five species (A. foveata, A. mellea, A. tuberculata, R. fasciculatum and S. calospora) were found in other studies on the Atlantic Forest (Jobim et al. 2018). The presence of the other eight morphospecies (three Acaulospora spp, four Glomus spp and one Scutellospora sp) could not be linked to other areas since they were not identified by species and might represent undescribed species.

The most abundant Glomeromycotina genus (Glomus) and the six most frequent species (Glomus sp. 1, Glomus sp. 3, A. mellea, Acaulospora sp1, Glomus sp4 and Glomus sp2), corroborated previous findings on the prevalence of Glomus and Acaulospora in Atlantic Forest soils (Souchie et al. 2006; Stürmer et al. 2006; Moreira et al. 2007; Pereira et al. 2014; Bonfim et al. 2016). Given that our data was based on morphological identification of soil spores, real diversity in the field could indeed be greater (Varela-Cervero et al. 2015).

**Determining factors of the AMF community and root colonization**

Some authors state that soil features such as chemistry and texture are predominantly responsible for shaping the AMF community (Oehl et al. 2010; Bonfim et al. 2016; Sousa et al. 2018), while other authors suggest that AMF community structure is modified by vegetation and soil types (Pagano et al. 2013; Oehl et al. 2010; Lovelock et al. 2003). Unlike previous studies on the Atlantic Forest, we used PCA and HP analysis to quantify how phytophysionomy and soil properties affect the variability of spore abundance, diversity indexes, AMF species and root colonization.

PCA indicated similarities and differences among the phytophysionogies, and thus showed that phytophysionomy affects the AMF community. Therefore, the three forest types were grouped separately. Specifically, the Restinga and Montane forests showed distinct AMF community, soil chemical and soil physical characteristics, while the Lowland forest showed intermediate AMF community composition and soil attributes that fit between the other two phytophysionogies. The HP method was also used to quantify the individual contribution of the explanatory variables (set to a maximum of nine variables) for each response variable (Walsh & Mac Nally 2015). HP
demonstrated that the phytophysiognomy and texture (clay content) had a greater determining effect on AMF variables than did soil chemical attributes (Tab. 3). In addition to phytophysiognomy and clay content, pH explained the occurrence of one AMF species and root colonization; B explained two diversity variables; and P, S and CEC explained one AMF variable each.

**Contribution of phytophysiognomy on the determination of the AMF community**

Phytophysiognomy most explained variability in spore density and five of the 13 AMF species (Tab. 3). Furthermore, variability in the most frequent AMF species (*Glomus* sp. 1, *Glomus* sp. 3, *Glomus* sp4 and *Glomus* sp2) was better explained by phytophysiognomy than by soil physical and chemical properties. These *Glomus* species were mainly associated with the Restinga forest. Interestingly, the Restinga forest showed the lowest plant-species richness among the forest types studied (Assis et al. 2011; Campos et al. 2011; Padgurschi et al. 2011); but had higher pH, Zn and sand content (Tab. 1). Some of these findings have already been reported: the adaptation of *Glomus* to different pH ranges (Coughlan et al. 2000) and greater abundance of *Glomus* spores with higher soil Zn content (Montiel-Rozas et al. 2017; Silva et al. 2014). However, contrary to Lekberg et al. (2007), we found that *Glomus* species were positively associated to soils with greater sand content.

Conversely, some authors have suggested that the plant community may alter soil characteristics that consequently affect the abundance of *Glomus* species (Lovelock et al. 2003; Mathimaran et al. 2005). Similarly, we found that plant community is the most important factor explaining variation in *Glomus* species.

**Contribution of individual soil properties on determining AMF community and root colonization**

Besides phytophysiognomy, physical and chemical soil properties also explained some of the variability in spore abundance, diversity variables and root colonization. After phytophysiognomy, clay content was the second most significant factor explaining variation in AMF species (three of the 13 AMF species: two *Scutellospora* species (*S. calospora* and *Scutellospora* sp1) and *Glomus* sp2).

Some authors have reported that vegetation type and soil texture have a greater influence on AMF community than soil chemical properties (Lovelock et al. 2003; Mathimaran et al. 2005; Silva et al. 2015a; Sousa et al. 2018). However, other studies have found that while soil chemistry and vegetation type do modify AMF communities, soil texture does not (Pagano et al. 2013; Gomide et al. 2014). Yet another study has shown that management, plant community, soil iron and fine sand influence AM fungal communities in protected and sustainably managed areas of the Atlantic Forest (Pereira et al. 2018). Nevertheless, recognizing the effect of clay content on the occurrence of an individual species can contribute to a better understanding of that species’ ecology.

Soil pH best explained variation in *A. foveata*. Although this was a positive association, all the soils in the current study were very acidic (pH of 4.17 - 4.50), which generally favors *A. foveata* sporulation (Klironomos et al. 1993; Moutoglis & Widden 1996). Therefore, this limited pH range could have influenced competition among AMF species and determined sporulation.

Phosphorus was positively associated with and most explained variation in *A. mellea*. Spore densities of some AMF species increase when low levels of phosphorus are applied, relative to no P applications (Mårtensson & Carlgren 1994; Kahliluoto et al. 2001).

Boron content was positively associated with and best explained variations in richness and estimated richness (ACE-1). Boron is not essential for microorganism growth (except for some cyanobacteria), but may affect microbial processes (e.g., quorum sensing and antibiotic production) (Bonilla et al. 1990; Semmelhack et al. 2004). However, further study is needed to understand how B content affects AMF communities.

Evenness was better explained by CEC than by phytophysiognomy and the other physicochemical properties. CEC was negatively associated with evenness but positively associated with organic carbon and pH. According to the theory of reciprocally-regulated resource exchange between AM fungi and plants (Walder & Heijden 2015), we hypothesized that higher CEC would favor more efficient AMF species that deliver more cationic nutrients to the plants. The plants, in turn, would favor these more efficient fungi, resulting in their dominance.

Root colonization variability was most determined by soil pH. The role of pH in mycorrhizal colonization is not well understood but it has been reported that increasing the pH of acidic soil improves root colonization (Siqueira et al. 1984; Clark 1997; Coughlan et al. 2000). Furthermore, pH seems to have distinct effects on root colonization given different AMF isolates (Medeiros et al. 1994).

**Final considerations**

Bonfim et al. (2016) also used AMF diversity to distinguish three areas at different altitudes in the Atlantic Forest. We broadened this scope by also determining to what extent phytophysiognomy, physical and chemical variables explained variation in AMF species, diversity and root colonization.

Other studies have shown that AM spores and colonization are influenced by the succession/restoration stage of the Atlantic Forest, but in non-mature and non-native phytophysiognomies (Stürmer et al. 2006; Zangaro et al. 2013). In protected and sustainably managed areas of Atlantic Forest, the management, plant community and...
soil iron and fine sand was found as factors influencing AM fungal communities (Pereira et al. 2018). Nevertheless, as in our study, relationships between AMF community structure and plant community have also been shown in land-use types and biomes other than those of the Atlantic Forest (Davison et al. 2011; Gomide et al. 2014; Johnson et al. 1992; Mueller et al. 2014). Thus, our study expands on this research by showing that variations in AMF indices and species are best explained by phytophysiology, followed by soil texture and then soil chemical characteristics.

We were able to add to current AMF research by using HP. To the best of our knowledge, this is the first time HP has been used to evaluate AMF community data. HP is a common statistical technique in animal ecology and water quality studies (Heikkinen et al. 2005; Varanka & Luoto 2012), but recently, its usefulness has been demonstrated in soil ecology studies (Bertini et al. 2014). Our study shows the potential of HP for analyzing ecological data of an AMF community in a tropical rain forest.

Despite differences in AMF species composition among different phytophysionomies, AMF diversity and root colonization remained the same throughout the three phytophysionomies. Given that we collected our soil and root samples only once at the beginning of the wet season, we were unable to estimate the effect of season on the AMF community. On the other hand, Pereira et al. (2018) found that season did not affect AMF community structure. Nevertheless, our findings expand on our understanding of the factors that determine AMF communities in the Atlantic Forest and contribute to the AM database for this environment, which may in turn be useful in future comparisons.

Acknowledgements

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support. SLS and MRL thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for a Research Assistantship. LMD thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Universidade Federal de Uberlândia for granting an undergraduate scholarship. Thanks to the staff of the Molecular Microbiology Lab at ESALQ, Universidade de São Paulo, specially to PhD. Sandra Patricia Montenegro Gomez.

References

Chevan A, Shen TJ. 2010. Program SPADE (Species Prediction And Diversity Estimation), Program and user’s guide. Hsinchu, National Tsing Hua University. http://chao.stat.nthu.edu.tw


Silva IR, Mello CMA, Neto RAF et al. 2011. Forces that structure arbuscular mycorrhizal fungal communities in soils under three phytophysionomies of the Brazilian Atlantic Forest.