Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Are microbial activity and arbuscular mycorrhizal fungal community influenced by regeneration stages? A case study in Southern Brazil coastal Atlantic Rain Forest

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ARTICLE INFO

Keywords:
Ecological succession
Microbial biomass
DGGE

ABSTRACT

Aiming to describe coastal Atlantic Rain Forest (ARF) regeneration dynamics, we evaluated attributes linked to soil biota and mycorrhizal symbiosis in two coastal ARF sites in Florianópolis, Southern Brazil. In each site, we selected forest transects in early regeneration (ER) and advanced regeneration (AR) stages, i.e., about 20 and 50 years without agricultural use, respectively. Soil total organic carbon (TOC) was higher in AR than in ER stage. Soil microbial biomass carbon (MBC) varied between sites and was higher in the AR than in the ER stage. Basal respiration and qCO_2 rates differed between sites and were, respectively, higher and lower in advanced regeneration stage areas. Arbuscular mycorrhizal (AM) root colonization and AM fungal spore density were higher in the ER stage, with no difference between sites. Principal Component Analysis (PCA) for correlation between soil microbiological and chemical attributes showed that the variables with the most influence on sample separation for the first component were total spore number, soil MBC, root mycorrhizal colonization, and soil TOC. Soil pH, available P, and qCO_2 had the highest influence on the second component. This analysis separated samples by regeneration stage, and sites differed only in AR stage. PCR-DGGE analysis on DNA extracted from soil samples showed that AMF community structure differed more between sites than between regeneration stages.

1. Introduction

The Atlantic Rain Forest (ARF) is a biodiversity hotspot, thus in need of urgent preservation (Ribeiro et al., 2009). Conservation areas and abandoned farmland or pastures in secondary succession generate forest fragments in varying regeneration stages, a process relevant for biodiversity restoration (Siminski et al., 2011). Early stages of regeneration have a few dominant pioneer plant species, and as succession progresses those plants are replaced by species better fit for more complex environments, with higher biodiversity and more structured forest strata. Such patterns occur in all Atlantic forest formations, including coastal ARF (Klein, 1980).

The rates and direction of successional stages are determined by soil characteristics, climate, use history, seed bank and plant interactions with other organisms (Siminski et al., 2011), including the soil microbiota (Bachelot et al., 2016). We have found a small number of reports relating successional stages to soil biological activity indicators or microbial community structure. That may be due to difficulties in directly assessing events taking place in the soil. Soil microbial biomass carbon (MBC), basal respiration (BR), and metabolic quotient (qCO_2) are indicators of processes linked to microbial growth, which may be used to evaluate the activity of soil microbial communities (Anderson and Domsch, 2010). As succession progresses, MBC and microbial activity increase due to soil organic matter accumulation, which results from increases in plant litter and rhizodeposition. This leads to enhanced nutrient cycling, increased soil aggregation, and higher soil microbial functional diversity (Bonfim et al., 2013; Nogueira et al., 2004).

Arbuscular mycorrhizal fungi (AMF) form a mutualistic association with more of 80% of plant species (Smith and Read, 2008), improving

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https://doi.org/10.1016/j.apsoil.2019.02.028
Received 26 February 2019; Accepted 28 February 2019

plant nutrition and alleviate biotic and abiotic stress (Pieterse and Dicke, 2007; Pozo and Azcón-Aguilar, 2007). Mycorrhizae are essential in early successional stages because they elicit processes favoring the establishment of pioneer plant species (Matsumoto et al., 2005; Zangaro et al., 2003), thus affecting plant community structures (Van der Heijden et al., 1998; Van Der Heijden et al., 2015). Many plant species in tropical forests have higher growth and survival rates when mycorrhizal (Janos, 1980), and fungus-plant reciprocal benefits depend on mechanisms each symbiont uses to recognize and reward the best performing symbiotic partner (Kiers et al., 2011). The AM fungal community may interact with tree taxonomic and functional community composition and create feedbacks that could influence tree successional dynamics (Peay et al., 2013; García De León et al., 2016). Diversity and community composition of both groups are therefore mutually conditioned (Klironomos, 2003). Ecological research has revealed processes and patterns involved, and molecular tools such as PCR-DGGE are useful to study soil AMF community structure (Gorzelak et al., 2012).

This research aimed to characterize microbial activity and AMF community structure in coastal ARF sites undergoing early and late regeneration stages. We sought to quantify microbial activity and growth in two regeneration stages, and to test whether AM colonization, spore density and AM fungal community species composition change as the succession advances.

2. Material and methods

2.1. Description of the sites

The study was carried out in two sites in a coastal ARF ecosystem in Florianópolis (SC, Brazil): the Desterro Environmental Conservation Unit (UCAD) and the Lagoinha do Leste Municipal Park (LLMP) (see Table S1). These sites, 34 km apart, were chosen because they are the sole areas undergoing regeneration located in public conservation units in the Island of Santa Catarina. They have controlled access, and therefore minimal anthropogenic disturbance. The climate is mesothermic subtropical humid (Cfa), soil is a Typic Hapludalf (Soil Survey Staff, 2014), mean annual precipitation of the 1462 mm, mean annual air temperature of the 20.1 °C and mean annual soil temperature of the 23.5 °C. At each site we selected forest fragments in two regeneration stages of secondary succession. Early regeneration stage areas (ER) have been in natural regeneration for about 20 years after agricultural use while advanced regeneration stage areas (AR) have been under natural regeneration for about 50 years. Plant community and forest strata differ between regeneration stages, but are similar between sites (Table S1). Sampling in the areas was done following a transect, each plot being collected every 25 m, totaling 5 plots of 25 m^2 per area. All areas were collected in only one transect except the ER area of the UCAD where they had two sets of transects, one with two plots and the second with three plots. UCAD ER and AR areas are approximately 0.5 km apart. At the LLMP site, early and advanced stage areas are about 1 km apart. In each plot, 20 10-cm deep soil samples were taken with an auger and pooled.

2.2. Chemical and microbiological analyzes of soil

Soil samples were sieved (2-mm mesh), and part was kept at 4 °C until microbiological analyses, part at −20 °C until molecular analyzes and part was oven-dried (50 °C) until constant weight. We measured soil total organic carbon (TOC, Walkley-Black), available phosphorus (P) (Mehlich-1 solution), and pH (1:1 water suspension), as by Tedesco et al. (1995). We estimated microbial biomass carbon (BMC) by the fumigation-extraction method (Vance et al., 1987) and basal respiration (BR) by the closed-jar method (Jenkinson and Powlson, 1976). Metabolic quotient (qCO_2) is BR/MBC ratio. Root samples were stained (Koske and Gemma, 1989) to assess mycorrhizal colonization (Giovannetti and Mosse, 1980). AMF spores were extracted (Gerdemann and Nicolson, 1963), counted, and separated into morphotypes for general characterisation. The identification followed morphological descriptions available in the INVAM page (International Culture Collection of Arbuscular Mycorrhizal Fungi) (http://invam.caf.wvu.edu).

2.3. PCR_DGGE of AMF community

The AMF community was characterized by PCR-DGGE, after amplification of the partial LSU rDNA region from the soil samples. Soil total DNA was extracted with Power Soil® DNA Isolation kit (MOBIO) and amplified with fungal universal primers LR1 and FLR2 (Trouvelot et al., 1999; Van Tuinen et al., 1998). Products from the first PCR were further amplified using Glomeromycota specific primers FLR3 and FLR4 (Gollotte et al., 2004) (see Table S2 for amplification conditions). PCR products from the second amplification (200 ng) were analyzed by DGGE (Øvreås et al., 1997) (see Table S2 for DGGE conditions). After electrophoresis, DNA was stained with Sybr Green (Life Technologies, São Paulo, Brazil) and recorded in a GelLogic 220Pro (Carestream Health, New York, USA). Amplicons were analyzed with the BIONUMERICS 7.10 software (BioSystematica, Wales, UK).

2.4. Statistical analysis

Soil microbial biomass, basal respiration, metabolic quotient, the extent of root colonization, and spore number data were tested with univariate analysis, with the permutational procedure for Permanova for only one variable, based in the Euclidean distance. A Principal Component Analysis (PCA) used microbiological variables and soil chemical attributes. All variables were standardized for PCA. Analyses were carried out with R software v 3.3.1 (R Development Core Team, 2008) using Vegan package (Oksanen et al., 2013) and with PRIMER v6 package (Clarke and Gorley, 2006). Banding patterns from the PCR-DGGE were compared in Non-Metric Multidimensional Scaling (NMDS) space, using the Jaccard similarity index. Mean similarity values were tested within and between groups, with an analysis of similarity (ANOSIM) using sites and regeneration stages as grouping variables, to discriminate differences between AMF communities. All tests were performed for a maximum of 9999 permutations.

3. Results

Soil TOC was different between regeneration stages but not between sites. It was higher in AR than in ER [3.5 ± 0.5 g kg^{-1} vs 2.7 ± 0.3 g kg^{-1}]. Soil available P differed between successional stages and between areas; UCAD soils had lower P than LLMP soils (1.01 ± 0.3 mg dm^{-3} and 1.5 ± 0.1 mg dm^{-3}, respectively); and soil P levels were higher in ER than on AR areas (1.4 mg dm^{-3} and 1.1 mg dm^{-3}). Soil pH was similar (5.4) between regeneration stages at UCAD while at LLMP soil pH was higher in ER (5.4) than in AR (4.5). In both sites, soil MBC in AR stages was 40% higher than in ER (Fig. 1A). Basal respiration (Fig. 1B) was similar in both stages at LLMP, but at UCAD, ER had higher values than AR areas. Values of qCO_2 in the LLMP area were higher in AR than in ER, while at UCAD no differences were detected (Fig. 1C). Root mycorrhizal colonization and spore density was higher in ER stages at both areas (Fig. 1D and E).

Principal Components Analysis (PCA) for correlation between soil microbiological and chemical attribute data showed that the first two components explain 77.7% of variability (Fig. 2). In the first component, the variables with the highest effect on sample separation were total spore number, soil MBC, root mycorrhizal colonization extent, and soil TOC. For the second component, the main variables were soil pH, soil available P, and qCO_2 (Table S3). This analysis separated samples by regeneration stage, and sites differed only in AR areas.

Morphological examination of the spores resulted in the

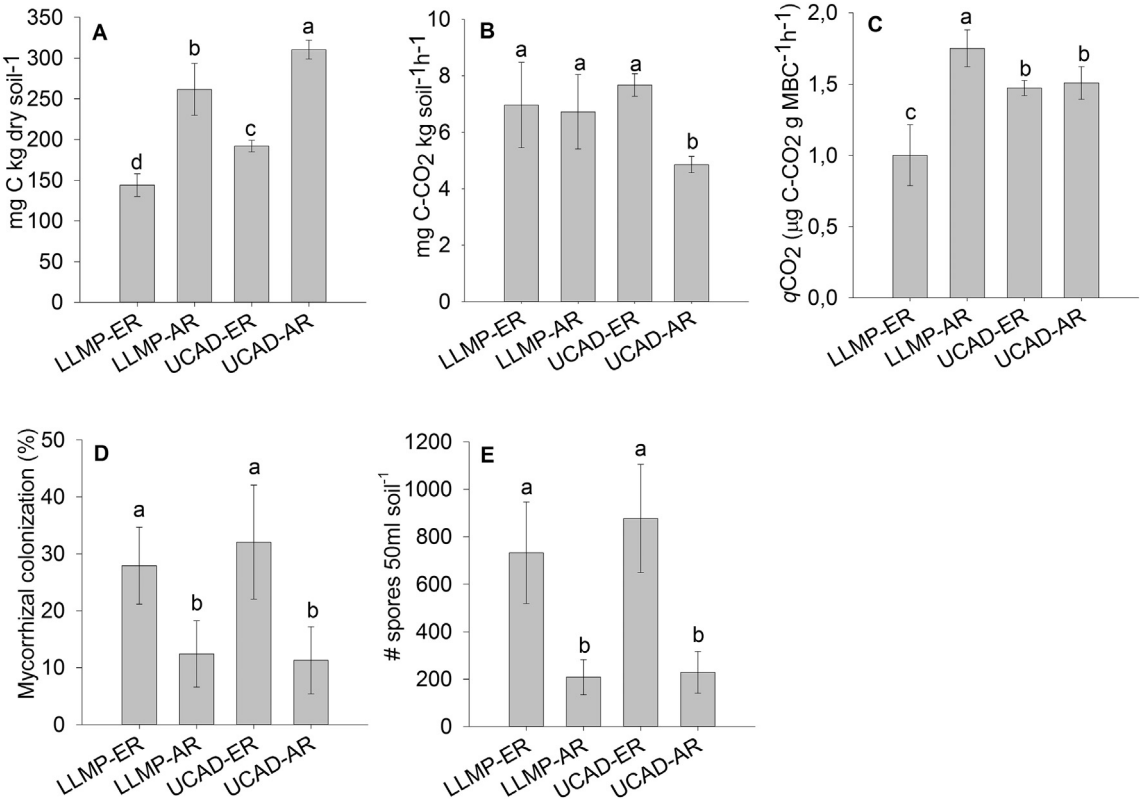

Fig. 1. Microbial biomass carbon (MBC) (A), basal respiration (BR) (B), metabolic quotient (qCO_2) (C), Colonization extent (Col) (D), and Spore density (Spo) (E) in soils from Lagoinha do Leste (LLMP) and Desterro Conservation Unit (UCAD) coastal Atlantic Forest fragments in early (ER) and advanced (AR) regeneration stages. Vertical bars representing the confidence interval, bars with the same letter do not differ by *t*-test ($p < 0.05$).

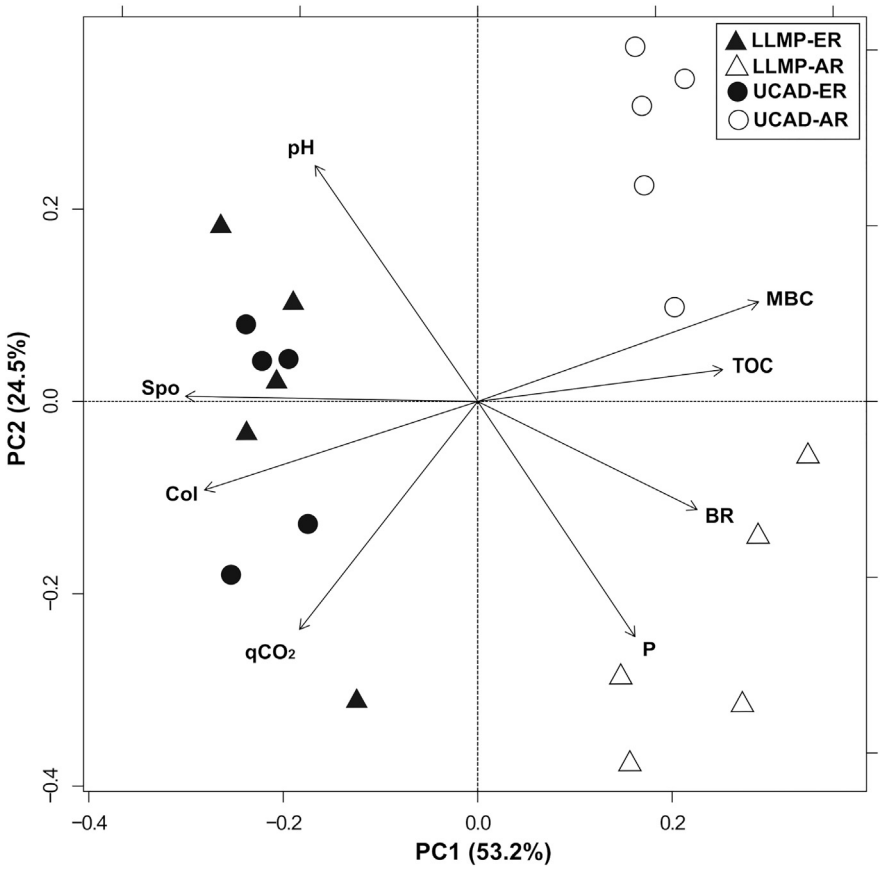

Fig. 2. Principal components analysis, including microbiological variables and soil chemical attributes in soils from Lagoinha do Leste (LLMP) and Desterro Conservation Unit (UCAD) Atlantic Forest fragments in early (ER) and advanced (AR) regeneration stages. Microbial biomass carbon (MBC); total organic carbon (TOC); basal respiration (BR); phosphorus (P); metabolic quotient (qCO_2); colonization extent (Col); spore density (Spo); soil pH (pH). Rotated loadings on the PC1 and PC2 of the PCA in Table S3.

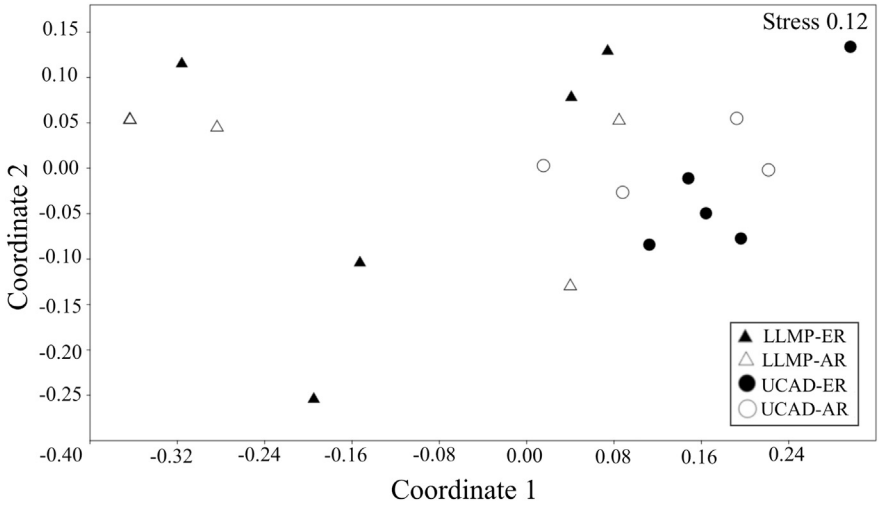

Fig. 3. Non-metric multidimensional scaling (NMDS) of PCR-DGGE banding patterns of soils from Lagoinha do Leste (LLMP) and Desterro Conservation Unit (UCAD) coastal Atlantic Forest fragments in early (ER) and advanced (AR) regeneration stages.

identification of ten morphotypes belonging to the genera Glomus sensu lato (3); Gigaspora (2); Dentiscutata (2); and Acaulospora (3). Assessment of AMF community composition using PCR-DGGE yielded a total of 38 bands. Samples from the LLMP site had fewer bands (6.3 ± 3.8), as compared to samples from the UCAD site (10.3 ± 3.4). On average the ER samples had 8.5 (± 4.4) bands, while AR samples had 7.9 (± 3.9) bands. AMF community structure was more heterogeneous at LLMP, and NMDS analysis showed that it was more scattered. Overall there were more differences in the AMF community between sites than between regeneration stages (Fig. 3). That was confirmed by ANOSIM, which showed significant differences between sites in banding patterns ($R = 0.39$; $p < 0.01$), with no difference between regeneration stages ($R = -0.03$; $p = 0.55$).

4. Discussion

Plant succession is a well-known process, but no general pattern in soil biota changes associated with it has been established (Fierer et al., 2010). Our results show differences in microbial activity indicators in coastal ARF fragments at contrasting regeneration stages. Soil MBC had the best ability to discriminate samples from different stages since this indicator was the most important contributor to group separation in the PCA. Nutrient mineralization and release from organic substrates are tightly linked to heterotrophic microbial activity in soil, which is modulated by climate and plant cover (Gessner et al., 2010). Soil litter decomposition by microbes makes nutrients available to plants, promoting an increase in plant biomass, which in turn forms new plant litter, activating a positive feedback mechanism (Begon, 2006). Such feedbacks are present in the areas undergoing advanced regeneration stages, which had higher values of TOC and MBC. Omeja et al. (2012) re-analyzed data from 57 experiments carried out in tropical forests and found higher levels of soil TOC in older forests. Older ARF fragments also had higher soil MBC (Bonfim et al., 2013), demonstrating the effect of continuous litter accumulation and rhizodeposition on soil microbial activity. Similarly to MBC, BR was generally higher in soils from forest fragments in advanced regeneration stages, which was associated with lower qCO_2. These results suggest that soil biota in older areas are both more active and more efficient in carbon use (Susyan et al., 2011). Our results support this theory, but in LLMP advanced stage area, BR points to high microbial activity while qCO_2, is higher than expected, indicating that a major fraction of metabolized carbon in soil at that specific area is not being used to generate new microbial biomass. That suggests a stressed microbial community, seemingly related to low soil pH.

AMF, a significant part of soil microbial biomass, is a biological indicator of plant community regeneration (Rillig et al., 2001). PCA showed that mycorrhizal variables had the highest contribution to the separation of both regeneration stages and the behavior we observed is in accordance with Zangaro et al. (2012), who demonstrated that AMF root colonization and spore production in ARF fragments are reduced as succession advances, a pattern also found in other searches (Stürmer et al., 2006; Stürmer and Siqueira, 2011; Zangaro et al., 2013). Plants from early regeneration stages adapt to soils with low organic matter content. Those species have small, fast-germinating seeds, and a short life cycle associated with high growth rates (Nogueira et al., 2004). Their high photosynthetic rates may stimulate root symbionts to establish, grow, and sporulate (Kaschuk et al., 2009). On the other hand, late succession plants grow slowly and have large seeds, with higher nutrient reserves (Nogueira et al., 2004). The mycorrhizal association is not an advantage for such plants since low growth rates are linked to low mineral nutrient demand and photosynthate production (Matsumoto et al., 2005).

In summary, our results showed a trend towards a high number of AMF spores, higher root colonization rates, and lower soil MBC and TOC in ER areas. Although those factors separated regeneration stages at both sites, they do not seem to be related to AMF community composition, which was more dependent on location, i.e., site, than on regeneration stage. AMF species number and composition change as ecosystem regeneration advances. Bonfim et al. (2013) showed that AMF spore diversity decreased and plant diversity increased in fragments of coastal ARF in regeneration stages varying from early to intermediate and advanced. Forested and deforested areas in the Amazon Forest had different AMF species composition, but species richness and diversity were similar in both conditions (Leal et al., 2013). However, these two studies relied on field-collected spore identification, a technique that excludes non-sporulating groups (Stürmer et al., 2006). In the present work, we used PCR-DGGE, a technique that allows for detection of AMF species not sporulating at sampling time, as well as other AMF propagules present, like root fragments or soil hyphae. This technique may improve the comparison of AMF communities in areas with varying conditions, such as regeneration stages.

AMF community composition and species richness are affected by abiotic conditions, interspecific competition, and dispersal ability of each species (Lekberg et al., 2007). Little is known about AMF dispersion mechanisms in ecosystems, but wind and animals may be important dispersers of these fungi. Since those dispersal agents are distance-dependent, adjacent areas have higher chances of sharing inoculum sources (Egan et al., 2014; Zaharick et al., 2015). The similar

results obtained from ER and AR areas in each site may be related to the short distance between sampling transects; 0.5 km at UCAD and 1.0 km at LLMP. Insects, small mammals, and wind currents may carry propagules across such distances, which would lead to homogenization. These results should nevertheless be taken as a trend. Establishment of AMF distribution patterns would benefit from a comparison of adjacent areas in sites under regeneration, to reduce irregularities produced by changes in pedo-climatic regimes.

5. Conclusions

This preliminary research shows that soil microbial biomass carbon is the most relevant indicator of changes in microbial responses to Atlantic forest regeneration, being higher in later stages of plant succession. Early regeneration stages appear more favorable to arbuscular mycorrhizal establishment probably because plant communities in this stage are more able to benefit from this symbiosis, although AMF community structure differs more between sites than between regeneration stages.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.02.028.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgements

This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and by CNPq. We thank Camila Guedes for collaboration with laboratory analyses and Marcia P. Hoeltgebaum for plant identification.

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