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Succession and environmental variation influence soil exploration potential by fine roots and mycorrhizal fungi in an Atlantic ecosystem in southern Brazil

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Abstract: Fast-growing plant species are plentiful at the early stages of succession and possess roots with greater capacity for soil exploration than slow-growing plant species of late stages. Thus, the dynamics of fine-root production, morphological traits and arbuscular mycorrhizal fungal (AMF) infection intensity were assessed monthly over 1 y in the grassland, scrub, secondary and mature forests of the Atlantic Forest ecosystem, amounting to 13 consecutive samplings. Fine roots were sampled in three 100 × 100-m plots at each study site. Each plot was subdivided in five 20 × 100-m subplots and 15 soil samples were randomly taken from a depth of 0–5 cm in soil within each plot. The average of the fine-root dry mass increased from 1.39 mg cm^{-3} soil in the grassland to 3.37 mg cm^{-3} in the secondary forest; fine-root tip diameter varied from 146 μm in the grassland to 303 μm in the mature forest; tissue density from 0.24 g cm^{-3} root in the grassland to 0.30 g cm^{-3} in the mature forest and fine-root length was 4.52 cm cm^{-3} soil in the grassland and 6.48 cm cm^{-3} soil in the secondary forest. On the other hand, fine-root specific length decreased from 43.9 m g^{-1} root to 18.3 m g^{-1} root in the mature forest; incidence of root hairs was 67% in the grassland and 30% in the mature forest; the length of root hairs was 215 μm in the grassland and 112 μm in the mature forest; and the intensity of AMF infection decreased from 66% in the grassland to 17% in the mature forest. In addition to AMF infection, the environmental variation also affected dry mass production and morphological traits of fine roots. During the cool season, fine-root dry mass, fine-root length, incidence and length of root hairs and intensity of AMF infection decreased compared with the warm season. We verified that the potential for soil exploration, that expresses the capacity for nutrient acquisition via fine roots and AMF infection intensity, decreased during the cool season and with the advance of the successional groups. These results indicate that fine-root traits and intensity of AMF infection are influenced by the intrinsic nutrient requirements of the plant species in each ecological group.

Key Words: arbuscular mycorrhizal fungi, carbon economy, nutrient acquisition, root dynamics, soil fertility, tropical forest

INTRODUCTION

Succession in a plant community is a continuous process that starts with the establishment of pioneer species and progresses to more advanced stages, in which competition and persistence in the environment control the substitution of species over time (Guariguata & Ostertag 2001). Fine roots are one of the main components of plant communities, representing an important portion of the primary productivity (Jaramillo *et al.* 2003, Norby & Jackson 2000, Vogt *et al.* 1996), and are responsible for the exploration and acquisition of resources from soil (Hertel *et al.* 2003, Holdaway *et al.* 2011). Species belonging to the early stages of succession

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grow fast and invest large amounts of fixed carbon into building tissues for acquisition of resources, such as leaves and fine roots (Guariguata & Ostertag 2001), compared with slow-growing species of later successional stages (Eissenstat *et al.* 2000). In turn, development of the root system and distribution in the soil are affected by physical and chemical properties of soil, micro-organisms, interaction with other roots, type of biome and seasonality (Brown & Lugo 1994, Dress & Boerner 2001, Hendrick & Pregitzer 1996, McMichael & Burke 1998, Robinson *et al.* 2003).

Limited nutrients in the soil induce plant adaptations, such as production of fine roots, alterations in morphological traits (Holdaway *et al.* 2011, Zangaro *et al.* 2005) and increases in lifespan (Comas *et al.* 2012, Eissenstat & Yanai 1997). The plasticity of fine roots changes with soil fertility (Hodge 2004), within (Gower 1987) and between tropical forests (Maycock & Congdon 2000), and also among woody species (Zangaro *et al.* 2007). In addition, fine roots associate with arbuscular mycorrhizal fungi (AMF) to improve the host's capacity for nutrient acquisition. Being ubiquitous in all successional stages, AMF are very important in tropical soils (Zangaro & Moreira 2010), but the intensity of mycorrhizal infection and plant response to AMF may differ among successional groups (Huante *et al.* 1993, Siqueira *et al.* 1998, Zangaro *et al.* 2000, 2003). In general, plant species of early-successional stages show higher root infection and are more effective in multiplying AMF compared with late-stage species (Zangaro *et al.* 2008). The high mycotrophic status of fast-growing plant species may be a combination of high nutritional demand, high requirement for light and high photosynthetic rates (Zangaro *et al.* 2012*a*,*b*, 2013). When compared with late-successional species, the early ones have fine roots with morphological traits adapted for greater nutrient acquisition, such as higher total and specific length, smaller diameter, lower tissue density and higher density of longer root hairs (Zangaro *et al.* 2005, 2007, 2008, 2012*a*,*b*, 2013).

Studies investigating the relationships between plant species of different ecological groups with their absorbing root morphological traits and AMF are rare, especially in the tropics. Thus, the aim of this work was to assess the dynamics of production of fine roots, morphological traits and AMF root infection intensity over 13 consecutive months in different successional stages in the Atlantic forest ecosystem. We hypothesized that (1) plants of early-successional stages use specially adapted fine-root morphological traits and higher AMF infection intensity for improved soil exploration compared with late-successional species; (2) low rainfall and temperature during the cool season negatively affect the production, morphological traits and intensity of mycorrhizal colonization of fine roots.

METHODS

Study sites

The study sites were located in the Atlantic Forest ecosystem in the municipality of Londrina, Paraná State, Southern Brazil (23°27′S, 51°15′W). The climate was classified as Cfa (mesothermic, subtropical humid) with an average annual temperature of 21 °C and 1600 mm total annual rainfall, which mostly occurred from October until March, however, the region has no well-defined dry season (Chagas e Silva & Soares-Silva 2000). The soil was classified as Rhodic Ferralsol and composed of 80% clay, originating from basalt rock (FAO 1994). Four sites at different successional stages were included in the study. The first site, located on the campus of the State University of Londrina, contained low-fertility soil that had the A and part of the B horizon removed 20 y ago (Zangaro *et al.* 2008). The grasses *Paspalum notatum* Flüggé and *Cynodon* sp. spontaneously regenerated and were prominent at the site. The second site comprised scrub vegetation spontaneously regenerated over a 5-y period on high-fertility soil of an abandoned agricultural area. The main species present were the grasses *Urochloa panicoides* P. Beauv. and *Urochloa decumbens* (Stapf) R.D. Webster, the shrubs *Baccharis dracunculifolia* DC. and *Mimosa invisa* Mart. ex Colla and the woody pioneer species *Solanum granulosoleprosum* Dun. and *Cecropia pachystachya* Trécul. The third site was an abandoned pasture, where a secondary forest spontaneously regenerated over 18 y. The most common woody species found were *Alchornea triplinervia* (Spreng.) Müll. Arg., *Anadenanthera colubrina* (Vell.) Brenan, *Croton floribundus* Spreng., *Parapiptadenia rigida* (Benth.) Brenan, *Tabernaemontana australis* Müll. Arg. and *Cedrela fissilis* Vell. The fourth site was a primary tropical, semi-deciduous forest. A wide diversity of plant species was observed with complex structures and canopies, as well as plants more than 40 m tall. The most common species were *Actinostemom concolor* Müll. Arg., *Aspidosperma polyneuron* Müll. Arg., *Balfourodendron riedelianum* Engl., *Cedrela fissilis* Vell., *Euterpe edulis* Mart., *Gallesia integrifolia* (Spreng.) Harms, *Sorocea bonplandii* (Baill.) Burg. Lanj. & Boer and species of *Guarea* and *Trichilia* (Chagas e Silva & Soares-Silva 2000). Data on rainfall and air temperature were obtained from the agro-climatologic website of the Instituto Agronômico do Paraná (IAPAR) (www.iapar.br), whereas day-length data were obtained from the National Observatory (www.on.br).

Field sampling and soil characteristics

Three 100 × 100-m plots were established at each study site. Each plot was subdivided in five 20 × 100-m subplots,

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and 15 sampling points were randomly placed within each plot, amounting to 45 samples per month from each study site. A steel auger (4.5 cm in diameter) was used to retrieve a soil core at a depth of 0–5 cm, because fine roots are most dense in the first few centimeters of soil and decline with depth (Zangaro *et al.* 2008, 2012*a*). After sampling, each individual core was stored at 5 °C until further analysis. Samplings were repeated monthly for 13 mo from October 2006 to October 2007. Before extraction of fine roots from cores, soil samples were taken and mixed to prepare a representative soil sample for each plot. The representative soil samples were then air-dried at room temperature and subjected to analysis. Content of organic carbon in the soil was obtained by oxidation with 2 M $Na_2Cr_2O_7$ in 5 M H_2SO_4, which was determined calorimetrically. Ca and Mg were extracted with 1 M KCl and quantified by titration. P and K were extracted using a Mehlich-1 solution and then measured using a colorimetric assay and flame photometry, respectively. Five samples containing 20 g of field-moist soil were dried at 104 °C for 24 h to determine soil water content.

Root extraction and measurements

Each soil core was soaked in tap water and root fragments were separated using a sieve with a 0.25-mm mesh. Retained material was hand-sorted in shallow dishes underwater, and the living fine roots (<2 mm diameter) were separated from coarse roots (>2 mm diameter), dead roots and organic debris. Using a stereomicroscope, living fine roots were distinguished from dead roots based on colour, elasticity, as well as degree of cohesion of the cortex and stele (Gower 1987). Only living fine roots (<2 mm diameter) were included in described analyses. Total length was determined by the gridline intersection method (Tennant 1975), while tissue density was determined by volume displacement upon immersion in water in a volumetric graduated burette. To obtain the dry mass, fresh fine-root fractions were dried at 60 °C until dry weight stabilized. Specific root length for each sample was determined based on the ratio between fine-root length and root dry mass. The fine-root mean diameter was calculated using the formula: $\text{diameter} = 2(W/L\pi)^{0.5}$, where W is the fresh root weight and L is the fine-root length (see Zangaro *et al.* 2013). The diameter of fine-root tips was measured 0.5 cm from the root cap. Root-hair length was determined in eight fine-root segments for up to 100 root hairs in each sample. Root-hair incidence was assessed by the presence or absence of root hairs on 100 fine-root intersections using the gridline method (Zangaro *et al.* 2005). Fine-root tip diameter and root-hair length were determined using a microscope at ×100 magnification with an ocular micrometer. Assessment of mycorrhizal infection intensity was carried out on fine roots after clarifying (10% KOH), acidifying (1% HCl), washing in tap water then staining (0.05% trypan blue) (Brundrett *et al.* 1996). For some darkly pigmented root fragments, 0.5% H_2O_2 was applied after KOH. Total AMF infection was estimated using the magnified intersection method (McGonigle *et al.* 1990) by observing the presence of different fungal structures at ×100 magnification. For the current study, only aseptate hyphae were considered to be AMF in the root cortex. For each site, 13 samples of dried fine roots were used for analysis of tissue nutrient concentration, amounting to 52 samples. Briefly, N was determined by the indophenol method after sulphuric acid digestion. The other nutrients were determined in nitric-perchloric digests. P was determined by the molybdenum blue method; K by flame photometry; Ca and Mg by atomic absorption spectrophotometry in the presence of lanthanum; Cu, Zn and Mn by atomic absorption spectrophotometry; and B by azometine-H colorimetry.

Data analyses

Fine-root traits and AMF root infection are shown as mean ± SD. Monthly means (15 samples mo^{-1}) within the same successional site and means among successional sites (representing 13 mo) were subjected to two-way ANOVA, according to a completely randomized design. The means of each successional site were also subjected to Tukey's test at $P \leq 0.05$. Both fine-root dry mass and fine-root length were log-transformed and percentages were arcsine-square root-transformed before analysis. Based on the data collected monthly in each site, we performed a multivariate analysis based on Principal Component Analysis (PCA) to have a general view of the behaviour of the fine-root morphological traits, nutrient concentration in root tissues and attributes relative to soil fertility in each sampling site. For this, we used the software Canoco 4.5 (ter Braak & Smilauer 1988).

RESULTS

Historical records between January 1976 and December 2009 show that rainfall in the Atlantic Forest ecosystem is not well defined throughout the year, but most predominantly occurs in October until March along with higher temperatures (Figure 1a), considered as the more favourable plant-growth period (warm season). During April to September the rainfall and temperature decreases, characterized as a less favourable plant-growth period (cool season). Similar climate patterns were observed during the study period with the exception of unexpectedly high precipitation in July. Day length averaged 12.8 h in the warm season and 10.9 h in the cool season (Figure 1b). The mean air temperature and

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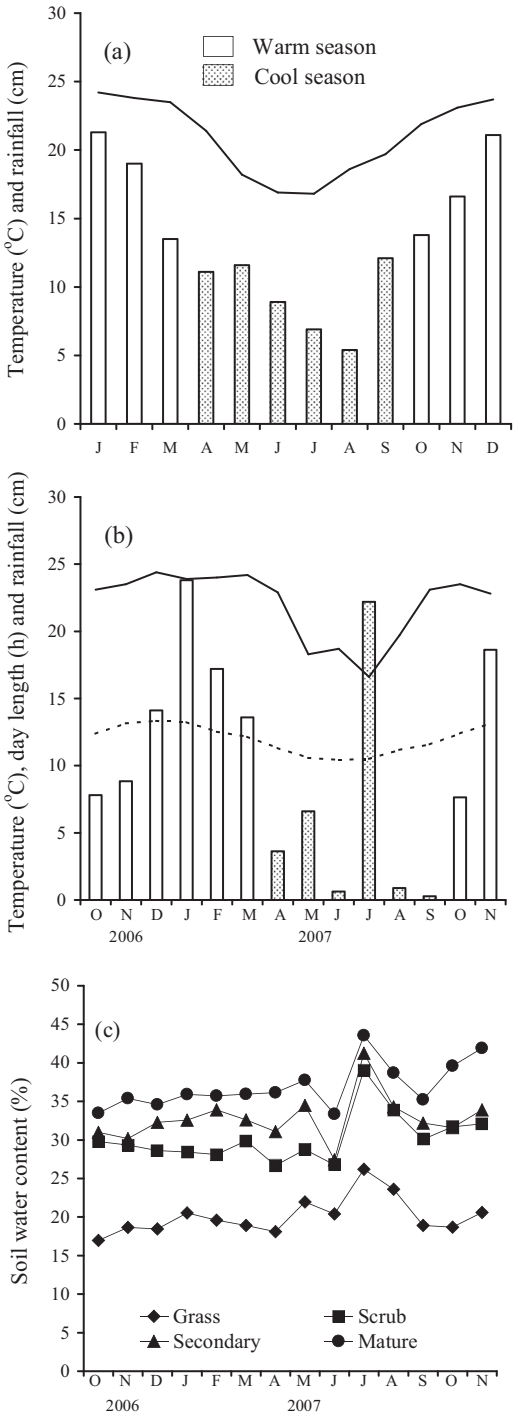

Figure 1. Environmental variables of the studied sites. Historical monthly means of rainfall (columns) and temperature (line) based on records from January 1976 to December 2009 (a). Monthly means of rainfall (columns), temperature (continuous line) and day length (dotted line) (b) and soil water content in the grassland, scrub, secondary and mature forests (c) that correspond to the period of sampling in an Atlantic rain-forest ecosystem, located at Londrina municipality, Paraná state, Southern Brazil.

rainfall were 23.9 °C and 14.2 cm, respectively, during the warm season, while 19.8 °C and 5.7 cm, respectively, during the cool season. Soil moisture varied depending on the vegetation, being 20.1% in the grassland, 30.2% in the scrub, 32.8% in the secondary forest and 36.9% in the mature forest (Figure 1c). However, soil moisture remained relatively well distributed in both growth periods, showing mild seasonality.

The average (± SD) fine-root dry mass was 1.39 ± 1.04, 1.34 ± 0.73, 3.37 ± 1.31 and 2.77 ± 1.06 mg cm^{-3} soil in the grassland, scrub, secondary and mature forest, respectively, showing an increase with succession (Figure 2a). Secondary forest differed significantly from mature forest, which also differed significantly from grassland and scrub (df = 3; F = 204; P < 0.0001). The last two were not significantly different. Fine-root mass was significantly greater in the months of the warm season compared with those of the cool season (df = 12; F = 9.52; P < 0.0001). We found a significant interaction between stage of succession and month (df = 36; F = 4.67; P < 0.0001). Fine-root lengths were 4.52 ± 3.11, 5.14 ± 1.90, 6.48 ± 2.35 and 4.67 ± 1.55 cm cm^{-3} soil in the grassland, scrub, secondary and mature forest, respectively (Figure 2b). Secondary forest differed significantly from mature forest (df = 3; F = 40.3; P < 0.0001), which was not different from grassland and scrub. Fine-root length showed a significant decrease during the months of the cool season compared with the warm ones (df = 12; F = 22.1; P < 0.0001). There was a significant interaction between stage of succession and month (df = 36; F = 7.95; P < 0.0001).

Specific fine-root length decreased with the advance of succession, averaging 36.0 ± 22.3, 43.9 ± 21.5, 20.9 ± 7.92 and 18.3 ± 6.90 m g^{-1} root in the grassland, scrub, secondary and mature forest, respectively (Figure 2c). Scrub vegetation differed significantly from grassland, which was different significantly from secondary and mature forests (df = 3; F = 126; P < 0.0001). The last were not significantly different between each other. There was a significant difference among months (df = 12; F = 3.36; P = 0.0001). We found significant interaction between stage of succession and month (df = 36; F = 3.91; P < 0.0001). Fine-root tissue density increased with succession, measuring 0.24 ± 0.06, 0.20 ± 0.05, 0.31 ± 0.07 and 0.30 ± 0.06 g cm^{-3} root in the grassland, scrub, secondary and mature forest, respectively (Figure 2d). Secondary and mature forests were not different between each other, which were different significantly from grassland and scrub (df = 3; F = 122; P < 0.0001). The last were not different between each other. Fine-root tissue density showed a significant decrease during the months of the cool season (df = 12; F = 7.90; P = 0.0001). There was significant interaction between stage of succession and month (df = 36; F = 2.34; P < 0.0001).

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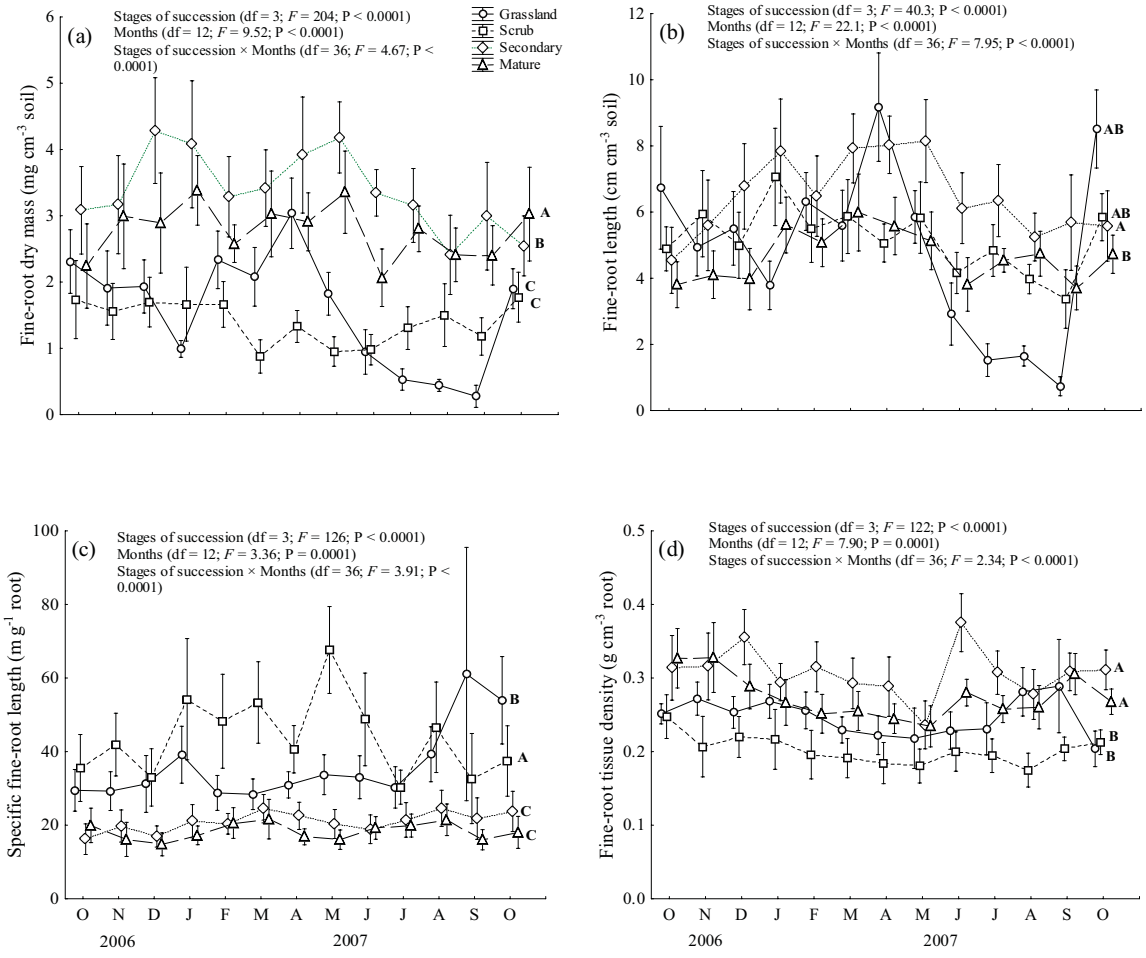

Figure 2. (Colour online) Dry mass (a), total length (b), specific length (c) and tissue density (d) of fine roots ($n = 15$; ± SD) collected at 0–5 cm of soil depth, in the sites under grassland, scrub, secondary and mature forests, located at Londrina municipality, Paraná state, Southern Brazil. Different letters among successional groups, considering the 13 months, mean significant differences by the Tukey's test at 0.05 level.

The mean fine-root diameter (Figure 3a) and root-tip diameter (Figure 3b) increased with the advance of succession. The means of fine-root diameter were 1.28 ± 0.24, 1.35 ± 0.36, 1.51 ± 0.34 and 1.70 ± 0.35 mm in the grassland, scrub, secondary and mature forest, respectively. The fine-root diameters of the mature forest differed significantly from secondary forest, which also differed significantly from grassland and scrub (df = 3; $F = 71.9$; $P < 0.0001$). The last two were not significantly different. There was significant difference among months (df = 12; $F = 4.00$; $P < 0.0001$). We found a significant interaction between stage of succession and month (df = 36; $F = 2.12$; $P = 0.0001$). The means of root-tip diameters were 146 ± 49.1, 171 ± 52.0, 214 ± 56.1 and 303 ± 68.2 μm in the grassland, scrub, secondary and mature forest, respectively. The root-tip diameter of the mature forest differed significantly from secondary forest, which also differed significantly from grassland and scrub (df = 3; $F = 253$; $P < 0.0001$). The last two were not significantly different. There was no significant difference among months (df = 12; $F = 0.19$; $P = 0.99$). We did not find any significant interaction between successional stage and month (df = 36; $F = 0.26$; $P = 0.99$).

The incidence of root hairs decreased with succession, measuring 67% ± 11.3%, 63% ± 10.1%, 46% ± 11.4% and 30% ± 9.28% in the grassland, scrub, secondary and mature forest, respectively (Figure 3c). The incidence of root hairs was not different between grassland and scrub, which differed significantly from secondary and mature forests. The last two differed significantly (df = 3; $F = 742$; $P < 0.0001$). The incidence of root hairs decreased significantly during the cool season compared with the warm (df = 12; $F = 29.6$; $P < 0.0001$). There was a significant interaction between stage of succession and month (df = 36; $F = 2.11$; $P = 0.0002$). Root-hair lengths also decreased with succession, measuring 215 ± 77.4, 227 ± 76.3, 147 ± 58.4 and 112 ± 31.7 μm

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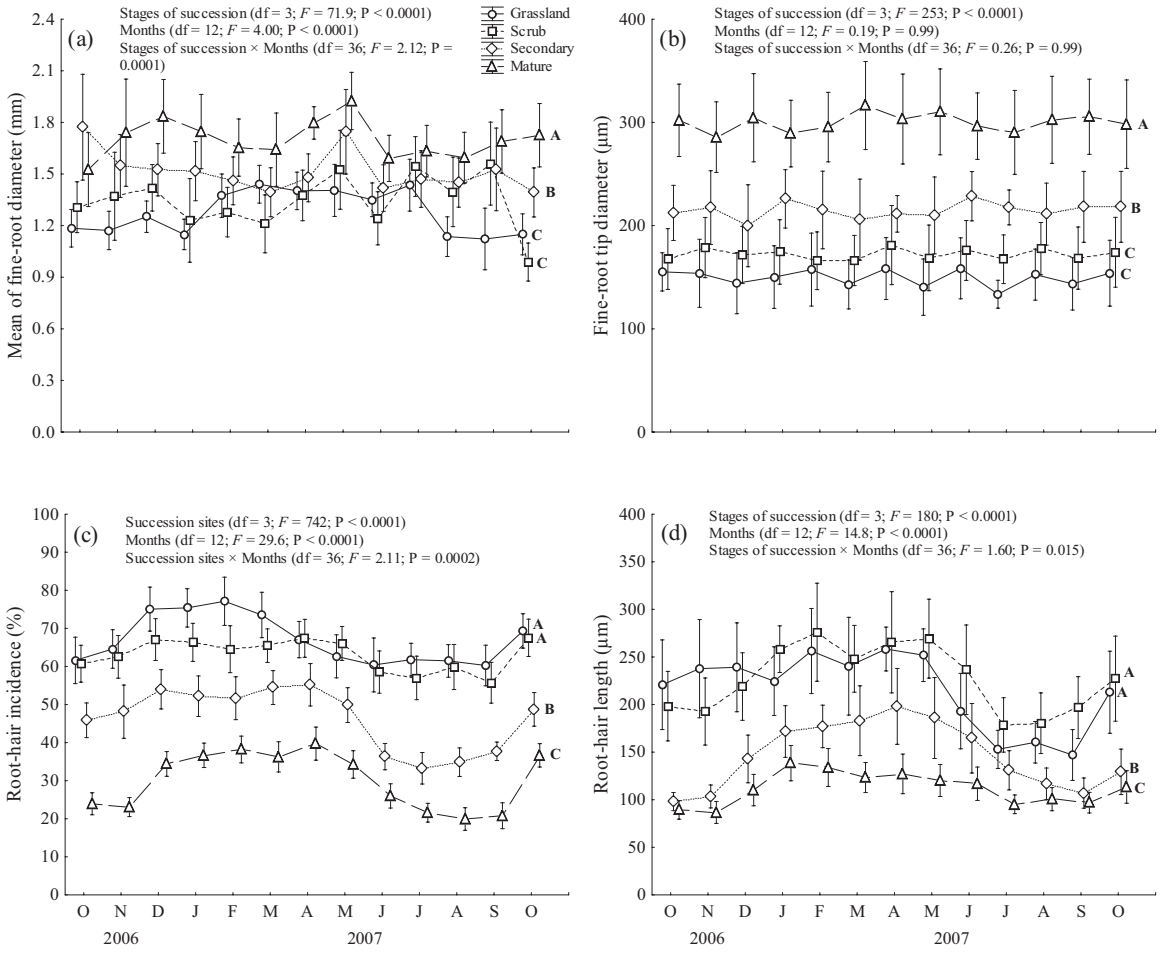

Figure 3. Mean of diameter (a), tip diameter (b), root-hair incidence (c) and root-hair length of fine roots ($n = 15$; ± SD) sampled at 0–5 cm of soil depth in the sites under grassland, scrub, secondary and mature forests, located at Londrina municipality, Paraná state, Southern Brazil. Different letters among successional groups, considering the 13 months, mean significant differences by the Tukey's test at 0.05 level.

in the grassland, scrub, secondary and mature forest, respectively (Figure 3d). The lengths of root hairs were not different between grassland and scrub, which differed significantly from secondary and mature forests. The last two differed significantly (df = 3; $F = 180$; $P < 0.0001$). The length of root hairs decreased significantly during the cool season compared with the warm one (df = 12; $F = 14.8$; $P < 0.0001$). We found significant interaction between stage of succession and month (df = 36; $F = 1.60$; $P = 0.015$).

Conversely, AMF infection intensity decreased over succession and averaged 66% ± 11.4%, 68% ± 8.36%, 52% ± 8.51% and 17% ± 6.34% in the grassland, scrub, secondary and mature forest, respectively (Figure 4). Grassland and scrub were not significantly different, but they both differed significantly from secondary and mature forests. The last two differed significantly (df = 3; $F = 287$; $P < 0.0001$). The AMF infection intensity did not show any significant difference among months (df = 12; $F = 1.41$; $P = 0.163$). We found no significant interaction between stage of succession and month (df = 36; $F = 0.334$; $P = 0.99$).

Regarding the attributes relative to soil fertility, the grassland site was clearly separated from the other three sampling sites as revealed by the PCA, showing the poorest soil fertility (Figure 5a), whereas the scrub, secondary and mature forests did not show any clear distinction. Considering the nutrients in root tissues, the grassland site differed from the other three sampling sites, showing lower concentrations as clearly separated along axis 1 (Figure 5b). However, no clear distinction was observed among scrub, secondary and mature forest along axis 1. Considering axis 2, however, the scrub appeared on the positive side, the secondary forest fitted in an intermediary position, while the mature forest appeared on the negative side. The roots taken in the scrub site showed higher concentrations of P and Zn, the secondary forest had more K, B, Mg, N and Ca, while the mature forest had higher Mn. The fine-root morphological traits showed clear distinction among sampling sites, forming

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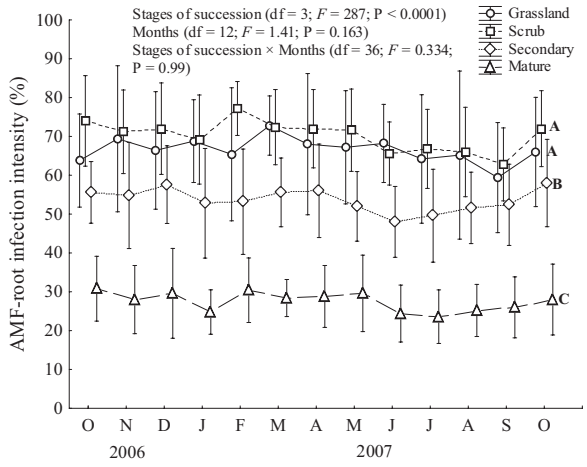

Figure 4. Arbuscular mycorrhizal infection of fine roots ($n = 15$; ± SD) sampled at 0–5 cm of soil depth in the sites under grassland, scrub, secondary and mature forests, located at Londrina municipality, Paraná state, Southern Brazil. Different letters among successional groups, considering the 13 months, mean significant differences by the Tukey's test at 0.05 level.

two distinct groupings (Figure 5c). Axis 1 indicated that the mature and secondary forest formed a grouping that had in common more of the following root morphological traits: root-tip diameter, fine-root diameter, root tissue density and root dry matter. Grassland and scrub, in turn, formed another grouping, having in common more of the following root traits: specific root length, arbuscular mycorrhizal infection, root-hair incidence and root-hair length.

Chemical attributes at sites with grassland indicate a low-fertility soil (Table 1). The scrub soil had the highest P concentration, but no strong differences were observed for other attributes in the scrub, secondary forest and mature-forest soils. Nutrient concentrations were lowest in the fine roots of the grassland site (Table 2). Fine roots from secondary and mature forests generally showed higher concentrations of nutrients, except P and Cu, which were higher in the scrub site. Root concentrations of N, P and Mg were higher in the cool season than the warm ones, with an opposite fashion for B.

DISCUSSION

Fine-root production

Fine-root mass increased with the progress of succession, ranging from grassland to mature forest. Similar results have also been observed around the world (Gower 1987, Hertel *et al.* 2003, Jaramillo *et al.* 2003, Maycock & Congdon 2000) with concomitant increase of root mass with age (Hertel *et al.* 2003), organic matter, nutrient cycling and soil quality (Cairns *et al.* 1997). In the

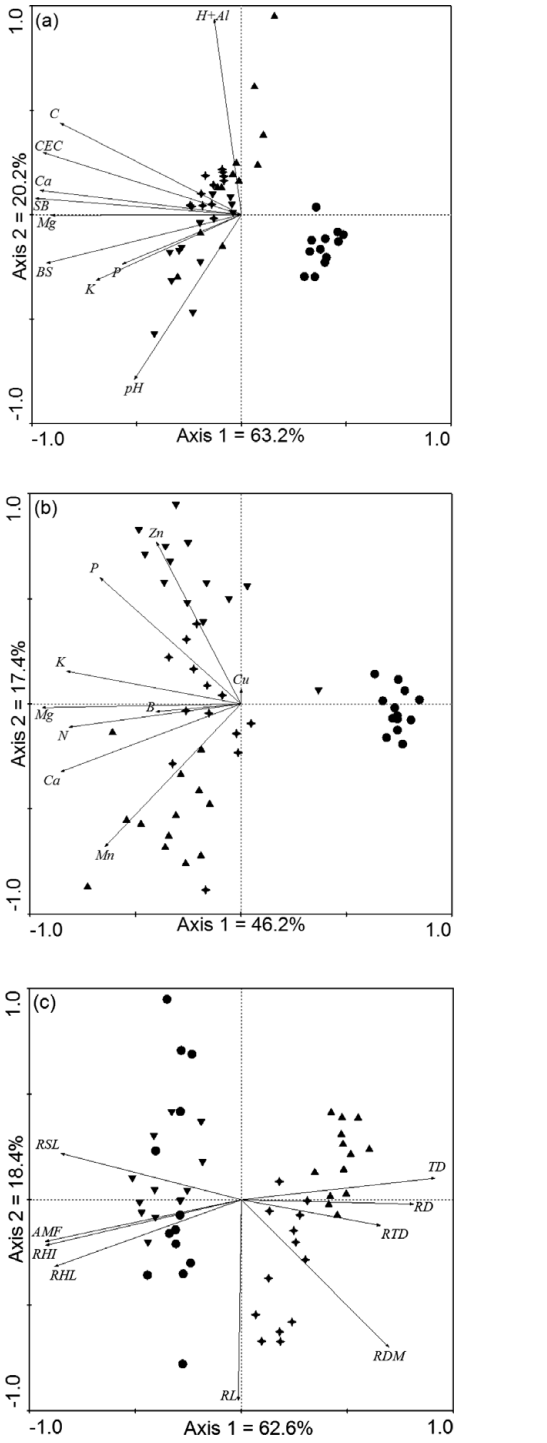

Figure 5. Principal component analysis (PCA) based on soil chemical attributes (a), concentration of nutrients in root tissues (b) and root morphological traits (c) from sites under grassland (●), scrub (▼), secondary forest (✦) and mature forest (▲), located at Londrina municipality, Paraná state, Southern Brazil. RDM = fine-root dry matter; RL = fine-root length; RSL = fine-root specific length; RTD = fine-root tissue density; TD = fine-root tip diameter; RD = fine-root diameter; RHL = root-hair length; RHI = root-hair incidence; AMF = arbuscular mycorrhizal infection intensity.

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Table 1. Annual mean values (± SD, $n = 13$) for chemical attributes in the soil from grassland, scrub, secondary and mature forests collected monthly in southern Brazil.

Chemical attribute	Grassland	Scrub	Secondary	Mature
P (mg L^{-1})	1.72 ± 0.24	16.5 ± 6.81	5.23 ± 1.02	4.66 ± 0.68
C (g L^{-1})	9.84 ± 3.19	44.6 ± 7.11	51.4 ± 1.73	51.7 ± 5.72
OM (g L^{-1})	46.8 ± 5.69	96.5 ± 13.4	107 ± 7.65	112 ± 10.4
pH ($CaCl_2$)	5.43 ± 0.18	5.76 ± 0.39	5.45 ± 0.18	5.47 ± 0.43
Al (cmol (+) L^{-1})	0	0	0	0
H+Al (cmol (+) L^{-1})	3.66 ± 0.35	4.42 ± 0.72	4.99 ± 0.56	5.51 ± 1.83
Ca (cmol (+) L^{-1})	2.53 ± 0.64	10.4 ± 2.48	10.9 ± 1.65	9.83 ± 1.93
Mg (cmol (+) L^{-1})	2.20 ± 0.21	4.13 ± 0.23	4.02 ± 0.35	3.29 ± 0.57
K (cmol (+) L^{-1})	0.38 ± 0.10	0.84 ± 0.23	0.64 ± 0.21	0.51 ± 0.13
CEC (cmol (+) L^{-1})	8.75 ± 0.93	19.8 ± 2.12	20.6 ± 1.46	19.1 ± 1.54
Base saturation (%)	58.1 ± 4.54	76.5 ± 6.05	76.4 ± 2.76	71.9 ± 9.68

Table 2. Mean values (± SD) for nutrient concentrations in fine roots on warm (W, $n = 7$) and cool (C, $n = 6$) season in grassland, scrub, secondary and mature forests in southern Brazil. Ratio between warm and cool seasons (W/C).

		Grassland	W/C	Scrub	W/C	Secondary	W/C	Mature	W/C
N (g kg^{-1})	W	3.78 ± 0.82		9.98 ± 1.70		15.8 ± 1.24		12.8 ± 1.45	
	C	5.78 ± 0.60	0.65	14.1 ± 1.45	0.71	18.3 ± 1.39	0.86	15.3 ± 0.77	0.84
P (g kg^{-1})	W	0.25 ± 0.02		0.90 ± 0.24		0.51 ± 0.08		0.54 ± 0.07	
	C	0.33 ± 0.03	0.76	1.17 ± 0.06	0.77	0.60 ± 0.10	0.85	0.61 ± 0.02	0.89
K (g kg^{-1})	W	2.00 ± 1.19		7.75 ± 2.71		5.25 ± 1.39		9.25 ± 2.60	
	C	1.40 ± 0.54	1.43	9.80 ± 1.64	0.79	5.40 ± 1.14	0.97	9.00 ± 1.87	1.03
Ca (g kg^{-1})	W	4.91 ± 1.04		8.47 ± 0.54		10.4 ± 1.25		11.6 ± 1.42	
	C	4.21 ± 0.74	1.17	8.53 ± 0.92	0.99	9.72 ± 0.34	1.07	11.7 ± 1.79	0.99
Mg (g kg^{-1})	W	0.85 ± 0.07		1.81 ± 0.44		2.02 ± 0.30		2.34 ± 0.27	
	C	1.13 ± 0.08	0.75	2.61 ± 0.20	0.69	2.36 ± 0.18	0.86	2.70 ± 0.22	0.87
Cu (mg kg^{-1})	W	134 ± 19.1		146 ± 32.7		114 ± 22.5		135 ± 27.5	
	C	136 ± 17.3	0.99	147 ± 3.06	0.99	122 ± 31.8	0.93	134 ± 12.2	1.01
Zn (mg kg^{-1})	W	19.9 ± 3.31		78.4 ± 10.9		69.2 ± 28.8		77.2 ± 21.4	
	C	24.0 ± 4.46	0.83	78.1 ± 13.9	1.00	67.0 ± 16.3	1.03	79.6 ± 18.9	0.97
B (mg kg^{-1})	W	31.7 ± 3.35		44.4 ± 11.0		49.1 ± 12.5		41.7 ± 10.6	
	C	21.3 ± 4.59	1.49	26.9 ± 2.65	1.65	27.4 ± 7.23	1.79	28.1 ± 2.08	1.48
Mn (mg kg^{-1})	W	117 ± 11.1		143 ± 21.6		166 ± 20.4		235 ± 23.0	
	C	119 ± 6.24	0.98	157 ± 6.94	0.91	190 ± 32.7	0.87	264 ± 31.8	0.89

present study, fine roots were mostly present in the young secondary forest compared with the mature forest, showing that the species in the former forest allocate more resources to building fine roots, which are critical for maintaining the high growth rates and metabolic demand (Cavalheiro & Nepstad 1996, Cavelier *et al.* 1996, Zangaro *et al.* 2008). When compared with earlier stages of the succession, higher rates of fine-root production in tropical forests may reflect the need for a high allocation of resources toward the roots as a consequence of high demands for water and nutrients for the large shoot biomass (Muthukumar *et al.* 2003).

Fine-root morphological traits

Fine-root dry mass is not always a good indicator of the potential for soil exploration of the fine-root system because alterations in root architecture and distribution may occur without changes in mass (Chen *et al.* 2004, Hodge 2004). Proliferation of the fine-root system facilitates nutrient acquisition in which adaptations in the morphology are directly involved (Eissenstat *et al.* 2000, Hodge 2004, Ryser & Lambers 1995, Wright & Westoby 1999, Zangaro *et al.* 2007), increasing the root surface area in contact with the soil (Bates & Lynch 2001, Comas *et al.* 2012, Föehse *et al.* 1991, Gahoonia *et al.* 2001, Zangaro *et al.* 2005). In the present study, fine roots in plants at the early stages of succession showed morphological adaptations indicative of greater nutrient acquisition than plants at later stages. These adaptations included increased fine-root length density and specific root length, as well as higher root-hair length and incidence. Root diameter is also important for nutrient acquisition, whereby thinner roots are more effective for soil exploration (Eissenstat 1992), for instance P influx increases as root diameter decreases (Itoh & Barber 1983). Furthermore, thinner roots usually have a more dense distribution in the soil (Holdaway *et al.* 2011), greater turnover rate and shorter lifespan than

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thicker roots (Comas & Eissenstat 2004, Comas *et al.* 2012). In this work and previous reports (Jaramillo *et al.* 2003, Zangaro *et al.* 2008, 2012*a*, *b*), an increase in fine-root diameter over succession was observed, suggesting that the potential for nutrient uptake by roots decreases with successional stage due to a decrease in both surface area and diameter. Thus, different plant communities in the successional stages have different nutrient exigencies and, thus, fine-root morphological traits are adapted to each particular demand. Species present in the early-successional stages showed fine-root morphological traits adapted for a high capacity to exploit soil and acquire resources. They were found in open environments with high light incidence, showing high photosynthetic potential, fast growth rates and high demand for nutrients (Brown & Lugo 1994, Zangaro *et al.* 2003). As the main function of fine roots is uptake of water and nutrients, fast-growing species must possess the described root morphological traits to ensure supply for the high metabolic requirements (Comas *et al.* 2002). Conversely, species that occupy the late-successional stages were under shaded conditions with lower rates of photosynthesis and growth (Reich *et al.* 1998), resulting in a root system less adapted for soil exploitation since there is less demand for nutrients.

Nutrients in soil and roots

Soil moisture increased over succession and was associated with an increase in soil organic matter. Concentrations of nutrients in both soil and fine-root tissues also increased with the advance of succession, which may be consequence of a progressive increase in mass, leading to accumulation of humus and nutrients resulting from mineralization (Guariguata & Ostertag 2001). Such an increase in soil nutrients and fine roots is followed by a decrease in the specific length, incidence and length of the root hairs, as well as an increase in the dry mass, tissue density and diameter. Similar results were previously observed over succession (Zangaro *et al.* 2008, 2012*a*). The availability of nutrients in soil may influence the number of absorbing roots (Powers *et al.* 2005, Son & Hwang 2003). The plasticity of the fine-root traits suggests that plants allocate biomass, alter morphology and increase the lifespan of the fine roots in response to limited nutrients (Hodge 2004, Eissenstat & Yanai 1997).

Influence of environmental variation on the production and morphological traits of fine roots

Environmental variation influenced the production of fine roots in the four studied sites, in which fewer fine roots were observed during the cool season, mainly in grass and scrub sites, in which the fine roots were more intensively renewed. In addition to moderate seasonality based on minimal variation in soil water content, mild temperatures, day length and the inherent short lifespan of species in the early-successional stages may lead to higher rates of fine-root turnover during the warm season. Production of fine roots (Dress & Boerner 2001, Hendrick & Pregitzer 1996) and rate of length extension (McMichael & Burke 1998) have been shown to increase with temperature. Increase in light availability is also associated with higher production of fine roots (Fitter *et al.* 1998). In both forests studied, moderate environmental variation had little influence during the cool season, and the production and rate of fine-root renewal was constant throughout the year, which is consistent with previous reports in tropical forests (Dress & Boerner 2001, Hendrick & Pregitzer 1996). On the other hand, maximum production of roots has been shown to occur during the rainy season in tropical forests under severe seasonality, followed by a decrease during the dry season (Chen *et al.* 2004, Guadarrama & Alvarez-Sanchez 1999).

Moderate environmental variation affected most root morphological traits in all successional stages. The fine-root length density, as well as the incidence and length of root hairs, were notably reduced during the cool season, coinciding with the lower metabolic demand of plants and the higher concentrations of some nutrients in the root tissues. This higher concentration of nutrients during the period of lower production of fine roots suggests lower demand for nutrients in the cool season, which can be used during the warm season, when plants make more investment in new tissues in roots and shoots. Son & Hwang (2003) also reported seasonal fluctuation in nutrient concentrations in roots and soil that were correlated with decomposition of litter.

Mycorrhizal root infection

The AMF root infection intensity decreased with the ecological succession, as previously observed in the Atlantic Forest in Brazil (Aidar *et al.* 2004, Zangaro *et al.* 2000, 2008, 2013). Zangaro *et al.* (2012*a*, *b*) reported a decrease in AMF root infection with the successional advance in three other Brazilian ecosystems, including the Atlantic Forest, *Araucaria* forest and pantanal. In this context, differences in the intensity of AMF infection in tropical plants may be attributed to the different ecological groups to which the plants belong (Huante *et al.* 1993, Lacerda *et al.* 2011, Matsumoto *et al.* 2005, Siqueira *et al.* 1998, Vandresen *et al.* 2007, Zangaro *et al.* 2000, 2003). Fine roots of plants from early-successional stages showed typically high AMF infection, whereas late-successional species showed low AMF infection. Therefore, a decrease

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in AMF infection in later stages of ecological succession has been frequently observed, contrary to earlier reports that mycorrhizal status increases with succession in tropical forests (Janos 1980, 1983).

AMF infection correlated positively with fine-root specific length, incidence and length of root hairs, but correlated negatively with root dry mass, diameter and tissue density. Thus, early-successional species showed higher AMF infection and root morphological traits that maximized the potential for soil mining. Conversely, late-successional species displayed lower AMF infection intensity and root morphological traits less effective for soil exploitation. As a result, AMF infection was highly influenced by the successional group to which the plant community belongs, as well as root morphological traits involved in soil exploitation and distribution in soil. Similar results were observed in seedlings (Zangaro *et al.* 2005, 2007) and under field conditions (Zangaro *et al.* 2008, 2012*a*, *b*, 2013). In general, AMF infection intensity correlated negatively with concentrations of nutrients in both roots and soil, suggesting that the lower concentration in soil and roots, besides leading to the development of root morphological traits for more effective soil exploration, also stimulates AMF infection.

Fine-root morphology of early-successional species provides greater surface area that may increase the probability of contact between AMF propagules and roots (Comas *et al.* 2012, Zangaro *et al.* 2005), resulting in higher root infection. Conversely, root morphological traits in late-successional species are less effective for soil exploitation and encounter with AMF propagules (Zangaro *et al.* 2008, 2012*a*, *b*). Lower AMF infection may also be attributed to high tissue density in fine roots, where cortical cells have thick walls, high suberization and lignification. Furthermore, high concentrations of tannins (Comas & Eissenstat 2004) may hinder AMF infection (Eissenstat 1992, Giovannetti *et al.* 1996).

High growth rates in early-successional communities are associated with high photosynthetic capacity and high light availability (Guariguata & Ostertag 2001, Lusk *et al.* 2008) that make possible higher amounts of photoassimilates to be exported to roots (Nielsen *et al.* 1998) and are also allocated for maintenance of higher AMF infection. In more advanced successional stages, plant communities show lower growth rates and lower demand for nutrients due to adaptation to shading (Reich *et al.* 1998). Lower light availability decreases the carbohydrate content in the fine roots (Gamage *et al.* 2004) and contributes to reduction of AMF infection. The lower demand for nutrients by late-successional species is in agreement with the lower AMF infection since high colonization levels would be energetically expensive for plants, which is disadvantageous in shaded environments where plants need to conserve energy (Zangaro *et al.* 2012*a*). Finally, environmental variation negatively affected AMF infection during the cool season. This effect may be a consequence of a reduced root system, in addition to lower temperatures and shorter day length, given that shorter days with less light available decrease the carbohydrate contents in roots for maintenance of AMF (Fitter *et al.* 1998, Nielsen *et al.* 1998).

In summary, different growth characteristics of plant communities in different successional stages have been observed. Fine-root adaptations for maximization of nutrient acquisition, such as morphological traits and higher AMF infection for more effective soil mining, are associated with fast-growing and highly metabolically active species that are predominant in the early succession. Conversely, slow-growing and less metabolically active species are most common in the late successional stages, showing morphological traits and AMF infection intensity with lower capacity for soil exploitation. This statement becomes stronger when the effect of environmental variation on the potential for acquisition of water and nutrients is taken into account in all successional stages. Specifically, when plants have a greater demand for resources from soil during the warm season, root morphological traits and AMF infection coincide with the maximum capacity for soil exploitation and vice versa.

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