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Root mycorrhizal colonization and plant responsiveness are related to root plasticity, soil fertility and successional status of native woody species in southern Brazil

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Abstract: Twelve native woody species were studied to investigate the influences of soil fertility and root morphology on colonization by arbuscular mycorrhizal (AM) fungi during seedling establishment and growth. Seedlings were grown in soils of low and high natural fertility, uninoculated or inoculated with AM fungi, under greenhouse conditions. The mycorrhizal root colonization and plant responsiveness were higher among early successional species than late successional ones. Among early successional species, in both soils, mycorrhizal colonization provided significant increase in total dry mass, growth rates of shoot and root, root length, density of root tissues, root surface area and P concentration and content in the shoot. Early successional species grown with AM fungi displayed significant decreases in carbon allocation to roots, specific root length and the length and incidence of root hairs. Mycorrhizal colonization did not affect the root morphology of the late successional species in either soil. The growth of these woody species was influenced by differences in soil fertility. There was positive correlation between the degree of plant responses to AM inoculation with the percentage of root colonized by AM fungi. In both soils, plant responsiveness and mycorrhizal root colonization correlated positively to root-hair incidence and root-hair length and correlated negatively to fine-root diameter. The results suggest that during the establishment of seedlings, the large responses to the inoculation and colonization of roots by AM fungi are related to both the successional status and root morphological plasticity of the host plant, regardless of soil fertility.

Key Words: arbuscular mycorrhizal fungi, biomass allocation, fine roots, root hairs, phosphorus uptake, root morphological traits, seedling growth, succession, tropical forest

INTRODUCTION

Root system effectiveness in taking up water and nutrients from soil depend on the way carbon is invested in fine roots (Comas *et al.* 2002). Plants originating from poor soils frequently display greater allocation of biomass to fine roots and, in order to increase the absorbing area (Hodge 2004, Tilman 1994), have fine roots with greater length per volume of soil (Eissenstat 1992), invest less biomass to produce root length and produce fine roots with greater specific root length (Comas *et al.* 2002). Fast-growing species, when compared with slow-growing ones, invest less carbon to acquire water and nutrients revealing greater specific root length (Bouma *et al.* 2000, Eissenstat 1992), have greater uptake capacity (Tilman 1994), display fine roots with smaller diameter (Wright & Westoby 1999) and low density of root tissues (Ryser & Lambers 1995). As an indicator of the root architecture, specific root length is influenced by the diameter and the density of the tissue, which reflect the potential for the acquisition of resources from the soil (Hodge 2004, Wright & Westoby 1999).

The uptake area of roots can be maximized by the production of root hairs, increasing the volume of soil explored (Gilroy & Jones 2000, Lynch & Ho 2005). Symbiosis with arbuscular mycorrhizal (AM) fungi also increases a root's uptake area (Marschner 1998). The smaller diameter of hyphae allows small soil pores to be

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explored and more nutrients to be taken up (Peterson & Farquhar 1996, Schweiger *et al.* 1995). Plants deficient in P increase root exudation thus promoting hyphal proliferation and an increase in colonization by AM fungi (Giovannetti *et al.* 1996, Graham *et al.* 1981, Schwab *et al.* 1991, Smith & Read 1997).

Plant species with shorter root length (Ryser & Lambers 1995), fine roots with greater diameter (Graham & Syvertsen 1985, Hetrick *et al.* 1992, Manjunath & Habte 1991), shorter and lower density of root hairs (Baylis 1975) benefit more from symbiosis with AM fungi than those with longer root length, small root diameter and roots covered with long root hairs. In contrast, Saif (1987) demonstrated that tropical grass species with long root hairs benefit more from colonization by AM fungi than leguminous species with shorter root hairs. Duponnois *et al.* (2001) demonstrated that leguminous woody species display a positive correlation between the degree of plant responses (responsiveness) to inoculation with AM fungi and the density of root hairs. Siqueira & Saggin-Júnior (2001) found that some native woody species in the south-east of Brazil, with thick roots and low density of root hairs, show low root AM colonization and low growth with AM inoculation, while other woody species with fine roots and a high density of root hairs displayed higher growth rate and colonization by AM fungi. Zangaro *et al.* (2005) confirmed positive correlation between plant responsiveness and root AM colonization with the root-hair incidence and root-hair length, and also obtained negative correlation with fine-roots diameter in 78 native woody species grown in soils deficient in P.

Early successional woody species with high metabolic rate dominate the initial stages of succession, occur in highly illuminated environments and usually exhibit high mycorrhizal colonization and responsiveness. In contrast, late-successional woody species with low metabolic rate dominant in the mature forest occur in an environment with low light incidence and exhibit low mycorrhizal colonization and responsiveness. In the present study we report some root and shoot attributes influencing the differences in root AM colonization and plant responsiveness of the 12 native woody species belonging to different successional groups in southern Brazil.

METHODS

Species studied

The 12 woody species studied are of natural occurrence in the Tibagi River Basin, State of Paraná, south of Brazil. Six are involved in the initial structuring of the forest and belong to the early stages of the tree succession (fast-growing species): *Schinus terebinthifolius* Raddi (Anacardiaceae), *Croton urucurana* Baill. (Euphorbiaceae), *Lafoensia pacari* A. St. Hil. (Lythraceae), *Tabernaemontana australis* (Mull. Arg.) Miers (Apocynaceae), *Casearia sylvestris* Sw. (Flacourtiaceae) and *Heliocarpus americanus* L. (Tiliaceae). The other six species are typical of the mature forest and belong to the late stages of succession (slow-growing species): *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae), *Enterolobium contortisiliquum* (Vell.) Morong (Mimosaceae), *Jacaratia spinosa* (Aubl.) A. DC. (Caricaceae), *Chrysophyllum gonocarpum* (Mart. & Eichler) Engl. (Sapotaceae), *Guarea macrophylla* Vahl (Meliaceae) and *Actinostemon concolor* (Spreng.) Mull. Arg. (Euphorbiaceae). The probable position in the different successional groups of these species was determined according to Chagas e Silva & Soares-Silva (2000), Dias *et al.* (1998) and Gandolfi *et al.* (1995). Some morphological and physiological traits of the early and late-successional woody species are from Zangaro *et al.* (2003). Seeds of woody species were weighed to obtain fresh mass, disinfected with sodium hypochlorite (1%) for 15 min, washed three times in distilled water and placed for germination in sterilized sand.

Substratum and AM fungi inoculation

Two types of substrate were used; the first consisted of 90% subsoil and 10% sand (infertile soil) and the second, 90% forest soil and 10% sand (fertile soil), obtained from a typical Hapludox. Substrates were placed in black plastic bags with 2.0 kg capacity and fumigated with methyl bromide. The nutrient concentrations in the substratum are presented in Table 1. The carbon was extracted with 2M $Na_2Cr_2O_7 + 5M$ H_2SO_4 and determined by colorimetry. Ca and Mg were extracted with 1M KCl and determined by titration. P was extracted by Mehlich-1 and determined by colorimetry. K was extracted by Mehlich-1 and determined by flame photometry.

One and a half grams of inoculum containing a mixture of different species of native AM fungi multiplied in the rhizosphere of *Croton urucurana*, a woody species, with approximately 1250 spores, along with colonized roots and hyphae were added in holes in the centre of each cultivation bag. Ten individuals of each plant species were

Table 1. Mean (± SE) nutrient concentrations in the substrates for seedling growth from subsoil (infertile) and forest soil (fertile) of the Mata dos Godoy State Park (n = 3).

	Infertile	Fertile
pH	5.1 ± 0.03	4.9 ± 0.04
C (%)	1.7 ± 0.04	4.3 ± 0.16
P (mg kg^{-1})	2.1 ± 0.02	8.6 ± 0.51
Al (meq per 100 ml)	0.08 ± 0.007	0.03 ± 0.006
Ca (meq per 100 ml)	5.41 ± 0.23	12.1 ± 0.31
Mg (meq per 100 ml)	2.17 ± 0.17	3.96 ± 0.17
K (meq per 100 ml)	0.44 ± 0.05	0.86 ± 0.07

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inoculated with AM fungi in each substrate. Another 10 individuals of each plant species did not receive AM fungi inoculum (control group). The seedlings were transferred to the bags between 6 and 15 d after emergence. For the microflora recomposition, 100 ml of filtrate from the original soil, without AM fungi propagules, were added in each bag. Seedlings were grown between 180 and 210 d in a greenhouse with 50% incidence of total sunlight. Plants were grown in the hot season and under uncontrolled temperatures. All seedlings were watered on a daily basis.

At the end of the experiment, plants were harvested and the roots and shoots placed in a drying chamber at 65 °C until they reached a constant weight to obtain dry mass. The degree of plant response (responsiveness) to AM fungi was calculated on the basis of the difference between the shoot dry mass of inoculated and uninoculated plants, and expressed as the percentage of dry mass of the inoculated plants (Plenchette *et al.* 1983).

Root measurements

For analysis of root morphological traits, 1 g of fresh fine roots of each species and treatments were taken from the extremities of the roots. For the early successional species grown in infertile soil, without AM inoculation, less than 1 g of fine fresh root samples was obtained. The segments of the fine roots were sampled in all the 10 individuals of each plant species and each treatment. Samples of fine roots were fixed in FAA (5 ml acetic acid, 5 ml formaldehyde and 90 ml 50% ethanol) and stored for up to 20 d before analysis. Morphological root traits were analysed in 12 segments in four random sites, for each sample. Accordingly, 48 measurements of fine-root diameter and root-hair length and diameter were carried out in each individual of each species of plant and in each treatment. Fine-root diameter was determined using a microscope at ×100 magnification with an ocular micrometer (Manjunath & Habte 1991). Root-hair length and diameter were also measured using a compound microscope at ×200 magnification with an ocular micrometer (Schweiger *et al.* 1995). The incidence of root hair (%) was assessed by the presence of root hair in 100 intersections between roots with a grid line (Siqueira & Saggin-Júnior 2001, Zangaro *et al.* 2005).

The total fresh root length was determined by the grid-line intersection method (Tennant 1975) by the formula: length $= \pi\ N\ A/2\ H$, where N is the total number of intersections, A is the Petri dish area and H the total length of lines in the grid. The root surface area was estimated by the formula $2\ \pi\ r\ L$, where r is the root's radius and L is the total root length (Manjunath & Habte 1991). From the primary data of the roots the following parameters were derived (Ryser & Lambers 1995): root dry mass per plant dry mass, root length per root dry mass (specific root length), root length per soil volume, root length per plant dry mass, root length per root volume ratio, and root dry mass per root fresh mass (root tissue density). According to Ryser & Lambers (1995), the specific weight of the root is similar to the water and root volume in cm^3 corresponds to root fresh weight in g. The root dry mass per root fresh mass ratio, as the root tissue density is calculated here, corresponds to root dry mass per unit root volume. The P concentration in the shoot was determined in the Instituto Agronômico do Paraná (IAPAR), Londrina, Paraná. The P content in the shoot was estimated by multiplying P concentration by the shoot dry mass. Shoot P-demand per unit of root mass was obtained as the ratio of total P uptake divided by root dry mass. Shoot P-demand per unit of root length was obtained as the ratio between total P uptake and root length. Shoot P-demand per unit of root mass or length reflects the efficiency of P uptake by the roots (Eissenstat 1992). P uptake per unit of root surface area was estimated as the ratio of total P uptake divided by root surface area (Manjunath & Habte 1991).

To estimate AM colonization, root segments used for morphological assessments were clarified with 10% KOH, acidified with 1% HCl, then washed in running water and stained with 0.05% trypan blue in lactoglycerol solution (Brundrett *et al.* 1996). The root colonization by AM fungi was calculated by the grid-line intersection method (Giovannetti & Mosse 1980).

Statistical analysis

The data were tested for normal distribution using the Kolmogorov–Smirnov test. The data for root dry mass, specific root length and root-hair length were log-transformed. Shoot dry mass was square-root-transformed and percentages were arcsine-square-root-transformed before analysis. Data were submitted to analysis of variance (ANOVA) and Pearson's correlation. In order to compare the effects of soil fertility and mycorrhizal status on each successional group separately, the means from each species belonging to each successional group were pooled and considered as a replicate. Thus, for this analysis, a 2×2 factorial (the combination of two levels of soil fertility with two levels of mycorrhizal status) with six replicates was considered and submitted to two-way ANOVA and Tukey's test (5%). According to Manjunath & Habte (1991), to determine the relationship between root morphology and plant responsiveness to AM fungi, it is essential to assure that (a) host species being compared are distinctly different in their response to AM fungi and (b) root morphology parameters are measured in the absence of AM colonization. Therefore, for correlation analysis the average of plant variables for each plant species grown

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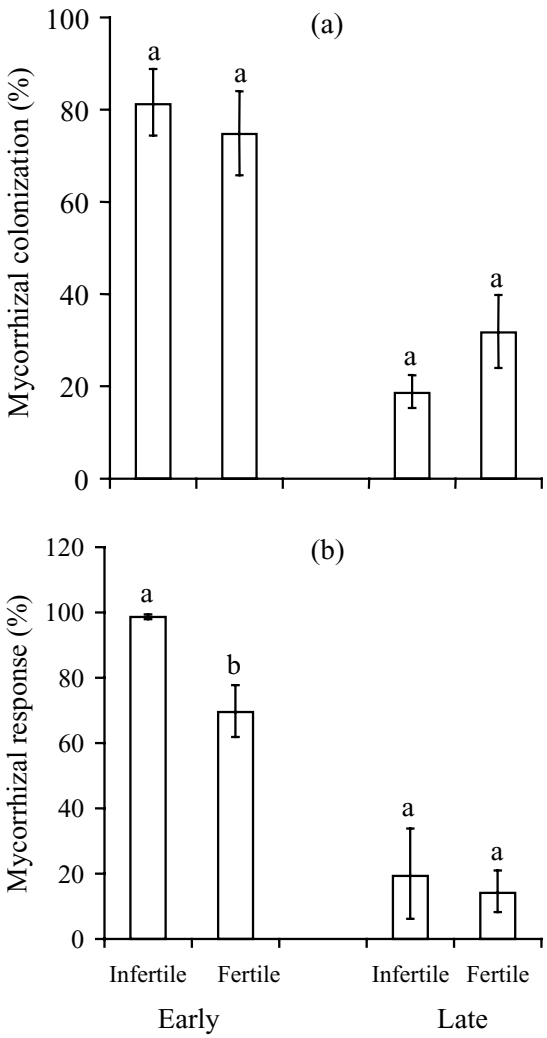

Figure 1. Root colonization by AM fungi (a) and plant mycorrhizal response (b) of early and late successional native woody species grown in infertile and fertile soils. The means were obtained from data of each plant species and treatments. Error bars represent ± 1 SE. Means followed by the same letter within a successional group are not different by Tukey's test at 0.05 level.

in the absence of AM fungi, in each soil, was considered separately (n = 12).

RESULTS

The AM fungi and soil fertility affected the growth and root morphology of seedlings of early successional species differently. In contrast, inoculation and soil fertility did not affect the measures of plant variables of late-successional species. The root colonization by AM fungi (Figure 1a) of the early successional species was 3.2 times greater than late-successional species in both soils. The responsiveness (Figure 2a) of early successional species was 5.2 times greater than late-successional species in both soils.

The shoot dry mass (Figure 2a) of the early successional species was significantly greater (Tukey HSD = 2.62, $P < 0.001$) in inoculated fertile soil, when compared with other treatments. Shoot dry mass did not differ among plants grown in inoculated infertile soil and uninoculated fertile soil. Shoot dry mass from uninoculated infertile soil was extremely low and differed significantly (Tukey HSD = 2.62, $P < 0.01$) from the other treatments. Root dry mass (Figure 2b) of the early successional species was significantly greater (Tukey HSD = 1.56, $P < 0.01$) in inoculated fertile soil, when compared to other treatments. Root dry mass did not differ among plants grown in inoculated infertile soil and uninoculated fertile soil. Root dry mass from uninoculated infertile soil was extremely low and differed significantly (Tukey HSD = 1.56, $P < 0.01$) from the other treatments. Root:shoot ratio (Figure 2c) of the early successional species was significantly greater (Tukey HSD = 0.34, $P < 0.001$) for plants grown in uninoculated infertile soil than other treatments. Shoot P concentration (Figure 2d) of the early successional species was significantly greater (Tukey HSD = 0.06, $P < 0.01$) in inoculated fertile soil, when compared to the other treatments. In both soils, plants presented significantly higher P concentrations (Tukey HSD = 0.06, $P < 0.05$) when inoculated with AM fungi. There was no significant difference between plants from inoculated infertile soil and uninoculated fertile soil. Specific root length (Figure 2e) of early successional species was significantly greater (Tukey HSD = 1.44, $P < 0.01$) in uninoculated infertile soil in relation to the other treatments, which did not differ from one another. Root tissue density (Figure 2f) of early successional species was significantly greater (Tukey HSD = 0.03, $P < 0.01$) in inoculated fertile soil, when compared to other treatments. There was no significant difference between plants from inoculated infertile soil and uninoculated fertile soil. Plants from uninoculated infertile soil showed the lowest means.

Root diameter (Figure 3a) of late successional species was 1.8 times greater than early successional ones. Among early successional species, plants in inoculated fertile soil had significantly greater (Tukey HSD = 0.04, $P < 0.05$) root diameter than in uninoculated infertile soil. The other treatments did not differ from one another. Root-hair incidence (Figure 3b) of early successional species was 5.8 times greater than late successional species. Root-hair incidence of early successional species was significantly greater (Tukey HSD = 11.49, $P < 0.01$) in uninoculated than in inoculated plants, both in infertile and fertile soil. Root-hair length (Figure 3c) of early successional species was 5.7 times greater than late-successional ones. The root-hair length of early successional species was significantly greater (Tukey HSD = 16.59, $P < 0.05$) in uninoculated infertile soil than inoculated fertile soil, while the other treatments did not

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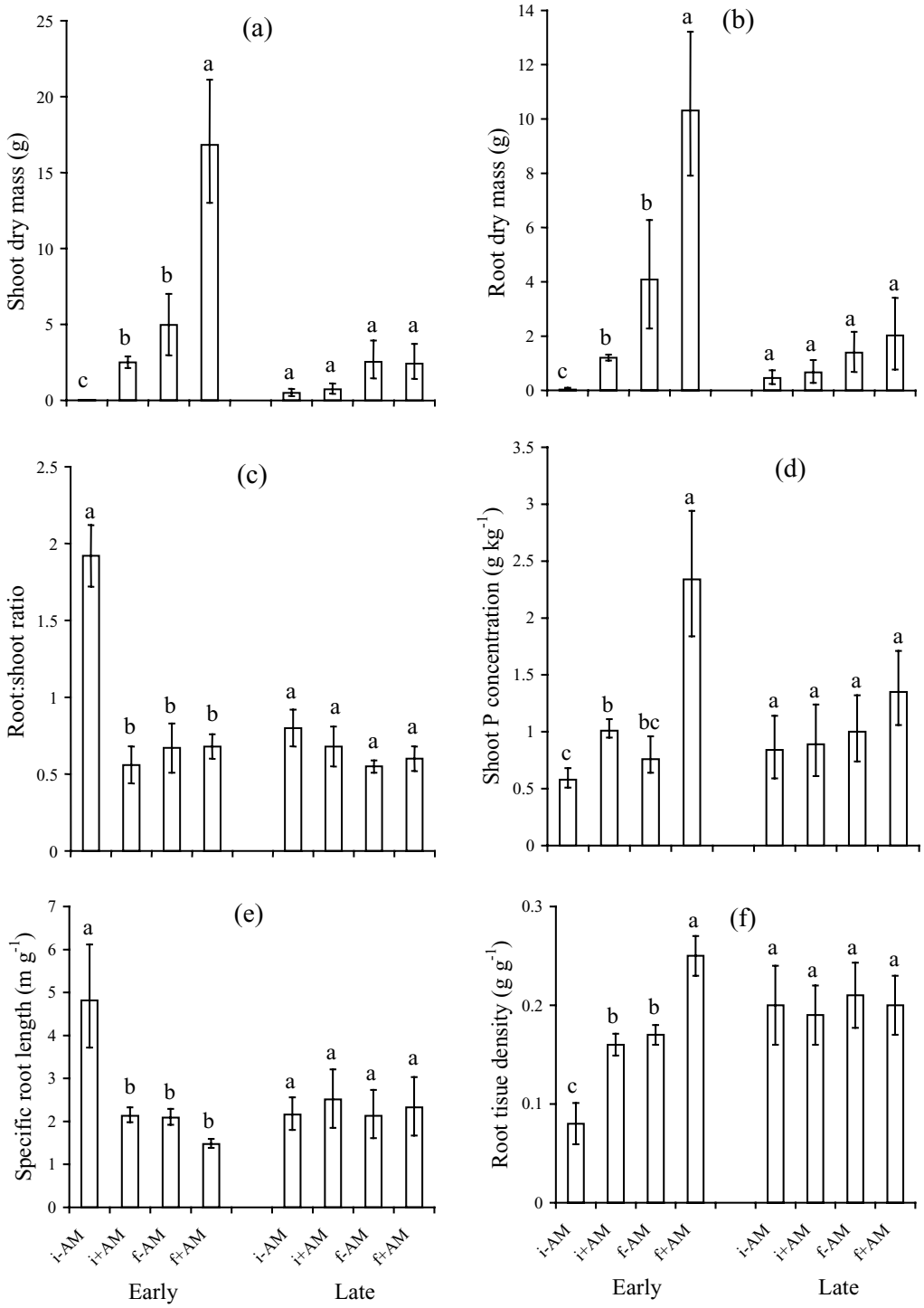

Figure 2. Shoot dry biomass (a), root dry biomass (b), root:shoot ratio (c), shoot P concentration (d), specific root length (e) and root tissue density (f) of early and late successional native woody species grown in infertile soil uninoculated (i–AM) and inoculated (i+AM) and fertile soil uninoculated (f–AM) and inoculated (f+AM) with arbuscular mycorrhizal fungi. The means were obtained from data of each plant species and treatments. Error bars represent ± 1 SE. Means followed by the same letter within a successional group are not different by Tukey's test at 0.05 level.

differ from one another. Root-hair diameter (Figure 3d) of early successional species was 1.4 times greater than early successional species. Root-hair diameter of early or late successional species did not differ among treatments. The summary of two-way ANOVA (Appendix) showed no significant interaction between soil fertility and AM fungi on plant variables, except for root:shoot ratio in the early successional species. This means that AM fungi effects on

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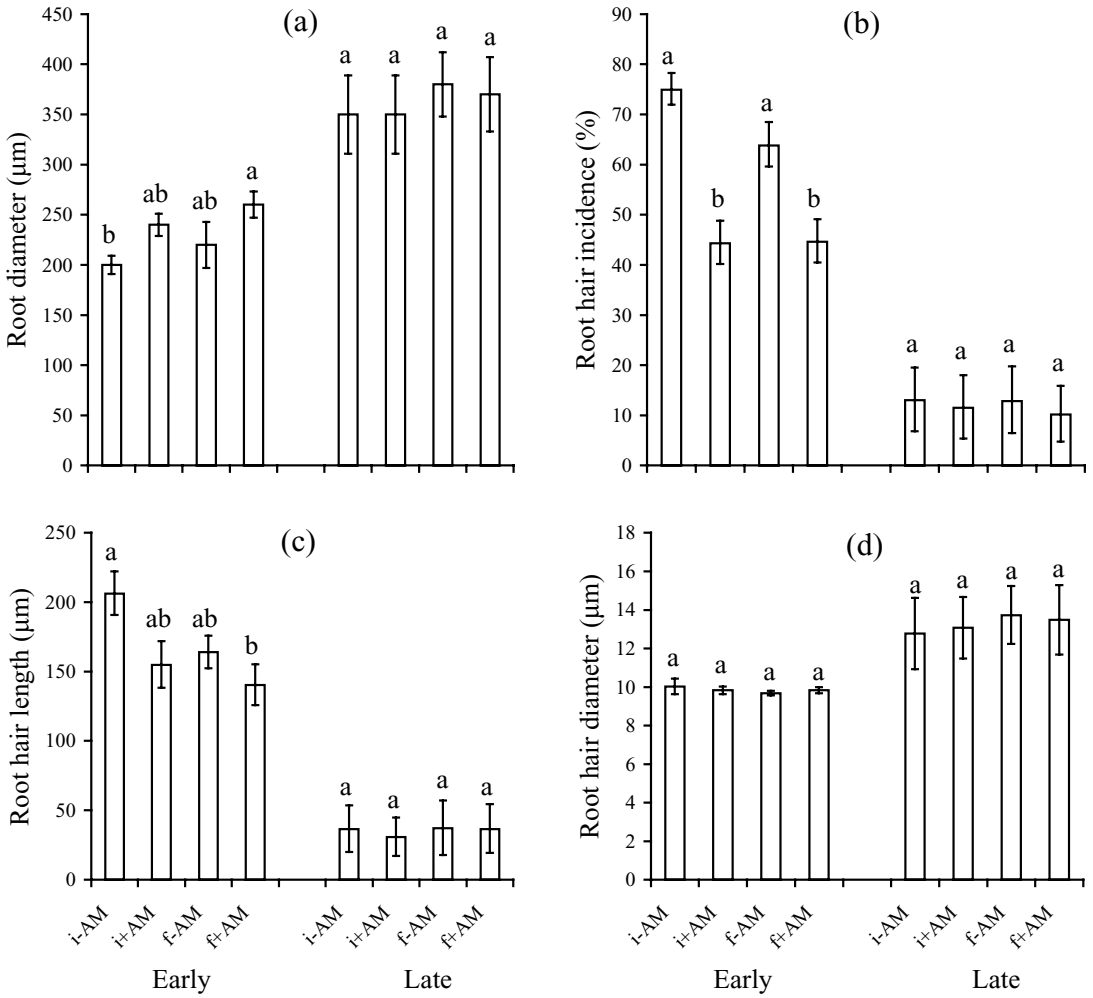

Figure 3. Fine-root diameter (a), root-hair incidence (b), root-hair length (c) and root-hair diameter (d) of early and late-successional native woody species grown in infertile and fertile soils inoculated and uninoculated with AM fungi (infertile soil uninoculated (i–AM) and inoculated (i+AM) and fertile soil uninoculated (f–AM) and inoculated (f+AM) with arbuscular mycorrhizal fungi). The means were obtained from data of each plant species and treatments. Error bars represent ± 1 SE. Means followed by the same letter within a successional group are not different by Tukey's test at 0.05 level.

early successional plant traits were not changed along the soil fertility level.

Plant responsiveness to AM fungi and AM root colonization were correlated with some plant traits obtained from seedlings grown in infertile and fertile soils only in the absence of AM fungi (Table 2). There was positive correlation between plant responsiveness and mycorrhizal root colonization in both soils. Among

Table 2. The Pearson's correlation coefficients (r) for plant responsiveness and root mycorrhizal colonization with variables of the uninoculated native woody species, grown in infertile (n = 12) and fertile (n = 12) soils. Levels of significance were corrected with Bonferroni procedure.

	Correlation coefficient			
	Infertile		Fertile	
Plant variables	Plant responsiveness	Mycorrhizal colonization	Plant responsiveness	Mycorrhizal colonization
Plant responsiveness	–	0.85***	–	0.77**
Seed fresh mass	−0.65*	−0.47 ns	−0.30 ns	−0.22 ns
Root:shoot ratio	0.69*	0.67*	0.08 ns	0.05 ns
Root diameter	−0.73**	−0.67*	−0.59*	−0.41 ns
Root-hair incidence	0.80**	0.87***	0.73**	0.69*
Root-hair length	0.77**	0.77**	0.68*	0.40 ns
Shoot P concentration	−0.66*	−0.33 ns	−0.57 ns	−0.36 ns

*P < 0.05; **P < 0.01; ***P < 0.001; ns P > 0.05.

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seedlings grown in infertile soil, plant responsiveness correlated positively to root:shoot ratio, root-hair incidence and root-hair length and correlated negatively with seed fresh mass, fine-root diameter and shoot P concentration. Mycorrhizal root colonization correlated positively to root:shoot ratio, root-hair incidence and root-hair length and correlated negatively with fine-root diameter. Among seedlings grown in fertile soil, plant responsiveness correlated positively to root-hair incidence and root-hair length and correlated negatively with fine-root diameter. Mycorrhizal root colonization correlated positively to root-hair incidence.

DISCUSSION

Early successional species and AM fungi

Seedlings of early successional species grown in infertile soil and an absence of AM fungi displayed changes in root dry mass allocation and root morphological traits when compared with plants from other treatments. These changes demonstrated that the roots of these species displayed plasticity during their initial stage of development (establishment stage). Shoot development was severely restricted in infertile soil without AM fungi, which coincided with an increase in the specific root length, incidence and length of root hair and decrease of the root diameter. These changes in root traits may be interpreted as an improvement in the root uptake potential. Nevertheless, this root adaptation was not enough to increase the nutrient uptake and promote shoot growth. Accordingly, it is suggested that the increase of root area for nutrient uptake may increase the likelihood of encountering AM fungi hyphae in soil, leading to contact between the root epidermis and root hairs with more AM fungi hyphae (Zangaro *et al.* 2005). In this work, this was verified by the positive correlation between mycorrhizal root colonization and plant responsiveness with length and incidence of root hair, and the negative correlation with root diameter. In addition, the root-hair incidence was the most important determinant of plant responsiveness to AM fungi and AM colonization among the early successional species. As the primary function of fine roots is the acquisition of nutritional resources for the plant (Comas *et al.* 2002), the formation of mycorrhizal symbiosis and substantial root morphological plasticity are two strategies that early successional species can use to acquire nutrients.

Root exudates stimulate AM colonization (Giovannetti *et al.* 1996, Peterson & Farquhar 1996) whose production is stimulated in P-deficient plants (Graham *et al.* 1981, Schwab *et al.* 1991, Smith & Read 1997). Seeds of early successional species have low nutritional reserves due to their small size, and this leads to seedlings with low P concentration (Allsopp & Stock 1992, Zangaro *et al.* 2000). Thus, these species are expected to exude more substances that stimulate AM colonization (Lynch & Ho 2005, Peterson & Farquhar 1996). The relation between P-deficient plants (high exudation) with AM colonization can be verified by the negative correlation between plant responsiveness with the P concentration in the shoot, shoot P-demand per unit root biomass and shoot P-demand per unit root length of seedlings.

Early successional species require a quick acquisition of nutrients due to high growth rates and metabolic demand (Bazzaz 1991, Zangaro *et al.* 2003). In addition, up to 20% of plant photosynthates are used to maintain AM colonization (Douds *et al.* 1988, Lynch & Ho 2005). Accordingly, adaptations of root traits that maximize the acquisition of nutrients, such as the increase in root surface area and the elevated colonization by AM fungi are related to fast-growing species, which dominate the early stages of the woody succession.

Late-successional species and AM fungi

In both soils, most late-successional woody species grew equally either in the absence or presence of AM fungi. This indicates that the reserve of their larger seeds could have been responsible for the similar initial growth of the seedlings. The inoculation with AM fungi did not influence the shoot and root dry mass production, dry mass allocation and the root morphological parameters, suggesting low root plasticity among seedlings of the late-successional species. Differences in root and shoot growth rates of late successional species were related to the availability of nutrients in the soil rather than the mycorrhizal symbiosis. These outcomes suggest that during the establishment of seedlings, most late successional species display limitations to use both AM fungi and the roots' plasticity as strategies for mineral acquisition.

Some traits of the late successional species can explain their lower AM colonization. (1) When grown in infertile soil without AM fungi, the early successional species displayed specific root length, root-hair length and root-hair incidence 2.4, 5.7 and 5.8 times greater than the late successional species. These aspects reduce the root spreading through the soil, decreasing the chances of contact between root surface of the late species and AM fungi hyphae. (2) The root tissue density of late-successional species was 2.5 times greater than the early species. This suggests that root-cortical cells of the late species display thick walls, which are more resistant to AM hyphae penetration (Giovannetti *et al.* 1996). In addition, more carbon was used in the construction of root tissue and, consequently, less carbon was available for AM fungi. (3) Late successional species displayed a

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root diameter about 1.8 times greater than the early successional species. This greater diameter may represent an obstacle to the movement of photosynthates through the phloem up to the root surface and may be an important anatomic aspect that may reduce the root exudation. Fine roots of greater diameter tend to be more suberized and more lignified than fine roots of lower ones (Eissenstat 1992), which also could decrease the exudation and limit the colonization by AM fungi. (4) The higher seed reserve of late successional species could supply the seedling during the initial growth period, decreasing the initial plant dependence on AM fungi. (5) The late successional species display low metabolic rates (Comas *et al.* 2002) and probably few photosynthates are available to the AM fungi due to lower growth rates. Therefore, root traits not favourable to nutrient acquisition, such as the smaller root surface area and the low colonization by AM fungi are related to slow-growing species, which dominate late stages of the woody succession.

CONCLUSION

Our results reveal that during the seedling establishment, early and late successional species display different strategies for acquiring nutrients from soil. Presumably, such strategies were selected by plant adaptations according to different conditions of light and nutrients availability in the soil. Thus, the establishment of seedling of early successional woody species in deficient P soils is largely dependent on AM fungi. Under these conditions, the root plasticity and the high AM fungi colonization are key to root functional efficiencies for the acquisition of nutrients and maintenance of high metabolic rates in early successional species.

The conditions for seedling establishment of late successional species are usually under low luminosity under the understorey and better soil fertility. The limited plasticity of their roots and the low colonization by AM fungi, both in fertile and infertile soil, may be related to the lower metabolic rate of these species. The root morphology of the late successional species not only displayed lower efficiency in obtaining nutrients, but also was less efficient in attracting the AM fungi. New research is needed to investigate the reason why these two successional groups respond differently to and are colonized differentially by AM fungi.

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Appendix. Summary of two-way ANOVA to test for significant effects of soil fertility and AM fungi on plant variables of early and late successional native woody species from southern Brazil.

		Early			Late		
Variable	Factor	df	F	P	df	F	P
Shoot dry biomass	Soil	1	16.1	<0.001	1	3.31	0.083
	AM	1	8.94	0.007	1	<0.01	0.955
	Soil × AM	1	3.82	0.064	1	0.03	0.857
	Error	20			20		
Root dry biomass	Soil	1	12.3	0.002	1	1.77	0.197
	AM	1	3.89	0.032	1	0.24	0.625
	Soil × AM	1	1.83	0.191	1	0.06	0.806
	Error	20			20		
Root: shoot ratio	Soil	1	12.1	0.002	1	2.45	0.132
	AM	1	16.9	<0.001	1	0.11	0.738
	Soil × AM	1	17.5	0.001	1	0.68	0.418
	Error	20			20		
Shoot P concentration	Soil	1	6.33	0.021	1	2.38	0.137
	AM	1	11.2	0.003	1	1.04	0.318
	Soil × AM	1	3.72	0.067	1	0.53	0.471
	Error	20			20		
Specific root length	Soil	1	5.96	0.024	1	0.02	0.867
	AM	1	5.69	0.027	1	0.20	0.658
	Soil × AM	1	2.20	0.153	1	0.01	0.907
	Error	20			20		
Root tissue density	Soil	1	18.0	<0.001	1	0.05	0.824
	AM	1	12.2	0.002	1	0.09	0.755
	Soil × AM	1	0.01	0.909	1	<0.01	0.989
	Error	20			20		
Root diameter	Soil	1	0.67	0.419	1	0.45	0.508
	AM	1	3.95	0.060	1	0.03	0.864
	Soil × AM	1	0.02	0.881	1	0.09	0.756
	Error	20			20		
Root- hair incidence	Soil	1	0.96	0.338	1	0.01	0.913
	AM	1	20.4	<0.001	1	0.09	0.756
	Soil × AM	1	1.08	0.310	1	<0.01	0.930
	Error	20			20		
Root- hair length	Soil	1	1.46	0.240	1	0.09	0.762
	AM	1	2.54	0.126	1	0.09	0.766
	Soil × AM	1	0.34	0.562	1	0.06	0.803
	Error	20			12		
Root- hair diameter	Soil	1	0.46	0.501	1	0.14	0.712
	AM	1	0.01	0.923	1	<0.01	0.989
	Soil × AM	1	0.47	0.512	1	0.02	0.880
	Error	20			12		

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