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DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

Comissão 2.1 - Biologia do solo

RELATIONSHIPS BETWEEN MICROBIAL ACTIVITY AND SOIL PHYSICAL AND CHEMICAL PROPERTIES IN NATIVE AND REFORESTED *Araucaria angustifolia* FORESTS IN THE STATE OF SÃO PAULO, BRAZIL[(1)]

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SUMMARY

Araucaria angustifolia (Bert.) O. Kuntze is the main component of the Mixed Ombrophilous forest and, in the State of São Paulo, it is associated with a high diversity of soil organisms, essential for the maintenance of soil quality, making the conservation of this ecosystem a major and pressing challenge. The objective of this study was to identify the physical and chemical properties that are most closely correlated with dehydrogenase enzyme activity, basal respiration and microbial biomass under native (NF) and replanted (RF) *Araucaria angustifolia* forests in three regions of the state of São Paulo, in winter and summer. The main differentiating factors between the areas were also determined. Each forest was represented by three true replications; at each site, from around the araucaria trees, 15 soil samples (0-20 cm) were collected to evaluate the soil physical, chemical and microbiological properties. At the same points, forest litter was sampled to assess mass and chemical properties. The following microbiological properties were evaluated: microbial biomass carbon (MBC), basal respiration (CO_2-C), metabolic quotient (qCO_2), dehydrogenase enzyme activity (DHA) as well as the physical properties (moisture, bulk density, macroporosity and total porosity), soil chemical properties [pH, organic carbon (org-C), P, Ca, K, Mg, Al, H+Al], litter dry mass, and C, N and S contents. The data were subjected to analysis of variance (*Two-way* ANOVA). A Canonical Discriminant Analysis (CDA) and a Canonical

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Correlation Analysis (CCA) were also performed. In the soil under NF, the values of K, P, soil macroporosity, and litter dry mass were higher and qCO_2 and DHA lower, regardless of the sampling period, and DHA was lower in winter. In the RF areas, the levels of moisture, porosity and qCO_2 were higher in both sampling periods, and DHA was higher in winter. The MBC was only higher under NF in the summer, while the litter contents of C, N and S were greater in winter. In winter, CCA showed a high correlation of DHA with CO_2-C, pH and H+Al, while in the summer org-C, moisture, Mg, pH and litter C were more associated with DHA and CO_2-C. The CDA indicated H+Al, available P, total porosity, litter S content, and soil moisture as the most discriminating variables between NF and RF, but moisture was the most relevant, in both seasons and CO_2-C only in winter. The combined analysis of CCA and CDA showed that the contribution of the microbiological variables to a differentiation of the areas was small at both samplings, which may indicate that the period after reforestation was long enough to allow an almost complete recovery of the microbial activity.

Index terms: multivariate analysis, soil quality, soil chemical properties, microbiological properties.

RESUMO: *RELAÇÃO ENTRE ATIVIDADE MICROBIANA E ATRIBUTOS FÍSICOS E QUÍMICOS DO SOLO EM FLORESTA DE* Araucaria angustifolia *NATIVA E REFLORESTADA NO ESTADO DE SÃO PAULO*

A Araucaria angustifolia *(Bert.) O. Kuntze é a principal componente da Floresta Ombrófila Mista no Estado de São Paulo e está associada à alta diversidade de organismos edáficos, que são importantes para manutenção da qualidade do solo, o que torna a preservação desse ecossistema tarefa urgente e inadiável. Este estudo teve como objetivo avaliar que atributos físico-químicos estão mais relacionados à atividade da enzima desidrogenase, à respiração basal e à biomassa microbiana do solo em floresta nativa (FN) e reflorestada (FR) com Araucaria angustifolia, em três regiões do Estado de São Paulo, no inverno e no verão, bem como quais desses são os principais fatores discrepantes entre as áreas. Cada floresta foi representada por três repetições verdadeiras, em que, em cada uma, foram coletadas, ao acaso, 15 amostras de solo (0-20 cm) para se avaliarem atributos físico-químicos e microbiológicos. No mesmo ponto, coletaram-se amostras de serapilheira para avaliar sua massa e seus atributos químicos. As coletas de solo e serapilheira foram realizadas no inverno e verão. Os atributos microbiológicos: carbono da biomassa microbiana (CBM), respiração basal (C-CO_2), quociente metabólico (qCO_2) e atividade da enzima desidrogenase (Desi); os atributos físicos do solo: umidade, densidade global, macroporosidade e porosidade total; e os químicos: pH, carbono orgânico (C-org), P, Ca, K, Mg, Al, H+Al, além da massa de matéria seca de serapilheira e C, N e S da serapilheira, foram submetidos à análise de variância (Two-way ANOVA). Realizaram-se também a Análise Canônica Discriminante (ACD) e a Análise de Correlação Canônica (ACC). O solo em NF apresentou maiores valores de K, P disponível, macroporosidade, massa de matéria seca de serapilheira e menores valores de qCO_2, independentemente da época de coleta, além de menor atividade da desidrogenase no inverno. Em RF, foram encontrados maiores valores de umidade, porosidade total e qCO_2, no inverno e verão, além da maior atividade da Desi no inverno. O CBM foi maior apenas na FN no verão, enquanto os teores de C, N e S da serapilheira foram maiores no inverno. No inverno, a ACC apresentou alta correlação da Desi com C-CO_2, pH e H+Al, enquanto no verão, C-org, umidade, Mg, pH e C da serapilheira se correlacionaram com a atividade da Desi e C-CO_2. A ACD discriminou os atributos H+Al, P disponível, porosidade total, teor de S da serapilheira e umidade do solo como os de maior importância na separação das áreas FN e FR, sendo a umidade o mais relevante, independentemente da época de coleta, e C-CO_2, apenas no inverno. Na avaliação da ACC e ACD em conjunto houve pouca influência das variáveis microbiológicas na separação das áreas, nas duas épocas de coleta, indicando que o período decorrido da implantação da área FR já tenha sido suficiente para propiciar a recuperação da sua atividade microbiológica.*

Termos de indexação: análise multivariada, qualidade do solo, atributos químicos do solo, atributos microbiológicos.

INTRODUCTION

Araucaria angustifolia (Bert). O. Kuntze is a typical arboreal species of the Mixed Ombrophilous forest in Brazil (Veloso et al., 1991). The area of occurrence of *Araucaria* forests was formerly huge in the south and southeast of the country, but inadequate exploration led to a significant shrinkage of the original area, which once covered approximately 253,000 km^2. Currently, there are only 32,021 km^2 (12.6 %) left, of which 981 km^2 are permanently preserved, representing only 0.39 % of the original forest area (Ribeiro et al., 2009).

For the preservation of this forest, the presence of a high diversity of arbuscular mycorrhizal fungi (Moreira et al., 2009) and diazotrophic bacteria (Lammel et al., 2007), heterotrophic bacteria and archaebacteria (Maluche-Baretta, 2007; Bertini, 2010), and soil invertebrates (Merlim, 2005; Baretta et al., 2010) is fundamental. Conservation is therefore an urgent issue that must be addressed without delay, since the soil quality is fundamental, not only for food, timber, fiber, and fuel production, but also for the maintenance of biodiversity and environmental quality (Doran & Zeiss, 2000; Bastida et al., 2006; Kaschuk et al., 2010).

The quality of a soil is related to its physical, chemical and biological properties, which are affected by management and land use type and can be measured by indicators that are sensitive to alterations (Doran & Parkin, 1994; Baretta et al., 2010). However, aside from the traditional physical and chemical properties, the indicators should include microbiological variables as well, which together can reflect the processes that influence soil quality (Tótola & Chaer, 2002; Bastida et al., 2008; Baretta et al., 2010). Among the microbiological indicators of soil quality, microbial biomass carbon (MBC) is one of the most promising and most commonly used, due to its immediate response to reflect environmental changes, i.e., more susceptible to variations than the physical and chemical properties, including soil organic carbon (Powlson & Jenkinson, 1981; Baretta et al., 2005; Nogueira et al., 2006; Baretta et al., 2010; Kaschuk et al., 2010).

The amount of mineralized carbon in the form of CO_2, due to the decomposition of organic matter, indicates whether climatic conditions, soil management or the presence of pollutants affect microbial activity and is used as soil quality indicator. For example, disturbed ecosystems tend to become sources of CO_2, as a result of the increased decomposition rate and reduction or interruption of organic residue input into the soil (Baretta et al., 2008). From the relationship between soil basal respiration and MBC, the metabolic quotient (qCO_2) can be calculated, which is an important index of the metabolic condition of the microbial community, reflecting the degree of environmental stress (Anderson & Domsch, 1989).

Soil enzymes are used as soil quality indicators due to their high sensitivity and fast response to soil management and use, as well as the ease of determination (Tabatabai, 1994). The enzyme dehydrogenase is sensitive to changes in the soil microbial community and is active in living cells, responsible for electron transfer reactions in the respiratory chain (Bastida et al., 2006), thus reflecting the oxidative activity of the soil microbial biomass.

The soil microbial community is fundamental in any ecosystem because it takes part in organic matter decomposition and nutrient cycling, influencing the chemical and physical properties of the soil and consequently, primary productivity. On the other hand, it is also affected by the physical and/or chemical parameters. For example, the pH affects both microbial activity as well as nutrient availability for microorganisms and plants. Organic carbon is a crucial component for soil fertility, be it as substrate for microorganisms or as a source of energy, nitrogen, sulfur, and other mineral nutrients (Idowu et al., 2008).

The most widely used physical indicators of soil quality, correlated with the chemical and microbiological variables, were bulk density, porosity, aggregate stability, aeration, infiltration, and water retention capacity, among others (Schoenholtz et al., 2000). To deepen the understanding of the soil quality in *Araucaria* forests, relating several indicators, the purpose of this study was to assess which physical and chemical properties are most closely related to dehydrogenase enzyme activity, basal respiration and microbial biomass under native and reforested forests with *A. angustifolia,* in two contrasting seasons (winter and summer), and which of these properties are the main discriminating factors between the areas.

MATERIAL AND METHODS

Description of the study areas

The study was conducted in areas of the Atlantic Forest biome, with Mixed Ombrophilous forest, in the state of São Paulo. Areas with native (NF) and reforested (RF) forests with *Araucaria angustifolia* were selected in three regions of the State (Figure 1). In each region, one 0.5 ha plot, considered a true replication, was marked in both forest types.

The first sampling area is part of the Ecological Station Bananal in the Serra da Bocaina, between the States of São Paulo and Rio de Janeiro. At a distance of 25 km away from the town of Bananal, the climate is humid mesothermal, with no dry season and mild summers (Cfb, according to the Köppen classification).

At the first sampling (August 2009), average temperature and rainfall were 17 ^{o}C and 40 mm,

respectively, while at the second (February 2010), the average temperature rose to 22 °C and more than 300 mm rainfall was recorded in January.

The NF area, at an altitude of 1,185 m, was demoninated Area 1, containing: 178 shrub and tree species distributed in 46 families and 89 genera. The most abundant families were Myrtaceae (28 species), Melastomataceae (19), Lauraceae (13), Rubiaceae (12) and Asteraceae (11). Four species are on the list of endangered species: *Euterpe edulis* Mart. (Juçara palm), *Raulinoreitzia leptophebia* (B.L. Rob) R.M. King & H. Rob, *Cedrella fissilis* Vell., and *Araucaria angustifolia* (Bert.) O. Kuntze. The floristic composition of the area was described in more detail by Santos et al. (2009).

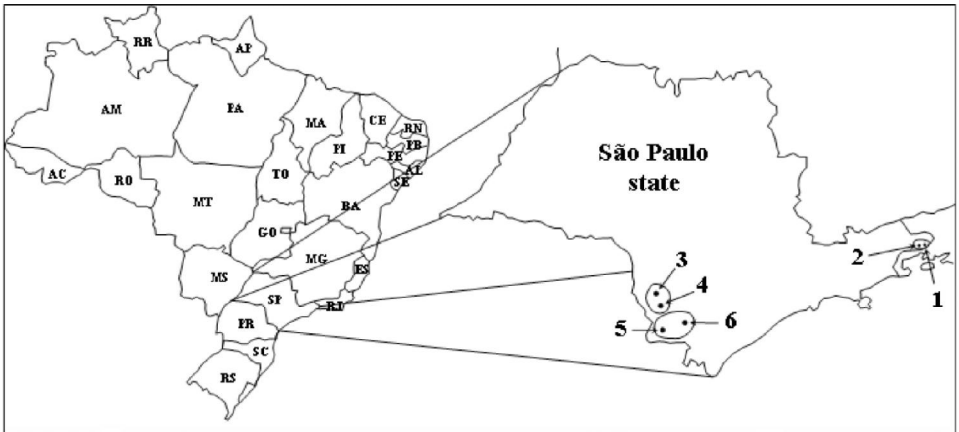
The RF area (Area 2; at 1,126 m) had been replanted about 38 years before and the forest is still in development, together with other species such as *Dicksonia sellowiana* Hook. (tree fern), *Euterpe edulis* Mart. (Juçara palm), *Myrcia rostrata* DC., among others, herbaceous and shrub flora and gramineae at sites of minor vegetation density. The soil in NF, area 1 and RF area 2 was classified as Dystric Haplic Cambisol (WRB, 2006).

The second sampling region (Area 3; NF at 678 m) is part of the Itaberá Ecological Station with 180 ha, in Itaberá-SP (Figure 1) covered with secondary forest with occurrence of native *A. angustifolia*. The climate is Cfb, humid mesothermal, with mild summers and no dry season, according to Köppen. In the first sampling season, the average temperature was 17 °C and rainfall 45 mm in August, while 250 mm rain fell in July, atypical for this time of the year. In summer, February was the warmest month (25 °C) and rainfall was highest in January (420 mm). The soil was classified as Alumic Vetic Ferralsol (WRB, 2006), with a slightly undulated relief (Figure 1).

Area 3 (NF at 678 m) was characterized by 134 shrub and tree species, belonging to 50 families and 91 genera. The largest families were Myrtaceae (12 species), Lauraceae (11), Rubiaceae (8), Euphorbiaceae (8), Salicaceae (6) and Melicaceae (6) (Santos & Ivanauskas, 2010). *Eugenia ligustrina* (Sw.) Willd., and *Sorocea bonplandii* (Baill.) W.C. Burger, Lanj. & Wess. Boer., are prevalent and very common in the understory and sub-canopy forest. *Syagrus romanzoffiana* (Cham.) Glassman also predominated in the intermediate stratum. The canopy consisted of *Matayba elaegnoides* Radlk., *Luehea divaricata* Mart., *Parapiptadenia rigida* (Benth.) Brenan, aside from *Araucaria angustifolia,* an emerging species (Santos & Ivanauskas, 2010).

Apart from *A. angustifolia,* the following threatened species were found: *Euterpe edulis* Mart. (Juçara palm), *Balfourodendron riedelianum* (Engl.) Engl. (guatambú), *Aspidosperma polyneuron* Müll. Arg. (Peroba Rosa) and *Cedrela fissilis* Vell. The flora of this area was described in detail by Santos & Ivanauskas (2010).

In the same region, the RF area (Area 4, 740 m) is part of the Experimental station of Itapeva, in the municipality of Itapeva, São Paulo, and had been planted 45 years before (Figure 1). Other plant species were observed, such as *Gochnatia polymorpha* (Less) Cabr. (Candeia), *Chusquea ramosissima* Lindm. (a bamboo type), as well as other herbaceous species in the understory, natural regeneration of *Araucaria* seedlings and scarce tree species, except for the



Areas	Forest Type - Location	Latitude (S)	Longitude (W)
1	Native *Araucaria* forest (NF) - Bananal, SP	22° 48'26"	44° 21'59"
2	Reforested site (RF) - Bananal, SP	22° 48'24"	44° 22'27"
3	Native *Araucaria* forest (NF) - Itaberá, SP	23° 50'27"	49° 08'40"
4	Reforested site (RF) - Itapeva, SP	24° 04'19"	49° 04'10"
5	Native *Araucaria* forest (NF)- Barra do Chapéu, SP	24° 28'40"	49° 01'07"
6	Reforested site (RF) - Iporanga, SP/PETAR	24° 20'14"	48° 36'14"

Figure 1. Map of the State of São Paulo with the georeferenced points of the study locations, São Paulo Brazil, 2013.

predominant *Araucaria*. The soil was classified as Eutric Ferralic Nitisol (WRB, 2006) (Figure 1).

The third forest replication was an area in the Parque Estadual Turístico of Alto do Ribeira (PETAR), in the municipality of Iporanga, São Paulo, and in the region surrounding the park, in Barra do Chapéu, São Paulo (Figure 1). The climate is mild and temperate without droughts, Cfb according to the Köppen classification (Souza, 2008). In the winter of sampling, April was the driest month (<50 mm) and June the coldest (on average 17 °C), while in the summer of evaluation, the wettest month was January (over 400 mm), with an average temperature of 24 °C. The soil was classified as Dystric Haplic Cambisol (WRB, 2006). The NF (Area 5,785 m) was covered by Mixed Ombrophilous forest (Figure 1). The vegetation of this area consists of *Dicksonia sellowiana* Hook, *Cordyline* sp. *and Eugenia cerasiflora* Miq., representative in the understory; *Allophylus edulis* (A.St.-Hil., Cambess. & A. Juss.) Radlk as typical species of the subcanopy; *Prunus myrtifolia* (L.) Urb, *Casearia sylvestris* Swartz, *Swartzia acutifolia* Vogel, *Matayba elaeagnoides* Radlk, *Casearia decandra* Jacq. and *Ocotea elegans* Mez are dominant in the canopy, while *Araucaria angustifolia* (Bertol.) Kuntze, *Cedrella fissilis* Vell., *Ocotea elegans* Mez, *Ocotea puberula* (Rich.) and *Matayba elaeagnoides Radlk* are emerging species. The floristic diversity of this area was described in detail by Souza (2008).

The corresponding RF (Area 6, 932 m), was represented by the "Núcleo do Areado", PETAR (Figure 1). Regenerating tree species, shrubs and herbs grow in this area, and grass on patches with lower plant density. The soil was classified as Dystric Haplic Cambisol (WRB, 2006). The physical-chemical properties of all sampled soils are listed in table 1.

Table 1. Physical and chemical properties of soil in the 0-20 cm layer in native *Araucaria* forest (NF) and reforested areas (RF), considering all the areas together, in the State of São Paulo, Brazil

Variable	NF	RF
pH ($CaCl_2$)	4.0 ± 0.37(1)	4.2 ± 0.7
Organic-C (g kg^{-1})	33 ± 12.9(1)	34.9 ± 7.9
P-resin (mg kg^{-1})	8.3 ± 4.01(1)	5.1 ± 2.1
K ($mmol_c$ kg^{-1})	2.0 ± 1.02(1)	1.4 ± 0.6
Ca ($mmol_c$ kg^{-1})	16.8 ± 17.6(1)	20.7 ± 28.8
Mg ($mmol_c$ kg^{-1})	9.0 ± 9.8(1)	7.6 ± 9.4
H + Al ($mmol_c$ kg^{-1})	115.5 ± 46.2(1)	132.3 ± 68.9
Bulk density(g cm^{-3})	1.08 ± 0.2(2)	1.03 ± 0.1
Macroporosity (m^3 m^{-3})	0.16 ± 0.07(2)	0.11 ± 0.1
Microporosity (m^3 m^{-3})	0.41 ± 0.06(2)	0.52 ± 0.1
Sand (g kg^{-1})	459.0 ± 157.6(3)	263.0 ± 165.1
Silt (g kg^{-1})	87.3 ± 40(3)	96.0 ± 75.3
Clay (g kg^{-1})	453.8 ± 136.5(3)	651.0 ± 119.0

(1) mean of 90 replications ± s.d.; (2) mean of 45 replications ± s.d.; (3) mean of 9 replications ± s.d. organic-C (organic carbon).

Soil and litter sampling

In plots of 0.5 ha, established in each sampling area, 15 trees of *A. angustifolia* were randomly selected, spaced at least 20 m apart. From around the trees (2 m away from the trunk) 15 soil and litter samples were randomly collected, to evaluate the microbiological, physical and chemical soil and the chemical litter properties, as well as dry litter in both seasons (winter - August 2009 and summer - February 2010). The soil was sampled five times per sampling point with a Dutch auger (0-20 cm layer), to form a composite sample. The litter of each tree was collected using a 25 x 25 cm frame. The samples were packed in polyethylene bags, refrigerated and transported to the laboratory in ice-cooled boxes. Thereafter the soil samples were sieved (2 mm), ground and stored in a refrigerator (4 °C) until analysis. Litter samples were oven-dried at 55 °C for 72 h and then ground in a mill.

Microbiological, physical and chemical evaluations of soil and chemical analysis of litter

The microbial biomass carbon (MBC) was determined by the fumigation - extraction method (Vance et al., 1987), using 10 g of soil (moisture was adjusted to 60 % of water holding capacity - WHC). The samples were fumigated with ethanol-free chloroform for 24 h, maintaining non-fumigated controls. Then, MBC was extracted from samples and controls with K_2SO_4 (0.5 mol L^{-1}) under stirring at 180 rpm for 30 min, oxidation with $K_2Cr_2O_7$ (66 mmol L^{-1}) and titration with ferrous ammonium sulfate ($[(NH_4)_2Fe(SO_4)_2.6H_2O]$ (33 mmol L^{-1}) in the presence of barium diphenylamine sulfonate. The correction factor Kc used for the calculations was 0.33.

Microbial activity was estimated by basal respiration (CO_2) released from 100 g incubated soil samples (soil moisture was adjusted to 60 % of WHC). The soil was incubated in a glass flask containing NaOH (0.5 mol L^{-1}), for 10 days in the dark, heated to 28 °C. The carbon released as CO_2 was measured every 24 h to determine the remaining NaOH by titration with a standardized HCl (0.5 mol L^{-1}) solution, using the indicators phenolphthalein and early carbonate precipitation by the addition of $BaCl_2$ (4 mol L^{-1}) (Alef & Nannipieri, 1995). The resulting CO_2-C and MBC values were used to calculate the metabolic quotient (qCO_2), which represents the CO_2-C release rate per unit of MBC, as described by Anderson & Domsch (1993).

The dehydrogenase activity (DHA) was determined in 5 g soil (natural moisture) with 5 mL of a 1.0 % 2,3,5 - triphenyltetrazolium chloride (TTC) solution, after incubation at 37 °C for 24 h. The enzyme activity was measured spectrophotometrically at 485 nm (Casida Jr. et al., 1964).

Determination of dry matter and C, N and S in litter

The litter dry mass was determined after drying at 55 ^{0}C to constant weight. Then the litter was ground, sieved (100 mesh) and the C, N, and S contents were determined by dry combustion in an elemental analyzer for C, N and S.

Determination of soil physical and chemical properties

The soil pH was determined by potentiometry in 0.01 mol L^{-1} $CaCl_2$ solution at a ratio of 1:2.5; P, Ca, Mg were extracted with ion exchange resin and K by Mehlich-1. Phosphorus was determined spectrophotometrically by the molybdenum blue complex, K by flame emission spectrometry, Ca and Mg by atomic absorption spectrometry, H+Al by potentiometry in SMP solution at pH 7.0; Al was extracted with KCl solution (1 mol L^{-1}) and determined by titration with 0.025 mol L^{-1} NaOH, and organic carbon (org-C) was determined by colorimetry, by oxidation of organic matter with $Na_2Cr_2O_7.2H_2O$ and H_2SO_4, according to Raij et al. (2001).

The moisture (moist) percentage was calculated by the difference between 10 g of fresh soil and of soil dried at 105 ^{0}C for 48 h. For particle size analysis, the sand fraction was obtained by sieving (0.053 mm), the clay fraction by the hydrometer method (Gee & Or, 2002), and silt from the difference between the quantities of soil and sand plus clay in the sample. In the winter, at the same points sampled for chemical analysis, undisturbed soil samples were taken (0-5 cm layer), using stainless steel rings (diameter 5 cm). The samples were wrapped in plastic film and taken to a laboratory, where they were refrigerated until analysis. In the laboratory, soil samples were saturated by capillary action, by gradually increasing the water depth up to two thirds the height of the rings. Then, they were subjected to a matric potential of -1 and -6 kPa using a tension table (Embrapa, 1997). When in hydraulic equilibrium, the samples were weighed and subsequently dried at 105 °C for 48 h to determine water content (Embrapa, 1997) and bulk density (Bd) (Blake & Hartge, 1986). Macroporosity (Ma) was computed as the difference between the water content of saturated soil and the water content after application of 6 kPa (Embrapa, 1997). Microporosity was estimated as the water content retained at a tension of 6 kPa. Total porosity (Pt) was calculated as the sum of macroporosity and microporosity. Particle density was determined by a helium pycnometer (Danielson & Sutherland, 1986).

Statistical analysis

The microbiological properties (MBC, CO_2-C, qCO_2, and DHA) were subjected to analysis of variance (*Two-way* ANOVA) and the means were compared by the LSD (Least significant difference) test ($p<0.05$). These properties were also subjected to Canonical Discriminant Analysis (CDA) to identify which results were more relevant to discriminate the studied areas (Cruz-Castillo et al., 1994; Baretta et al., 2005). In case of significant differences between areas, the values of the standardized canonical coefficients (SCCs) were subjected to the mean comparison test LSD ($p<0.05$) as described by Cruz-Castillo et al., (1994). Additionally, the microbiological and chemical properties of soil [(pH $CaCl_2$, org-C, P, K, Mg, Al, H+Al] and litter (litter C, litter N, litter S), as well as soil physical properties [(Bd, Ma, Pt and Moist) and litter dry mass (LDM) were subjected to Canonical Correlation Analysis (CCA). Univariate statistical analyses were performed using SAS version 8.2 (SAS, 2002).

RESULTS AND DISCUSSION

Significant differences were found for some of the physical, chemical and microbiological properties in both sampling seasons (Table 2). Regardless of the sampling time, the levels of potassium (K), phosphorus (P) and macroporosity in the soil of native *Araucaria* forests were higher than in the reforested areas, whereas in soil of reforested *Araucaria* areas, total porosity and moisture were higher (Table 2).

In summer, there was a reduction in the available K and P levels, coinciding with higher soil moisture, which may have accelerated waste decomposition because of greater use of these elements by the biological community of the soil. Aside from the differences between forests, significant differences of some of the properties between seasons were also observed (Table 2). The levels of C and N in litter in winter were higher for NF than RF.

Litter dry mass was also highest in NF soil, regardless of the sampling time. Most likely, this reflects the conditions of the more complete soil cover and plant diversification and the greater input of plant residues in NF (Table 2). The contribution of crop residues to soil is usually related to the soil C content, but in this case it did not differ significantly between areas, reflecting the recovery of vegetation in RF areas and the low fluctuations of this variable in a short period (season). However, the C content can increase at certain times of the year, in response to the increased organic matter and nutrient cycling, triggering a cycle of stimulation of the soil biological activity (Maluche-Baretta et al., 2007; Correia & Andrade, 2008).

In general, the soils are acidic under both forest types and, together with the other chemical properties, are within the range of values reported by Bertini (2010) and Carvalho et al. (2012) for soils under native and replanted A*raucaria* forest, in the State of São Paulo. Macroporosity was higher in the preserved native than the reforested area and the values found in the two ecosystems (≥ 0.10 m^3 m^{-3}) (Table 2) were

Table 2. Soil physical and chemical and chemical litter properties in native *Araucaria* forest (NF) and reforested (RF)

Environmental variable[(1)]	Winter		Summer	
	NF	RF	NF	RF
pH ($CaCl_2$)	4.0 a	4.2 a	4.0 a	4.1 a
H + Al ($mmol_c\ kg^{-1}$)	113.2 a	127.0 a	117.80 a	137.69 a
K ($mmol_c\ kg^{-1}$)	2.3 a	1.5 b	1.6 a	1.2 b
Al ($mmol_c\ kg^{-1}$)	23.2 a	25.84 a	14.4 b	21.34 a
Mg ($mmol_c\ kg^{-1}$)	8.8 a	8.0 a	9.2 a	7.2 a
P-resin ($mg\ kg^{-1}$)	8.9 a	5.6 b	7.6 a	4.4 b
Organic carbon ($g\ kg^{-1}$)	32.6 a	35.5 a	34.3 a	33.3 a
Moisture (%)	28.5 b	40.7 a	31.9 b	46.3 a
Bulk density ($g\ cm^{-3}$)	1.09 a	1.02 a	1.09 a	1.02 a
Total porosity ($m^3\ m^{-3}$)	0.57 b	0.63 a	0.56 b	0.62 a
Macroporosity ($m^3\ m^{-3}$)	0.15 a	0.11 b	0.15 a	0.11 b
Litter dry mass ($kg\ m^{-2}$)	1.12 a	1.10 b	1.14 a	0.93 b
Litter C (%)	43.8 a	40.0 b	33.03 a	34.44 a
Litter N (%)	1.7 a	1.3 b	1.39 a	1.25 a
Litter S (%)	0.18 a	0.14 b	0.20 a	0.17 a

[(1)] LSD Test ($p<0.05$).

suitable for adequate oxygen diffusion and water infiltration into the soil. This shows a good structural quality, favorable for the successful development of the biological community (Grable & Siemer, 1968; Alves et al., 2007). Total porosity was higher under RF than NF, probably due to the higher microporosity and clay content in the RF soil (Table 1).

The levels of microbial biomass carbon (MBC) only differed between areas ($p<0.05$) in the summer (Figure 2). In this period, the highest and lowest MBC values

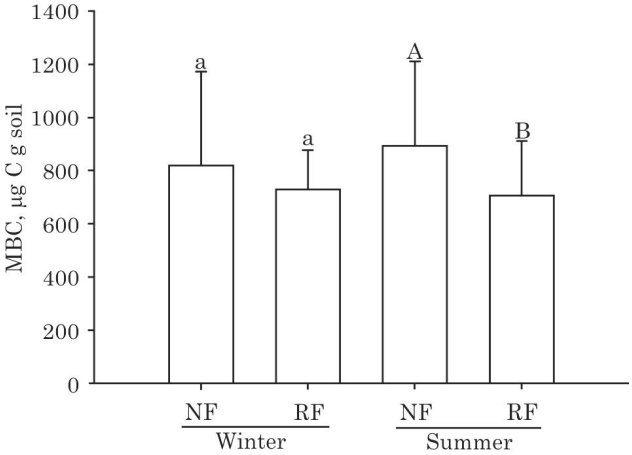


Figure 2. Levels of microbial biomass carbon (MBC) in soil discriminating native *Araucaria* forest (NF) and reforested areas (RF) in August 2009 (winter) and February 2010 (summer). Lowercase and uppercase letters compare between forests in the same season. LSD test at 5 %. Vertical bars represent the standard deviation (n=45).

were detected (893 μg C g^{-1}) in NF, which was approximately 21 % higher than in RF (706 μg C g^{-1}). This indicates that the maintenance of native vegetation ensures the best conditions (macroporosity, litter dry mass and K and P levels), with a positive influence on the development and establishment of the soil microbiota (Wardle, 1992) (Table 2). In addition, seasonal changes in the microbial activity may be due to the increase in temperature and rainfall, particularly in tropical regions, accelerating metabolic processes and promoting the activity of the soil microbial community (Joergensen et al., 1990; Wardle, 1992; Bastida et al., 2006).

In winter, the MBC values of NF and RF areas did not differ (818 and 723 μg C g^{-1} soil), respectively (Figure 2). The MBC indicates changes in the soil ecosystem balance (Baretta et al., 2005, 2008; Silva et al., 2009; Baretta et al., 2010; Kaschuk et al., 2010). The MBC values found in this study were almost double those reported by Baretta (2007) and Bertini (2010) and are within the range of values found by Carvalho et al. (2012) in soils under native and reforested *Araucaria* forests in the State of São Paulo, and within the range found for ecosystems of Atlantic forest (683 - 1520 mg C kg^{-1} soil) (Kaschuk et al., 2010).

Unlike MBC, microbial basal respiration (CO_2-C) did not differ between areas and sampling seasons (Figure 3). In the winter, NF and RF values were 64 and 76 μg CO_2-C g^{-1} soil d^{-1}, respectively, and in summer, 64 and 68 μg CO_2-C g^{-1} soil d^{-1}, respectively. The similarity of the CO_2-C values in the different areas suggested similar microbial activity. The CO_2-C values were within the same range as those found

by Bertini (2010) and Bini et al. (2013a), in soil under native and reforested *Araucaria* forest in the State of São Paulo and Paraná, respectively.

The respiration rates can increase when the soil microbiota uses more readily degradable carbon substrate or due to the rapid oxidation of this carbon (Islam & Weil, 2000). Other authors found no significant differences either, in relation to soil basal respiration between native and reforested *Araucaria* forest, under similar experimental conditions (Baretta, 2007; Bertini, 2010; Carvalho et al., 2012).

The metabolic quotient (qCO_2) indicates the changes in microbial activity between natural and disturbed ecosystems more clearly (Islam & Weil 2000; Baretta et al., 2005; Bastida et al., 2008). The only microbiological variable that differed ($p<0.05$) between sampling sites in both seasons was qCO_2 (Figure 4). The higher qCO_2 values in RF may indicate a higher consumption of carbon readily assimilated by the microbial community, requiring more energy for their support, associated with a condition of disorder, with greater CO_2 losses, a characteristic of ecosystems in development (Anderson & Domsch, 1993). Higher qCO_2 values in cropped soils indicate greater stress of the microbial community compared to soils with more stable systems, such as mature forests (Islam & Weil, 2000), similarly to healthy *Araucaria* forests harmed by fire in comparison with native *Araucaria* and planted *Araucaria* forests (Baretta, 2007; Bertini, 2010), or replanted *Araucaria* forests in the rainy and dry season (Carvalho et al., 2012), or agricultural cultivation with annual crops compared with Mixed Ombrophilous forest, Bini et al. (2013a). This tendency to higher qCO_2 values may indicate an environment of ecological stress and degradation or a high level of productivity in this area, which is in line with the history of the RF area, more exposed to human intervention and to heavier impacts on the soil-climatic factors.

In contrast, qCO_2 values in NF soil were lower, indicating more stable ecosystems, with greater efficiency of microorganisms to convert organic waste into microbial biomass and with greater sustainability, which is consistent with the history of the NF area and corroborates results published elsewhere (Tótola & Chaer, 2002; Gil-Sotres et al., 2005; Baretta, 2007).

The use of qCO_2 is based on the theory of ecological succession proposed by Odum (1969), according to which the qCO_2 value is reduced during the succession or regeneration, caused by some disturbance. In the climax stage however, as in NF, the soil microbial community would tend to become more efficient in conserving soil organic carbon.

The activity of the enzyme dehydrogenase (DHA) differed significantly ($p<0.05$) between the NF and RF areas in winter only (Figure 5). In this season, the DHA in NF and RF were 10.9 and 10.1 μg TTF g^{-1} soil d^{-1}, respectively, and in summer 10.3 and 9.8 μg TTF g^{-1} soil d^{-1}. The higher activity in RF than NF was related to the metabolism of viable soil microorganisms, indicating the oxidative activity of the microbial community which, together with MBC, CO_2-C and qCO_2, has been used as general biochemical parameter to measure the influence of management on soil quality (Gil-Sotres et al., 2005; Bastida et al., 2006; Carvalho et al., 2012; Bini et al., 2013b).

In winter, the thinner canopy and greater soil exposure in RF than NF may have resulted in greater temperature and soil moisture fluctuation, as well as of other factors that influence the increase of microbial

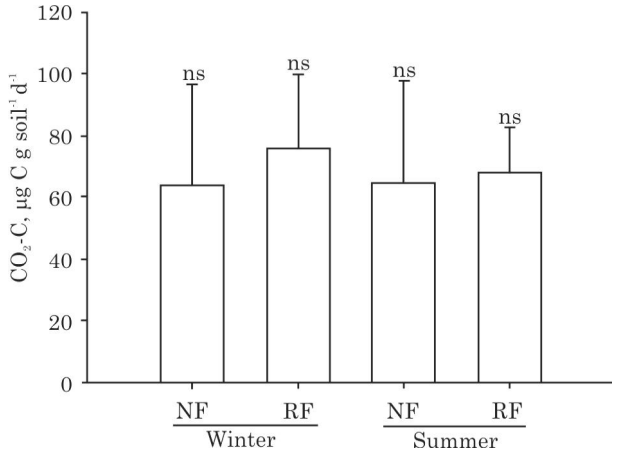


Figure 3. Microbial basal respiration (CO_2-C) in soil native *Araucaria* forest (NF) and reforested (RF) in August 2009 (winter) and February 2010 (summer). LSD test at 5 %. ns: not significant. Vertical bars represent the standard deviation (n=45).

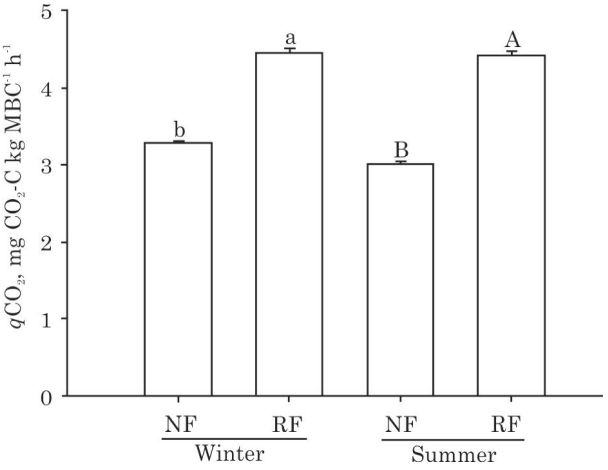


Figure 4. Metabolic quotient (qCO_2) in soil native *Araucaria* forest (NF) and reforested areas (RF) in August 2009 (winter) and February 2010 (summer). Lowercase and uppercase letters compare forests at the same season. LSD test at 5 %. Vertical bars represent the standard deviation (n=45).

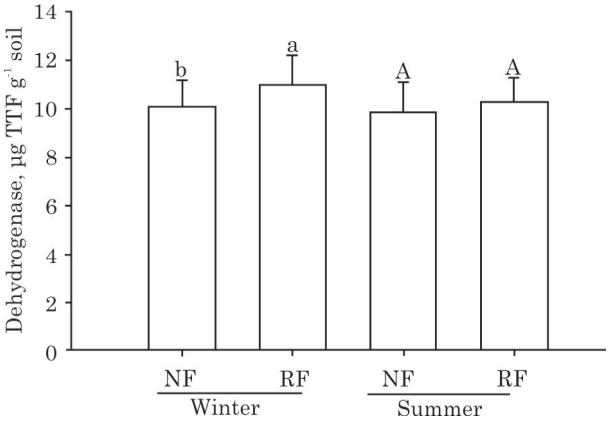


Figure 5. Dehydrogenase enzyme activity in soil native *Araucaria* forest (NF) and reforested areas (RF) in August 2009 (winter) and February 2010 (summer). Lowercase and uppercase letters compare forests in the same season. LSD test at 5 %. Vertical bars represent the standard deviation (n=45).

activity, represented by the higher DHA, in line with the qCO_2 (Fig. 5).

Bini (2009) and Bini et al. (2013a) found higher DHA in soils of an agricultural area (16.46 µg TTF g^{-1} soil d^{-1}), than of *Araucaria* forest (11.28 µg TTF g^{-1} soil d^{-1}) and in an area reforested with *Araucaria* (10.65 µg TTF g^{-1} soil d^{-1}) in the state of Paraná. The possibility of increased metabolic stress in RF, indicated by higher qCO_2 values, was confirmed by the higher DHA, especially in winter (Figures 4 and 5).

Canonical Correlation Analysis (CCA)

Four canonical correlations of microbiological properties (MBC, qCO_2, basal respiration, and DHA) were included in the CCA and 12 environmental variables [pH ($CaCl_2$), H+Al, K, Al, org-C, available P, Mg, and soil moisture], and litter dry mass, and litter C, N and S levels. The microbiological variables were called biological (BIO1) and environmental (physical and chemical) were denominated chemical variables (CHEM1). The soil physical properties bulk density, macroporosity and total porosity were not used in the CCA because they were only evaluated in winter and may decrease the reliability of the correlations. Only the first canonical correlation of data was evaluated, with a higher value and significance level ($p<0.0001$) than of the other canonical correlations.

In winter, the first canonical correlation between the physical - chemical (CHEM1) and biological properties (BIO1) was 0.91 ($p<0.0001$), with 81 % of the variance of scores of the first canonical variable of the BIO1 properties explained by the scores of the first canonical variable of the CHEM1 properties (Figure 6a). The high value of canonical correlation (CC) between microbiological and physical-chemical properties was due to the higher CC between

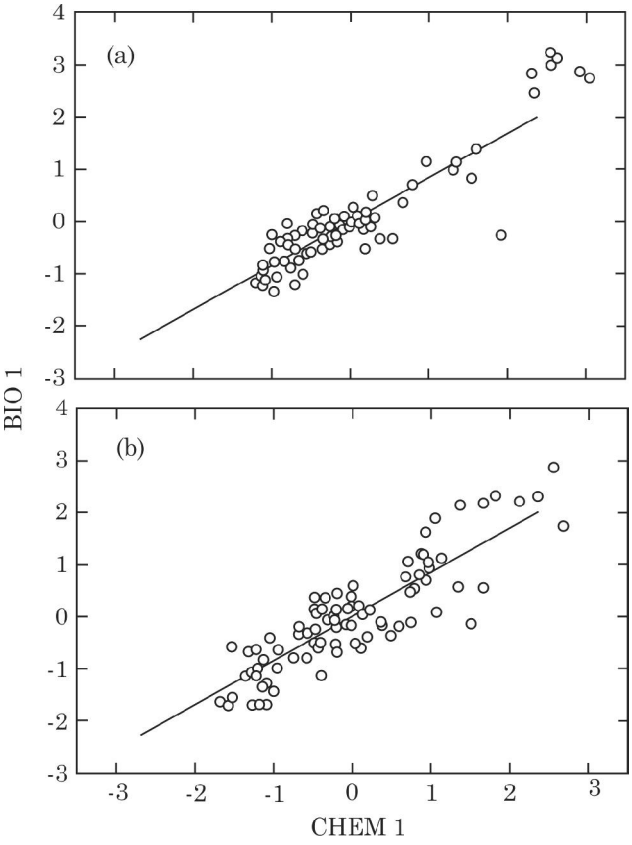


Figure 6. Canonical Correlation Analysis between the soil physical and chemical properties + chemical attributes of litter (CHEM1) and soil microbiological properties (BIO1). Soil microbiological (CO_2-C, MBC, and DHA qCO_2) and chemical [pH($CaCl_2$), org-C (H+Al), K, Al, Mg and P] properties, chemical litter (C, N and S) properties, and soil moisture + litter dry weight sampled in winter (a) and summer (b) in native *Araucaria* forest (NF) and reforested areas (RF), (n=45).

microbiological properties, especially of DHA (0.7410) and chemical properties such as pH (0.5936), Mg (0.3466), org-C (0.1970) and litter N (0.1306), aside from physical properties, e.g.: soil moisture (0.2652) and litter dry mass (0.1756) (Table 3).

The first biological canonical variable had higher standardized canonical coefficients (SCC) for the microbiological property dehydrogenase (1.0683), followed by basal soil respiration (0.4683) (Table 3), since the CC was highest for DHA (0.7470). The positive signs of DHA and CO_2-C (SCC and CC) indicated high values of DHA and CO_2 emission and high sensitivity to the related physical-chemical properties (Table 3). Several physical and chemical properties were correlated with DHA and soil microbial basal respiration (CO_2-C) in winter.

In winter, the SCC values of pH (1.2746) and exchangeable acidity (H+Al) (0.6320) were noteworthy.

Table 3. Standardized canonical coefficients (SCC) and canonical correlation (CC) as the microbiological properties of the biological canonical variables 1 (CVB1) and physical-chemical canonical variables (CVPC1) of soil and litter in native *Araucaria* forest (NF) and reforested areas (RF), São Paulo, Brazil. (n=45)

Environmental variable	Winter		Summer	
	SCC	CC	SCC	CC
	CVB1		CVB1	
Microbial biomass carbon (MBC)	-0.6796	0.0111	-0.4769	0.0055
Respiration of soil (CO_2-C)	0.4683	0.0852	0.3149	0.3567
Metabolic quotient (qCO_2)	-0.2349	0.0841	0.0694	0.4086
Dehydrogenase (DHA)	1.0683	0.7470	0.7940	0.5952
	CVPC1		CVPC1	
pH ($CaCl_2$)	1.2746	0.5936	0.5452	0.5483
H+Al	0.6320	0.0963	-0.3017	0.1996
K	-0.1805	0.0006	-0.0992	0.0837
Al	-0.0191	0.0746	-0.0521	0.1490
Organic carbon	0.0850	0.1970	0.2220	0.2070
P	0.0271	0.0001	-0.0695	0.0001
Mg	0.0274	0.3466	0.2727	0.3856
Litter dry matter	-0.0384	0.1756	-0.2119	0.0188
Moisture	0.0717	0.2652	0.4561	0.0402
Litter C	0.0794	0.0290	0.1131	0.0181
Litter N	0.0119	0.1306	-0.0542	0.0605
Litter S	-0.2574	0.0898	-0.0899	0.0515

In summer, SCCs were highest for pH (0.5452), moisture (0.4561), Mg (0.2727) and organic carbon (org-C) (0.2220) and litter carbon (0, 1131), which were associated with increased DHA (0.7940) and CO_2-C (0.3149) (Table 3). In this sense, it was suggested that these physical-chemical parameters are season-dependent and the metabolic behavior of microorganisms and the system functionality can be modified according to the time of the year.

The qCO_2 value for SCC was negative in winter and positive but low in summer, with few positive correlations with the soil chemical properties. The SCC values of microbial biomass carbon (MBC) were negative, both in winter (-0.6796) and summer (-0.4769). This attribute was considered a suppressor property with little influence on the chemical properties of soil and litter (Table 3). However, both MBC and qCO_2 have been reported as important microbiological properties, reflecting soil quality in different ecosystems (Baretta et al., 2010; Kaschuk et al., 2010). The differences found for the physical-chemical properties indicate that these, after more than 25 years since the implementation of RF, still differ from NF soil (Figure 7).

Considering the first physical-chemical canonical variable, the properties pH and H+Al showed high levels of SCC, indicating that these are directly related to the microbiological properties, with a positive relationship, in particular with CO_2-C and DHA (Table 3). Negative values of SCC and CC, both in the first physical-chemical canonical variable as in first biological canonical variable indicate that these properties were similar in the studied areas and were therefore considered suppressor properties, with low discrimination capacity of areas.

In the summer, the first canonical correlation between physical and microbiological properties was 0.86 ($p<0.0001$), whereas the microbiological variation was mostly explained by the first canonical variable of physical and chemical properties (0.73) (Figure 6b). This high value of canonical correlation (0.86) between microbiological and physical-chemical properties is a result of the higher CC values of the microbiological (DHA, CO_2-C and qCO_2) and the chemical properties (pH, H+Al, Al, org-C, and Mg) (Table 3).

The first biological canonical variable in summer was similar to that observed in winter, since the standardized canonical coefficients (SCC) were higher for the microbiological attribute DHA, followed by soil respiration CO_2-C (Table 3). In addition, the canonical correlation (CC) was highest for DHA (0.60). The positive signs of DHA, CO_2-C and qCO_2 indicate a high DHA and microbial respiration in the areas, most marked in summer. In the first physical-chemical canonical variable, SCC was highest for the soil properties pH, organic-C, Mg, moisture and litter C content, indicating a direct relation with the microbiological properties (Table 3).

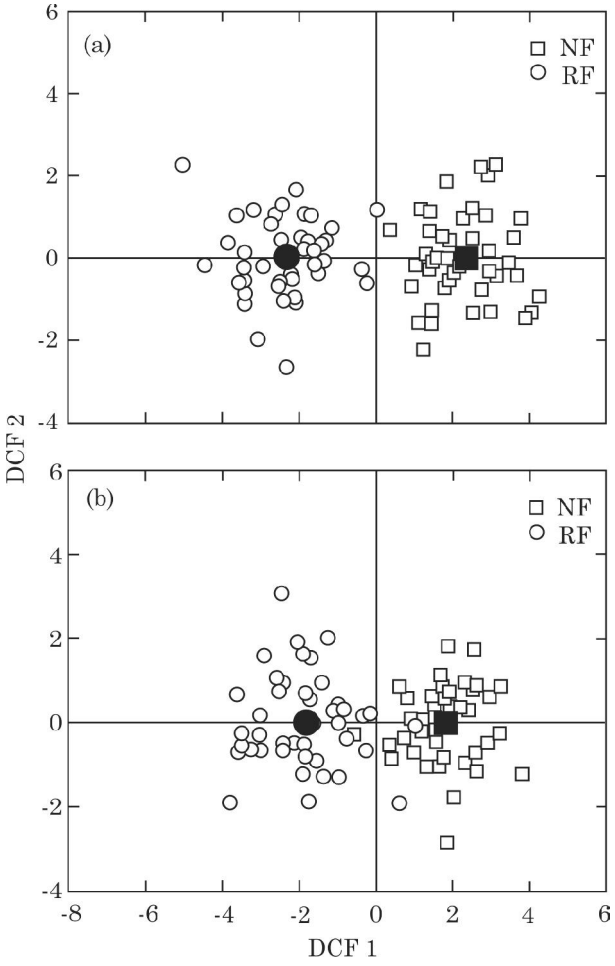


Figure 7. Standardized canonical coefficients (SCC) of canonical discriminating function 1 and 2 (DCF1and DCF2), discriminating native *Araucaria* forest (NF) and reforested area (RF), considering the microbiological and physical-chemical soil properties and chemical litter properties of samples collected in winter (August 2009) (a) and summer (February 2010) (b). São Paulo, Brazil. The full symbol represents the average value of SCC (centroid) for each area (n=45).

Canonical Discriminant Analysis (CDA)

The individual contribution of each property to the area differentiation was expressed by the parallel discrimination rate coefficient (PDRC), which is the product of the standardized canonical coefficients (SCC) by the respective canonical correlation (*r*) (Baretta et al., 2010). Thus, the coefficient of PDRC is considered the most suitable parameter to evaluate the effect of discrimination by soil properties analyzed in the areas. Positive PDRC values indicate effects of separation between the areas, that is, differences in the analyzed property, while negative values indicate similarities (Cruz-Castillo et al., 1994; Baretta et al., 2010). The *r* values reflect univariate information and show the isolated contribution of each property analyzed in the separation of the areas studied (Table 4 and Figure 7).

In general, the PDRC value of each property was different for each sampling time (Table 4). In this case, only variables with PDRC > 0.1 were discussed, which can be considered good quality indicators to distinguish areas (Baretta et al., 2010).

Regardless of the sampling time (winter and summer), the properties available P (0.162 and 0.138), moisture (0.304 and 0.237), S litter (0.116 and 0.178) and total porosity (0.152 and 0.236) were mainly responsible for the discrimination between areas in both seasons, due to their higher PDRC (parallel discrimination rate coefficient) values (Table 4). The microbial basal respiration also contributed in winter (0.111) and potential acidity (H+Al) in summer (0.121) (Table 4).

The first canonical discriminating function (DCF1) clearly separated the NF from the RF, with higher SCC values. The polarized distribution of the SCC values confirmed the high dissimilarity in the microbiological and physical-chemical properties between the areas (Figure 7a,b).

Soil moisture was the most discrepant property (highest PDRC) in the areas of discrimination, regardless of the season, since this property is greatly influenced by environmental conditions (temperature and rainfall) and vegetation cover, with little variation in less impacted areas (Zornoza et al., 2007). In addition, changes in vegetation cover induce changes in the residues that constitute the litter, as observed in winter in NF, where higher levels of nutrients such as N and P were related to a better litter quality and quantity produced in NF (Table 2). The level of available P in the soil was also relevant in the discrimination of the areas (Tables 2 and 4). Phosphorus, together with N, is considered one of the most limiting elements for biological activity, especially in tropical soils, where P and N availability are naturally low. In ecosystems where P fixation in the solid phase is possible, the conditions given by the chemical-physical characteristics of soil colloids, the pH, cations associated with phosphate (Ca, Al and Fe), and a low organic matter content are often a reason for difficulties in this nutrient cycling, as a result of changes in the type of management or land use, as in the case of reforestation with *Araucaria* (Table 2).

Total porosity was an important property in the discrimination of the areas (Table 4). Normally, porosity is related to the oxygenation capacity and moisture retention of the soil, determining the conditions for the development of organisms and the root system as well as for the functioning of the soil chemical and biological processes (Dexter, 1988). Although relevant in the discrimination of the areas, in this study total porosity was not correlated with other physical-chemical and microbiological properties,

Table 4. Correlation Coefficients (r), Standardized Canonical Coefficients (SCC) and Parallel Discriminating Rate Coefficient (PDRC) of first Canonical Discriminating Function 1 and 2 (DCF1 and DCF2) discriminated the native *Araucaria* forest (NF) and reforested areas (RF), considering the microbiological and physical-chemical of soil and chemical litter properties; samples taken in the winter (August 2009) and summer (February 2010) (n=45)

Environmental variable	DCF1 - Winter			DCF1 - Summer		
	r	SCC	PDRC	*r*	SCC	PDRC
pH ($CaCl_2$)	-0.076	-0.812	0.062	-0.050	-1.089	0.054
H+Al	-0.052	-0.856	0.044	-0.088	-1.367	0.121
K	0.214	0.385	0.082	0.174	0.477	0.083
Al	-0.030	0.440	-0.013	-0.136	0.598	-0.081
Organic carbon	-0.050	0.008	0.000	-0.030	0.092	-0.003
P available	0.214	0.760	0.162	0.285	0.483	0.138
Mg	0.017	0.085	0.001	0.062	-0.078	-0.005
Mass of dry Litter	-0.006	0.196	-0.001	0.133	0.190	0.025
Moisture	-0.435	-0.698	0.304	-0.388	-0.612	0.237
Litter C	0.099	-0.763	-0.075	0.131	-0.624	-0.082
Litter N	0.329	0.228	0.075	0.405	-0.013	-0.005
Litter S	0.212	0.550	0.116	0.277	0.643	0.178
Bulk density	0.073	-0.359	-0.026	0.091	-0.654	-0.060
Total porosity	-0.229	-0.665	0.152	-0.287	-0.824	0.236
Macroporosity	0.140	0.203	0.028	0.175	0.384	0.067
Microbial biomass carbon	0.071	1.103	0.079	0.188	0.300	0.056
Soil basal respiration	-0.088	-1.264	0.111	-0.038	-0.420	0.016
Metabolic quocient (qCO_2)	-0.202	0.511	-0.103	-0.256	-0.132	0.034
Desidrogenase (DHA)	-0.160	-0.012	0.002	-0.112	0.097	-0.011

but its importance for soil microbiota has already been emphasized elsewhere (Schoenholtz et al., 2000; Idowu et al., 2008).

An analysis of the biological variables showed that they contributed little to the discrimination of the areas in the two seasons, with the exception of basal respiration in winter (Table 4). Thus, although the CDA demonstrated the difference between the two areas, mainly by means of the physical-chemical variables discussed above, little could be shown by the microbiological variables, given their limited influence to discriminate the areas in both seasons. The microbiological activity of the reforested area was shown to be similar to that in the native *Araucaria* forest, probably resulting from a metabolic redundancy of micro-organisms as well as the similar organic matter content in the two ecosystems. However, we emphasize the importance of an integrated assessment of physical-chemical and microbiological soil properties, for a more accurate assessment of soil quality, in view of the close interaction between them (Sparling et al., 2004; Baretta et al., 2008).

The small differences in microbiological activity observed between RF and NF suggest that not only the time in the replanted Araucaria area, but also the presence of other plant species had contributed to a recovery of the soil microbial community in RF, since the location of the planted *Araucaria* forest, inserted in Atlantic forest, favored the development of other plant species, thus contributing to a recovery of the soil microbial community in RF, in spite of the discrepant physical-chemical conditions of the soil.

Another important aspect is the contribution of organic carbon content of soil and leaf litter, stimulating soil microbiota, aside from other factors such as soil pH. These are key parameters determining the soil biota, rather than the variables with lower canonical correlation, since these parameters contribute to more accurate interpretations from the microbiological point of view of soil health and recovery processes.

The above discussion reinforces the need to examine the relationships between physical-chemical and microbiological properties for a better understanding of a distinction of the two systems studied. For example, the activity of some enzymes such as dehydrogenase, evaluated together with soil basal respiration, microbial biomass and some physical-chemical parameters, may contribute to the interpretation of the functional status of different ecosystems (Matsuoka et al., 2003; Baretta et al., 2008)

Since to date there are no publications in Brazil addressing the physical-chemical and microbiological

properties in the same sampling area and using true replications, our results can be important for the formulation of new hypotheses about the quality-related soil properties that best assess this quality when used together, due to relations between physicochemical and microbiological properties, as demonstrated in this study.

CONCLUSIONS

1. Among the microbiological properties analyzed individually, the metabolic quotient was the most sensitive indicator to assess the state of conservation of forests, regardless of season, although microbial biomass carbon and dehydrogenase activity in summer and winter, respectively, were also clearly discriminating.

2. There were significant correlations between the soil chemical and microbiological properties, particularly with regard to dehydrogenase activity and basal respiration, for the microbial properties, and for pH and H+Al with regard to the chemical properties, in winter, whereas organic carbon, soil moisture, soil Mg, pH and litter carbon were most closely correlated with dehydrogenase activity and respiration, in summer.

3. The canonical correlation analysis (CCA) identified the properties available P, total porosity, litter S content and soil moisture as the most sensitive to discriminate the native *Araucaria* forest (NF) from the areas of *Araucaria* reforestation (RF), of which soil moisture was the most relevant, regardless of the sampling season.

4. Evaluating the results of CCA and canonical discriminant analysis together, similar microbiological activity was observed in NF and RF, while the differences in physical and chemical properties indicated a recovery in RF, mainly due to the formation of a soil cover and increasing plant diversity in this area.

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