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Eucalyptus grandis and *Acacia mangium* in monoculture and intercropped plantations: Evolution of soil and litter microbial and chemical attributes during early stages of plant development

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ABSTRACT

Soil microorganisms and microbial processes are influenced by the quality and quantity of plant waste entering the soil, by its seasonal and spatial distribution, by the ratio of above- to below-ground inputs, and by changes in nutrient inputs. Soil management strategies sometimes promote mixed-species plantations to mitigate the loss of soil nutrients and improve biogeochemical cycling. The objective of this study was to explore changes in microbiological and chemical attributes of soils and litter in the early stages of the second rotation of mixed and pure plantations of *Eucalyptus grandis* and *Acacia mangium*, and to look for correlations between attributes. Soil samples at 0–10 cm depth were collected two, seven, 14, and 20 months after planting in the following treatments: monocultures of *A. mangium* and *E. grandis*, a monoculture of *E. grandis* with N-fertilizer, and an intercropped plantation with *E. grandis* and *A. mangium*. Microbial soil attributes varied dramatically between treatments 20 months after planting. Total C, N and P contents in litter showed the strongest correlations with microbial biomass C and N (C_{mic} and N_{mic}), microbial respiration, and dehydrogenase activity in all sampling periods. Lower C/N and C/P ratios in litter and lower C/N and C_{mic}/tC ratios in soils after 20 months in the intercropped plantation illustrated the system's capacity for supplying inputs of high-quality organic matter rich in N and P, but this did not result in higher contents of these elements or greater microbial activity in soils. An implication of this finding is that, at least in the initial growth phase of these plantations, chemical attributes of the litter and variation in those attributes govern microbial processes and, consequently, are mostly responsible for plant development. Canonical discriminant analysis revealed changes in the microbiological and chemical attributes of soil in the intercropped plantation due to the plants growth and the leaf litter accumulation. Twenty months after planting, the different plantations could be discriminated by differences in litter chemistry (C, N, and P), total soil C, N_{mic}, and dehydrogenase activity, which were very similar in intercropped plantations and *E. grandis* with N-fertilizer. These results from the early stages of plantation development are important for understanding the dynamics of soil attributes in these systems, and especially in intercropped plantations. In intercropped areas the cumulative effect of microbial attributes reflects a more sustainable system. Long-term studies are needed to identify patterns that develop after 20 months, during the growth period of these plantations.

1. Introduction

As decomposers in the food chain, microorganisms play a key role in the formation, maintenance, and cycling of nutrients, as well as in the bioremediation of ecosystems. Their sensitivity to changes in humidity, temperature, and nutrient availability (Fierer et al., 2003; Sinha et al., 2009) makes them good indicators of soil quality and health (Bastida et al., 2006; Nogueira et al., 2006). However, other parameters that vary with local vegetation can also regulate microbial activity in soils; these include C and nutrient contents, and the quality of organic matter in the litter (Bonanomi et al., 2010). There is increasing interest in studying the relationship between plant diversity and ecosystem function, since it is well known that biodiversity strongly affects functions

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such as productivity and stability (McGrady-Steed et al., 1997; Tilman et al., 2001). Understanding these relationships is especially important for commercial forestry systems involving multiple tree species. Novel management schemes require careful research to maintain production levels, reduce the need for fertilizers, and develop higher-quality organic matter. For example, some foresters have proposed planting eucalyptus trees together with leguminous species (e.g., *Acacia mangium*) in order to circumvent the problems posed by depleted N and P fixation (Forrester et al., 2004), especially in tropical soils (Bouillet et al., 2008; Jourdan et al., 2008; Laclau et al., 2008).

Generally, *Eucalyptus grandis* is a fast-growing tree with clear-felling estimated in Brazil at six to seven years for cellulose and 12–14 years for timber. Other advantages, such as high species diversity and good adaptation to various regions and climates have led to the common use of *Eucalyptus* trees in reforestation. While *E. grandis* is considered to have high commercial value, its drawbacks include high nutrient exportation and depletion of N and P stocks with successive rotations. These factors can decrease productivity, since the species shows little to no response to N fertilization (Gonçalves et al., 1997). *A. mangium* has become one of the leading candidates for intercropping with eucalyptus in Brazil. According to studies, *A. mangium* rapidly accumulates biomass, is stable and highly competitive (Coelho et al., 2007; Laclau et al., 2008). *A. mangium* also forms symbioses with N-fixing soil bacteria and mycorrhizal fungi and is commonly used to restore degraded areas (Mendes-Filho et al., 2009), in addition to being commercially valuable. Its leaves can be used as animal fodder and its wood is valued in the paper, cellulose and particleboard industries and can be used for charcoal, firewood, and other products (Xie and Hong, 2002).

Various studies in Australia (Khanna, 1997; Forrester et al., 2004), Africa (Traoré et al., 2007; Isaac et al., 2011), Asia (Khanna, 1998; Yamashita et al., 2008), and Brazil (Bouillet et al., 2008; Jourdan et al., 2008; Laclau et al., 2008; Voigtlaender et al., 2012) have described patterns of tree growth, plant biomass, nutrient content, root development, biological N fixation, and soil water content in monoculture and intercropped plantations of *Eucalyptus* and *Acacia*. While these studies illustrate the advantage of mixed forest systems, they also raise many questions regarding biogeochemical soil processes. For example, how do intercropped plantations affect and interact with soil microbes? Soil microbial activity is closely related to biogeochemical cycling, which is crucial for the sustainability and growth of forestry systems.

The conversion of organic N and P to inorganic or available forms is related to microbial groups and processes, which play a central role in the ecosystem. Microbial biomass represents the labile fraction of soil organic material, which is naturally dynamic and easily modified by biotic and abiotic factors. Its sensitivity to disturbance and changes in land management is well documented, and it is considered an excellent indicator of soil quality (Nogueira et al., 2006; Kaschuk et al., 2010). Quantifying C and N in soil microbial biomass is an attractive alternative to quantifying total C and N in soils. Soil chemistry attributes often change more slowly and are much less sensitive to disturbance than microbiological attributes (Dick and Tabatabai, 1993). However, soil chemistry attributes (e.g., total C, total N, and available P) are important for correlation studies with soil microbial data, especially when the goal is to evaluate different management and planting strategies. In this case, the microbial coefficient, defined as the ratio between the microbial biomass C and total soil C (C_{mic}/tC), is a valuable indicator of organic material quality in an ecosystem (Wardle, 1992). Also, indicators of microbial activity such as basal respiration (microbial respiration), dehydrogenase activity, and metabolic coefficient (qCO_2) reflect the oxidative capacity and efficiency of the metabolism of soil microorganisms, and are often correlated with the quantity and quality of organic matter (Madritch and Hunter, 2003; Nogueira et al., 2006; Sinha et al., 2009; Prescott, 2010).

The theoretical rationale for intercropping plantations with leguminous plants is to provide greater inputs of N and P into soils, via higher quality and more diverse plant litter. Microbiological factors play an important role in maintaining the biogeochemical cycling of these elements, both through decomposition and as a sink and/or source of soil C and nutrients (Kaschuk et al., 2010). The objective of this study was to document how soil chemical and microbial attributes vary during the early stages (up to 20 months after planting) of different forestry management systems, with a special focus on C, N and P in soil and litter, in order to learn more about their dynamics and sustainability.

2. Materials and methods

2.1. Site description

The study was carried out at the Itatinga Forestry Sciences Experimental Station (23°02′01″S 48°37′30″W, ~830 m), which is managed by the Forestry Sciences Department of the Escola Superior de Agricultura Luiz de Queiroz, in the municipality of Itatinga, São Paulo state, Brazil. The coldest, driest months are June and July (means of 16 °C and 45 mm rainfall, respectively) and the warmest, rainiest months are December and January (means of 24 °C and 360 mm, respectively). The soil in this area has been classified as Ferralsol (FAO classification), with 84% sand and 12% clay. After the first clear-felling the soil contained 23 g organic matter and 11 and 52 $mmol_c$ Al^{3+} and CEC per kg soil, respectively.

The experiment was established in an area previously planted with *Eucalyptus saligna*, managed as a coppice without fertilizer application, during the period 1940–1998. After clearing the vegetation at the site, seedlings of *E. grandis* were planted in 1998 with a relatively small amount of fertilizer (300 kg ha^{-1} of NPK 10:20:10), and the adult trees were harvested in December 2002. A complete randomized block design was established in May 2003 and the trees were clear-felled in May 2009. This period corresponds to the first rotation of monocultures and intercropped plantations of *E. grandis* and *A. mangium*. Each plot measured 30 m × 30 m, in which only an inner plot of 18 m × 18 m with two buffer rows was considered for sampling. A complete description of the experimental layout is given by Laclau et al. (2008).

2.2. Experimental design

The same experimental design of the first rotation was used for the second, with the treatment layout of the second rotation following exactly the same position of the first one. Our study covered the period of the second rotation of monocultures and intercropped plantations of *A. mangium* and *E. grandis*, spaced 3 m × 3 m, installed in the same area in November 2009 (minimum-tillage system). The experiment had a completely randomized block design with three blocks and the following treatments: a monoculture of *A. mangium* (treatment A) (0E:100A); a monoculture of *E. grandis* (100E:0A) (treatment E) with no N-fertilizer; a monoculture of *E. grandis* with application of 120 kg ha^{-1} N (100E:0A) (treatment EN); and an intercropped plantation of *E. grandis* and *A. mangium* (50A:50E) with no N-fertilizer. This intercropped area was subdivided such that some soil samples were sampled around *A. mangium* trees (treatment $A_{(A+E)}$) and others around *E. grandis* trees (treatment $E_{(A+E)}$), in order to compare the rhizosphere effects of both species within this treatment.

2.3. Soil and litter sampling

For all treatments, we sampled soil at a depth of 0–10 cm in four sampling periods: two in the dry season (July 2010/June 2011) and two in the rainy season (January 2010/December 2010). These sampling periods correspond to two, seven, 14, and 20 months after the trees were planted.

Nine representative trees were randomly selected in each plot (treatment). At each tree three soil subsamples were taken at different positions around the trunk (between the rows) at a maximum distance of 1.5 m from the base. Each composite sample was comprised of nine independent subsamples (one from each tree). We ended up with three composite soil samples per plot. Three composite samples were collected from the monoculture treatments and six from the mixed plantation (three close to *E. grandis* and three close to *A. mangium*) (50E:50A). Thus, for each sampling period there were nine composite samples for each treatment. These samples were sieved and stored at 4 °C for up to six days before microbial and biochemical analyses. For chemical analyses we used air-dried soils. Litter samples were taken inside nine 0.25 m^2 squares at the same sampling points. The sampled litter included twigs, branches, and leaves, and was oven-dried at 45 °C and weighed to estimate dry mass, for a total of nine samples per plot.

2.4. Analytical methods

Total C and N contents in litter and in soils were quantified in an elemental analyzer. Total P content in litter was quantified following Murphy and Riley (1962). C, N, and P concentration in litter were standardized based on an analysis of ash content determined after ignition at 550 °C until a constant weight was reached. Available P in soils was extracted using the ion-exchange resin method described by Van Raij et al. (2001). Soil pH was determined in a soil suspension with 0.01 mol L^{-1} $CaCl_2$ at a 1:2.5 soil:solution ratio.

Microbial biomass C and N were estimated by the fumigation–extraction method following Vance et al. (1987). Microbial biomass C (C_{mic}) was calculated based on the difference between the C in the fumigated and non-fumigated samples, using a K_C factor of 0.40 (Roscoe et al., 2006). Microbial biomass N (N_{mic}) was determined in a Kjeldahl distiller (Bremner and Mulvaney, 1982) using a K_N factor of 0.54 (Kaschuk et al., 2010). We determined dehydrogenase activity following Casida et al. (1964), where 5 g of field-moist soil samples were incubated with 5 mL of 1% triphenyl tetrazolium chlorine (TTC) solution, and incubated at 37 °C for 24 h. After incubation, the triphenyl tetrazolium formazan (TTF) was extracted with methanol, filtered, and the red color in the extract was read with a spectrophotometer at 485 ηm. Basal respiration was measured following Alef (1995), during 21 days of incubation. The ratio of the basal respiration and C_{mic} data was used to estimate the qCO_2. The microbial coefficient was calculated from the ratio of the C_{mic} and tC (C_{mic}/tC) and of the ratio of the N_{mic} and tN (N_{mic}/tN).

2.5. Statistical analyses

We used analysis of variance (ANOVA), and compared means with Duncan's test ($p < 0.05$) and Pearson's correlation test. Soil chemistry and microbiological data for soils and litter were subjected to canonical discriminant analysis (CDA) in order to identify which attributes were most important for distinguishing between the sampled areas, and the areas were compared with the LSD test ($p < 0.05$).

3. Results

In most cases soil water content was lowest in the *E. grandis* monoculture (E) and highest under *A. mangium* (A and $A_{(A+E)}$) (Table 1). Soil pH and total C and N in soils showed little variation between sampling up to 14 months and among treatments. Total C in soils differed strongly between treatments after 20 months, when the lowest C concentrations were observed in the intercropped plantation ($A_{(A+E)}$ and $E_{(A+E)}$) (Table 1). The C/N ratio in soils remained similar up to 14 months after planting but, at 20 months, $A_{(A+E)}$, $E_{(A+E)}$, and A showed lower C/N ratios than the *E. grandis* monocultures (E and EN) (Table 1). Available P was highly variable within treatments. After 20 months available P was highest in the EN treatment, but it differed only from the $A_{(A+E)}$ value (Table 1).

Litter C varied little across the four sampling periods in the intercropped plantation and in the *A. mangium* monoculture. By contrast, E and EN showed increased C concentration in litter after seven and 20 months (Table 2). N concentration in litter varied strongly between treatments and sampling periods, and was highest in A. However, the presence of *A. mangium* in the intercropped plantation did not lead to significantly higher litter N than the *E. grandis* monocultures (Table 2). P concentration in litter was higher in $A_{(A+E)}$ and $E_{(A+E)}$ than in EN for almost all sampling periods. After 14 months the P concentration in litter of the intercropped plantation was approximately twice as high as that in the monocultures (A, E, and EN) (Table 2). The *E. grandis* monoculture showed the highest C/N and C/P ratios in litter for all sampling periods. The C/N ratio in litter showed a consistent pattern between treatments for all sampling periods, as follows: $E > EN > E_{(A+E)} > A_{(A+E)} > A$. By contrast, the C/P ratio in litter declined in all treatments until 14 months after planting (Table 2).

In all treatments, the highest respiration values observed in the four sampling periods were observed after 14 and 20 months. The lowest CO_2 emissions in all treatments occurred at seven months. Between treatments there was an inversion of CO_2 emission by microorganisms as plants grew (Fig. 1). This resulted from the fact that the high CO_2 emissions in the early stages in A (at two and seven months) were not maintained 20 months after planting. However, CO_2 emissions were low in the early stages of the intercropped plantation (at two and seven months), but increased in the later stages (at 14 and 20 months), reaching levels that were intermediate among the other treatments (Fig. 1). Dehydrogenase activity also changed as the trees grew (Fig. 1). In the early stages (at two and seven months) activity was highest in A and lowest in $A_{(A+E)}$ and $E_{(A+E)}$, but at 20 months activity was highest in $A_{(A+E)}$ and $E_{(A+E)}$.

Microbial biomass C and N (C_{mic} and N_{mic}, respectively) increased up to 14 months after planting in all treatments (Fig. 1), and declined in the samples taken after 20 months. At seven and 14 months $A_{(A+E)}$, $E_{(A+E)}$ and A showed higher C_{mic} than E and EN (Fig. 1). N_{mic} was highest in A in almost every sampling period (Fig. 1). In $A_{(A+E)}$ and $E_{(A+E)}$ there was high N_{mic} only at 2 and 7 months. The highest qCO_2 values were observed seven and 14 months after planting for all the treatments (Fig. 2). Between treatments the C_{mic}/tC and N_{mic}/tN ratios showed high variation along the periods, especially after seven and 20 months, when $A_{(A+E)}$ and $E_{(A+E)}$ showed the highest C_{mic}/tC ratio (Fig. 2).

Correlation analysis revealed stronger relationships between microbial biomass, respiration, and dehydrogenase enzyme activity and levels of C, N, and C/N in litter than between these variables in soil, especially at two and 20 months (Table 3). N_{mic} was the biochemical variable most strongly correlated with physical and chemical attributes in soil and litter, and showed an especially strong correlation with the C/N ratio in litter for all sampling periods.

Table 1
Soil chemical attributes under monoculture and intercropped cultures of *E. grandis* and *A. mangium* plantations. A (*A. mangium*), E (*E. grandis*), EN (N-fertilized *E. grandis*) and an intercropped plantation of *A. mangium* ($A_{(A+E)}$) and *E. grandis* ($E_{(A+E)}$).

	Period	A	E	EN	$A_{(A+E)}$	$E_{(A+E)}$
Moisture (%)	2 months	11.5 (0.7)* a	10.7 (0.7) a	11.2 (0.8) a	11.2 (1.0) a	11.3 (1.1) a
	7 months	8.2 (0.9) b	7.8 (0.9) c	9.0 (1.2) ab	9.3 (0.5) a	7.9 (0.5) b
	14 months	8.9 (0.8) a	7.5 (0.6) c	7.8 (0.8) bc	9.1 (1.4) a	8.6 (1.0) ab
	20 months	8.1 (0.9) a	6.9 (1.1) b	7.1 (1.1) ab	7.4 (1.2) ab	7.1 (1.1) ab
pH ($CaCl_2$)	2 months	3.7 (0.1) a	3.7 (0.1) a	3.7 (0.1) a	3.6 (0.1) a	3.7 (0.2) a
	7 months	3.8 (0.3) a	3.8 (0.1) a	3.7 (0.1) ab	3.6 (0.1) b	3.7 (0.1) ab
	14 months	3.8 (0.04) d	4.0 (0.02) c	4.1 (0.03) b	4.0 (0.08) c	4.3 (0.05) a
	20 months	3.9 (0.1) b	4.0 (0.1) a	4.0 (0.04) a	3.9 (0.02) b	3.9 (0.03) b
Total C (%)	2 months	4.1(1.1) a	3.7 (0.5) a	4(0.7) a	4.3 (0.6) a	4.1 (0.9) a
	7 months	4.5 (0.8) a	3.6 (0.4) b	4.2 (0.8) ab	4.2 (0.8) ab	4.4 (0.8) a
	14 months	5.0 (2.1) a	4.5 (0.4) a	4.6 (0.5) a	4.5 (1.1) a	4.7 (0.9) a
	20 months	4.5 (0.9) a	4.2 (0.5) ab	5(0.7) a	3.5 (1.0) b	3.5 (1.1) b
Total N (%)	2 months	0.20 (0.05) a	0.17 (0.02) a	0.19 (0.03) a	0.21 (0.03) a	0.20 (0.04) a
	7 months	0.19 (0.04) ab	0.15 (0.02) c	0.16 (0.02) b	0.20 (0.04) a	0.19 (0.04) ab
	14 months	0.23 (0.07) a	0.20 (0.02) a	0.20 (0.01) a	0.20 (0.05) a	0.20 (0.04) a
	20 months	0.25 (0.02) a	0.20 (0.02) a	0.25 (0.02) a	0.23 (0.04) a	0.21 (0.01) a
C/N	2 months	21 (1.2) a	21 (2.0) a	21 (1.0) a	21 (1.8) a	20 (2.2) a
	7 months	23 (2.2) ab	23 (2.8) ab	26 (4.2) a	21 (1.7) b	23 (3.1) ab
	14 months	21 (1.8) a	23 (1.1) a	23 (1.9) a	22 (1.0) a	23 (1.3) a
	20 months	18 (1.6) bc	22 (2.4) a	20 (1.7) b	15 (3.4) c	18 (2.6) bc
Available P ($\mu g\,g^{-1}$)	2 months	4.8 (0.4) c	4.9 (0.3) b	5.5 (0.7) a	5.7 (0.7) a	5.4 (0.7) ab
	7 months	5.7 (0.9) a	5.8 (1.0) a	5.8 (0.7) a	5.3 (0.8) a	5.8 (0.7) a
	14 months	5.5 (0.7) a	5.8 (1.0) a	5.8 (0.7) a	5.6 (1.5) a	5.7 (0.7) a
	20 months	5.3 (0.5) ab	5.2 (0.4) ab	5.8 (0.4) a	4.9 (0.8) b	5.1 (1.0) ab

* Line means sharing the same letter are not statistically different (Duncan $p < 0.05$). Values in parentheses correspond to standard deviation.

CDA in the first and second canonical discrimination functions (CDF1 and CDF2) showed a strong canonical correlation for all the sampling periods (Fig. 3). The highest values were used to better explain the variation in the biological, physical, and chemical variables. These variables showed significant values in the multivariate statistical Wilk's Lambda test, Pillai's test, the Hotelling-Lawley test, and Roy's largest root test due to the low colinearity between them and the highly significant differences between areas ($p < 0.0001$) (data not shown).

The CDA scatterplot at two months shows three well-defined clusters: (1) A, (2) EN and E, and (3) $A_{(A+E)}$ and $E_{(A+E)}$ (Fig. 3A). The individual contribution of each variable in the distribution of the clusters is expressed by the parallel discrimination coefficient (PDC), since positive values greater than 0.1 are considered highly relevant (Baretta et al., 2010). The biological variable dehydrogenase activity and the chemical variables N and P in litter and available P in soils were the most important for separating clusters, especially for CDF1 (Table 4). In the case of CDF2, CO_2 evolution and C_{mic} were the biological factors that helped separate clusters. At seven months there were four well-defined clusters: (1) A, (2) E, (3) EN, (4) $A_{(A+E)}$, and (5) $E_{(A+E)}$ (CDF1) (Fig. 3B). In this sampling period biological variables such as CO_2 evolution, N_{mic}, and dehydrogenase

Table 2
Litter C, N and P under monoculture and intercropped cultures of *E. grandis* and *A. mangium* plantations. A (*A. mangium*), E (*E. grandis*), EN (N-fertilized *E. grandis*) and an intercropped plantation of *A. mangium* ($A_{(A+E)}$) and *E. grandis* ($E_{(A+E)}$).

	Period	A	E	EN	$A_{(A+E)}$	$E_{(A+E)}$
Total C (%)	2 months	54.9 (13.7)* a	52.1 (9.3) a	52.3 (7.1) a	50(6.9) a	52.3 (4.7) a
	7 months	54.5 (7.0) b	70.8 (6.0) a	75.8 (6.8) a	55.6 (3.0) b	56.4 (6.2) b
	14 months	56.1(5.6) b	54.1 (2.4) b	61.8 (3.4) a	52.0 (5.0) b	53.7 (5.1) b
	20 months	57.6 (2.9) b	62.7 (4.7) a	63.8 (9.5) a	50.2 (3.6) c	51.6 (3.2) c
Total N (%)	2 months	2.2 (0.5) a	1.4 (0.4) b	1.4 (0.4) b	1.6 (0.4) b	1.5 (0.3) b
	7 months	2.5 (0.4) a	2.4 (0.1) ab	2.6 (0.3) a	2.2 (0.2) bc	2.1 (0.2) c
	14 months	2.6 (0.3) a	1.7 (0.2) c	2.0 (0.3) b	1.9 (0.2) bc	1.9 (0.2) bc
	20 months	2.6 (0.2) a	1.3 (0.1) c	1.6 (0.5) b	1.7 (0.1) b	1.5 (0.1) bc
Litter C/N	2 months	24(3.3) c	39(11.1) a	38(5.5) a	32(5.5) b	33(5.0) b
	7 months	21(1.3) c	29(2.8) a	30(1.5) a	25(2.1) b	27(2.8) a
	14 months	21(1.4) c	33(2.9) a	31(3.1) a	28(2.1) b	29(2.5) b
	20 months	21(0.7) e	48(5.3) a	41(1.9) b	28(1.6) d	34(1.2) c
Total P (%)	2 months	0.34 (0.04) b	0.28 (0.07) c	0.29 (0.06) c	0.41 (0.08) a	0.40 (0.06) ab
	7 months	0.47 (0.13) ab	0.53 (0.17) ab	0.57 (0.14) ab	0.69 (0.22) a	0.40 (0.10) b
	14 months	0.62 (0.06) b	0.64 (0.12) b	0.68 (0.08) b	1.23 (0.62) a	1.41 (0.60) a
	20 months	0.91 (0.04) a	0.50 (0.06) d	0.57 (0.08) c	0.79 (0.1) b	0.64 (0.05) c
Litter C/P	2 months	161 (32) a	193 (42) a	184 (29) a	129 (35) b	124 (22) b
	7 months	121 (24) ab	142 (35) a	140 (36) a	92 (41) b	148 (42) a
	14 months	91 (14) a	88 (18) a	91(6.9) a	57 (35) b	46 (24) b
	20 months	63 (2) c	121 (25) a	109 (10) a	67 (10) c	86 (20) b

* Line means sharing the same letter are not statistically different (Duncan $p < 0.05$). Values in parentheses correspond to standard deviation.

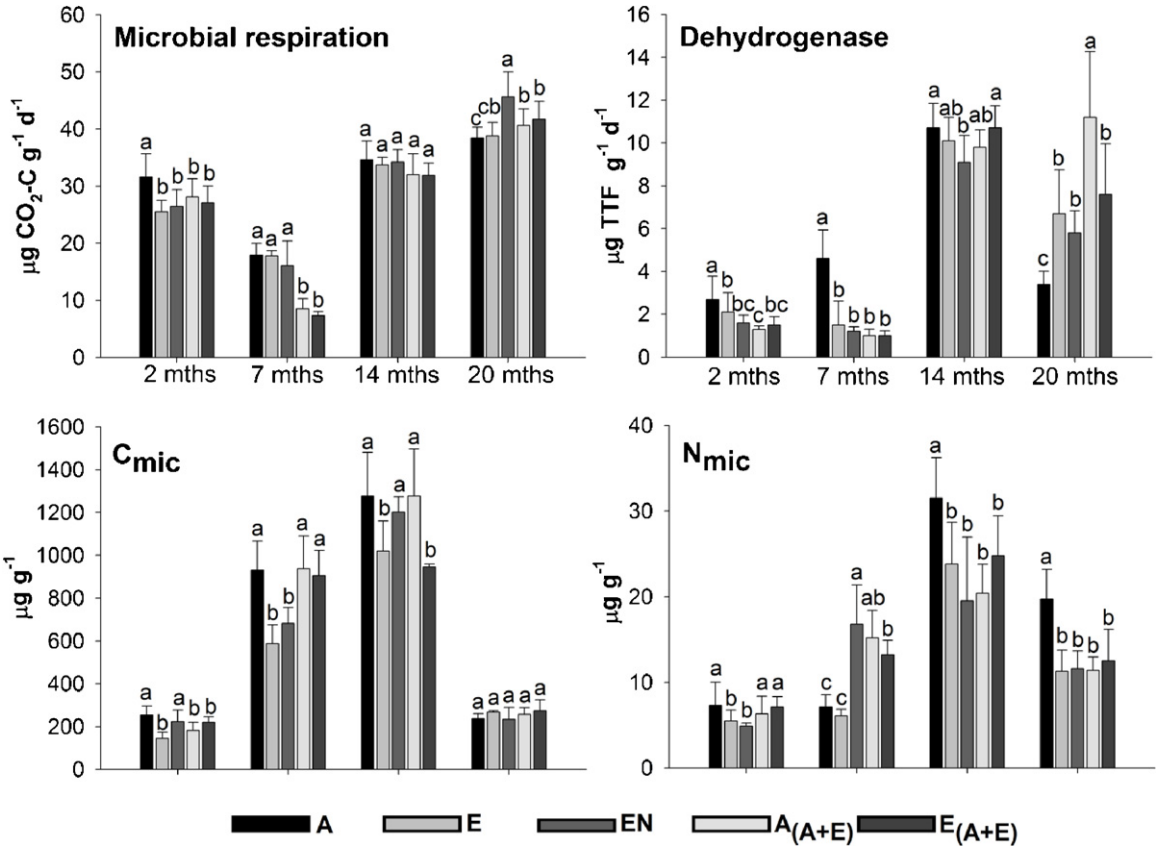

Fig. 1. Soil microbial attributes under monoculture and intercropped cultures of *E. grandis* and *A. mangium* until 20 months (mths) after planting. C_{mic} (C microbial biomass); N_{mic} (N microbial biomass); TTF (triphenyl tetrazolium formazan); A (*A. mangium*), E (*E. grandis*), EN (N-fertilized *E. grandis*) and intercropped plantation of *A. mangium* ($A_{(A+E)}$) and *E. grandis* ($E_{(A+E)}$). Means (with standard deviation) with same letter are not statistically different (Duncan $p < 0.05$).

activity strongly distinguished between treatments in CDF1. Dehydrogenase activity, C_{mic}, and C in litter were the best variables at separating clusters in CDF2. After 14 months biological variables were not the primary ones separating treatments (Fig. 3C); pH was the variable that best separated the four clusters in CDF1 (Table 4): (1) A, (2) E and $A_{(A+E)}$, (3) EN, and 4) $E_{(A+E)}$. According to the PDC values of CDF2, total N in soils and litter were responsible for separating treatments. Twenty months after planting the clusters were separated by C, N and P in litter for CDF1 and by N_{mic}, dehydrogenase, total C and C in litter for CDF2. Together, these variables distinguished five distinct clusters: (1) A, (2) $A_{(A+E)}$, (3) $E_{(A+E)}$, (4) EN, and (5) E (Fig. 3D).

4. Discussion

4.1. Plant development and microbial attributes

In general, plant development may have been responsible for variation in soil microbial variables in all the sampling periods. In this case, as the seedlings' root systems developed, a more

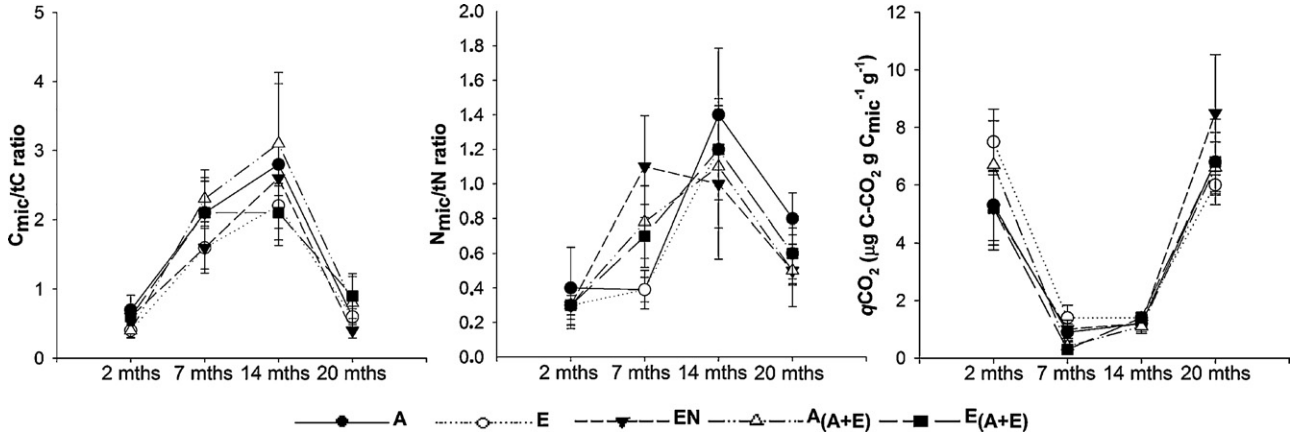

Fig. 2. Means (with standard deviation) of the evolution of the ratios: C microbial biomass/total soil C (C_{mic}/tC); N microbial biomass/total soil N (N_{mic}/tN) and metabolic coefficient (qCO_2) under intercropped and monoculture plantations until 20 months (mths) after planting. A (*A. mangium*), E (*E. grandis*), EN (N-fertilized *E. grandis*) and intercropped plantation of *A. mangium* ($A_{(A+E)}$) and *E. grandis* ($E_{(A+E)}$).

Table 3
Correlation coefficients between soil chemical, litter chemical and microbial properties at four periods.

	2 months				7 months				14 months				20 months			
	AR[a]	C_{mic}[b]	N_{mic}[b]	DASE[c]	AR	C_{mic}	N_{mic}	DASE	AR	C_{mic}	N_{mic}	DASE	AR	C_{mic}	N_{mic}	DASE
Moisture	–	–	–	–	–	–	–	–	–	–	–	–	–	−0.32*	–	–
pH	–	–	–	0.44*	–	–	–	0.46*	–	−0.35*	−0.29*	–	–	-	–	–
Soil C	–	–	–	–	-	0.32*	–	–	–	–	–	0.31*	–	–	–	–
Soil N	–	–	–	–	–	0.41*	–	–	–	–	0.35*	0.29*	–	–	–	–
Soil C/N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	−0.55***	–
Litter C	–	–	–	–	0.38*	−0.55***	–	–	0.38*	–	–	–	–	–	–	−0.38*
Litter N	0.49**	–	0.31*	–	0.51***	–	–	0.31*	–	–	0.41**	–	–	–	0.59***	−0.40**
Litter C/N	−0.36*	–	−0.34*	–	–	−0.48**	0.32*	−0.51***	–	–	−0.52***	–	–	–	−0.55***	–

[a] AR: accumulated basal respiration.
[b] C_{mic} and N_{mic}: carbon and nitrogen of microbial biomass, respectively.
[c] DASE: dehydrogenase.
* $p < 0.05$.
** $p < 0.001$.
*** $p < 0.0001$.

pronounced rhizosphere effect became apparent in the plantations. This was especially apparent at seven, 14, and 20 months, when root growth and the accumulation of senescent leaves certainly stimulated the soil microbiota. By contrast, two months after planting root development remained incipient, and much of the data recorded at that time reflects the previous plantation cycle, the resting time between cycles, and the management strategy (minimum tillage). For that reason, a knowledge of the plantation history and related details is vital for understanding the behavior of the soil microbiota in agroforestry systems (Varennes and Torres, 2011; Zhao et al., 2011), since rhizosphere effects of individual plants can favor or deter microbial activity in soils (Sinha et al., 2009).

4.2. Microbial biomass C and N

At seven and 14 months, C_{mic} and N_{mic} in soil of all treatments were higher than has been previously reported in the literature for soils planted with *E. grandis* and/or *A. mangium* (Traoré et al., 2007; Barreto, 2008; Xiong et al., 2008; Araujo et al., 2010; Kaschuk et al., 2010) (Fig. 1). However, the cited authors did not follow plant development from the seedling stage on, as we did. The changes with plantation age illustrate the capacity of microbial biomass to serve as a sink or source of soil C and soil nutrients, and reflect its sensitivity to environmental change (Nogueira et al., 2006; Kaschuk et al., 2010). In our study, levels of C_{mic} varied with

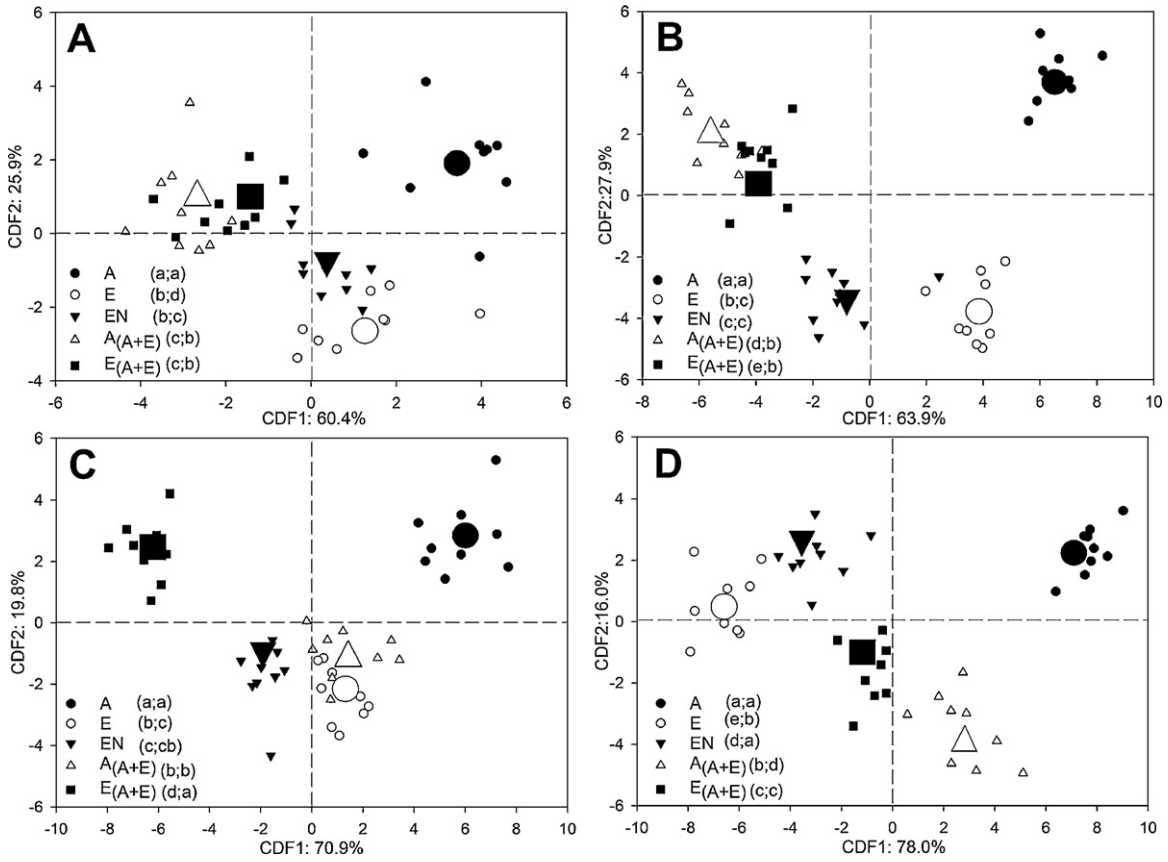

Fig. 3. Canonical discriminant analysis. A (2 months); B (7 months); C (14 months) and D (20 months). Large symbols represent the centroids of each area. A (*A. mangium*), E (*E. grandis*), EN (N-fertilized *E. grandis*), and intercropped plantation of *A. mangium* ($A_{(A+E)}$) and *E. grandis* ($E_{(A+E)}$). Same letters do not differ in columns by LSD test at $p < 0.05$, the first letter representing CFD1 and the second letter CDF2 (CDF1; CDF2).

Table 4
Parallel discrimination coefficient (PDC) in relation to the canonical discriminating function 1 (CDF2) and function 2 (CDF2), based on chemical and microbiological soil attributes in the four sampling periods.

	2 months		7 months		14 months		20 months	
	CDF1	CDF2	CDF1	CDF2	CDF1	CDF2	CDF1	CDF2
	Soil microbial attributes							
AR[a]	−0.044	0.228	0.481	0.055	0.017	0.000	−0.007	0.000
C_{mic}[b]	0.025	0.300	0.008	0.207	0.052	0.017	0.018	0.038
N_{mic}[b]	−0.001	0.079	0.245	−0.008	0.008	0.051	0.077	0.117
DASE[c]	0.345	−0.031	0.201	0.213	0.001	0.042	−0.006	0.432
	Soil chemical attributes							
Moisture	0.005	−0.009	0.034	0.000	0.006	−0.066	0.017	−0.019
pH	−0.023	0.001	−0.004	−0.005	0.965	0.013	0.016	0.013
C	−0.024	−0.018	−0.020	−0.013	0.001	−0.058	0.000	0.112
N	0.053	0.049	0.036	0.072	−0.017	0.149	0.046	0.028
Available P	0.241	0.002	0.001	0.001	0.001	−0.002	0.002	0.025
	Litter chemical attributes							
C	−0.009	−0.005	−0.014	0.519	0.002	0.075	0.140	0.148
N	0.164	0.266	0.021	−0.040	−0.011	0.744	0.440	0.082
P	0.269	0.137	0.011	−0.001	−0.024	0.034	0.257	0.024

[a] AR: accumulated basal respiration.
[b] C_{mic} and N_{mic}: carbon and nitrogen of microbial biomass, respectively.
[c] DASE: dehydrogenase.

plant development, acting as a carbon sink up to 14 months. We suggest that after 20 months cycling of C_{mic} predominated, acting as a source of C and other nutrients in the system. This indicates the importance of the soil microbiota and its dynamics, providing evidence that it must be considered the most sensitive and reactive organic matter, with faster cycling rates than soil C and N (Dick and Tabatabai, 1993; Kaschuk et al., 2010).

In addition, the rhizophere of *A. mangium* (A and $A_{(A+E)}$) showed the highest values of C_{mic} at seven and 14 months, indicating a positive correlation with higher levels of soil N, a lower C/N ratio in litter, and higher soil pH, suggesting that the presence of leguminous trees in the system improved conditions for microbial biomass development. Generally, microbial biomass is strongly affected by the rhizospheres of tree species growing in the system (Chabrerie et al., 2003; Sinha et al., 2009). Sinha et al. (2009) also reported that the quality and quantity of rhizodepositions specific to each tree species may be as important for the microbiota as climatic and soil conditions. In fact, climatic conditions did not show great influences on microbial and chemical attributes at 20 months for all treatments.

4.3. Soil and litter chemical attributes

In general, plant development and rhizosphere exudates showed an effect on soil microbial biomass, basal respiration, and dehydrogenase activity data. Generally, variation in levels of C, N, and P in soils and litter is associated with variation in microbial attributes (Badiane et al., 2001; Nogueira et al., 2006; Allison et al., 2006; Wang et al., 2010). However, total C and N and available P in soils did not vary strongly over the duration of our study (Table 1), and thus were only weakly correlated with microbiological attributes. Soil chemistry showed moderate changes over time, and a longer experiment might have revealed significant differences (Dick and Tabatabai, 1993). In this case, the fact that our plantations were at the establishment stage is reflected in the relatively low variation in soil chemistry and the relatively higher variation in microbial biomass and activity caused by the new conditions associated with management strategy and plant growth (Kaschuk et al., 2010). In this way, the variation of C, N, and P in litter between treatments and sampling periods offered valuable information on changes in soil microbial activity and biomass (Table 2). Some authors have reported a stronger relationship between microbial soil attributes and the content and quality of litter (Allison et al., 2006; Wang et al., 2008, 2010). This appears to be confirmed by our results (Table 3), which illustrate the need for additional studies on the soil microbiota–litter relationship.

The intercropped plantation showed lower total C in litter than the monocultures (A, E, and EN) from 14 months onwards (Table 2). By contrast, higher total P in litter was found in $A_{(A+E)}$ and $E_{(A+E)}$ up to 14 months after planting. These conditions resulted in lower C/N and C/P ratios in the litter than in EN and E. This indicates that *A. mangium* is responsible for the difference, since total C and P contents in litter of the *A. mangium* monoculture were equal to or greater than those in the intercropped plantation. These results disagree with those obtained by Khanna (1997), who did not find any increase in the litter P concentrations during the three first years of a mixed plantation of *Eucalyptus globulus* and *Acacia mearnsii*. On the other hand, other authors have reported an increase in the cycling and amounts of litter N and P in mixed plantations of *Eucalyptus* sp. and N-fixing leguminous trees (Binkley et al., 1992; Parrota, 1999; May and Attiwill, 2003; Forrester et al., 2005a). These data are indicative of a stimulus to recycle and make P available more rapidly due to the presence of legumes (Forrester et al., 2005a), besides favoring a decrease in the litter C/N ratio (Forrester et al., 2005b).

However, higher levels of total P in litter did not lead to higher levels of available P in soils, perhaps due to the mineralogical attributes of tropical soils, in which P is strongly fixed to Ca, Al, and Fe oxides. Special mechanisms for the uptake of P from litter and soils, such as modified roots and mycorrhizal fungi, may be especially important in these poor-soil systems (Vance et al., 2003; Mendes-Filho et al., 2009). However, the increased supply of P can be considered a future source of the nutrient since, with adequate management and in time, this adsorbed P may be slowly released into the soil solution in a form available to plants.

4.4. Basal respiration and litter C/N and C/P ratios

The lower C/N and C/P ratios in the litter of the intercropped plantation and the *A. mangium* monoculture would suggest that litter there is more susceptible to microbial degradation. However, this was not what we observed. Microbial respiration after 20 months was highest in the N-fertilized *E. grandis* monoculture (Fig. 1), which also had the highest C/N and C/P ratios. Recently

deposited *A. mangium* litter has a higher N concentration, and thus has also a lower C/N ratio. However, these are not the only characteristics that lead to faster breakdown of litter. Fresh litter of *A. mangium* contains significant amounts of tannins and lignin, which are difficult to breakdown (data not shown). Knowledge about the quality of plant waste is crucial for understanding microbial activity in soils, and it may be that the breakdown of leguminous plant waste is not significantly different from that of non-legumes (Sanborn and Brockley, 2009; Prescott, 2010). This may be the real reason to explain the slower biodegradation of *A. mangium* litter, although it is not exclusive to this species. According to Wedderburn and Carter (1999) decomposition of *Acacia melanoxylon* was slower than that of *Eucalyptus nitens*, due to a greater amount of lignin in the residues of this species. Nonetheless, these results depend on the legume species employed, since in Australia *A. mearsii* had a faster decomposition rate than *E. globulus* (Forrester et al., 2005b).

While N fertilization of plants with low lignin content facilitates degradation of plant waste, this may not be the case for N fertilization of plants with high lignin content, since fertilization may change the microbiota and lower the activity of enzymes that breakdown lignin and other recalcitrant compounds (Knorr et al., 2005; Zhang and Wang, 2012). Madritch and Hunter (2003) reported increased levels of microbial respiration in the presence of higher N content in litter and higher litter diversity, and this was also seen in the EN treatment and the intercropped plantation 20 months after planting. Eucalyptus leaves generally have fine, weakly lignified veins, which facilitates their breakdown (Barlow et al., 2007), especially in the presence of an easily available N source.

Twenty months after planting, the intercropped plantation showed intermediate levels of soil respiration, which suggests that the mixture of species and their effects via rhizodeposition and litter resulted in greater activity of soil microbes than in the non-fertilized monocultures (A and E). The same trend was observed for the dehydrogenase enzyme. It may be that measures of litter quality, such as lignin content or total phenols, explain patterns of soil microbial activity better than measurements of C, N, and P content in litter (Madritch and Hunter, 2003). We also noted a decrease in microbial activity (respiration and dehydrogenase) after seven months. While this could reflect reduced microbial activity during the dry season, that pattern would explain a broad decline across all treatments and not diminished activity between the pure and mixed plantation. In this case, the litter initially introduced into the soil may contain toxic defensive substances that inhibit microbial activity. It may be that the intercropped plantation had the lowest basal respiration values due to the mixed species litter. However, after 14 months, these effects may have been reduced, leading to increased decomposition due to greater interactions of microorganisms and available substrates, since microbial respiration increases significantly as organic material is added to the system (Geisseler et al., 2011).

4.5. Metabolic and microbial coefficients

qCO_2 values were good indicators of the efficiency of C use by the microbial biomass between sampling periods (Fig. 2). Values were high at two and 20 months, which, together with the low C_{mic}/tC ratios, indicates the inefficiency of microbial biomass and the poor quality of litter in these periods. At seven and 14 months the situation was inverted, reflecting a greater efficiency of C immobilization of the microbial biomass. This suggests the presence of a *K*-type microbial community (greater diversity of species, higher biomass and lower growth rates, as well as qCO_2 indices) (Insam, 1990). On the other hand, high qCO_2 suggests the presence of a *r*-type microbial community (fewer species with high growth rates and greater CO_2, as observed at two and 20 months after planting). In this case, differences in qCO_2 may indicate changes in the soil microbial community and activity (Sinha et al., 2009), in accordance with the initial development of the plants.

At 20 months the total C/N ratio in soils was lowest in the intercropped plantation and the *A. mangium* monoculture, which suggests that the organic matter deposited in those sites was converted by the soil microbiota more efficiently than in the *E. grandis* monocultures (E and EN). As discussed previously, microbial respiration in the soils of the intercropped plantation was not greater than that of EN after 20 months, but it was sufficient to breakdown the organic matter, resulting in an input to the soil of more diverse and processed organic matter and a long-term stimulus for microbial activity. This argument is supported by the observation of higher C_{mic}/tC values in the intercropped plantation in this sampling period (Fig. 2). In general, the C_{mic}/tC and N_{mic}/tN ratios are indirect estimates of the quality of organic matter. Lower ratios indicate poorer quality organic matter (Wardle, 1992) and consequently a lower efficiency of the microbial biomass in immobilizing C and N. Chaer and Tótola (2007) report that the diversity and quantity of litter on the soil surface is an important driver of C_{mic} in soils. This could potentially favor organisms with an economical metabolism, which fix more C in microbial cells and use less for energetic metabolism (Anderson and Domsch, 1989), thereby increasing the C_{mic}/tC ratio. While we expected positive correlations between C_{mic} and total C in soils, this was not observed. This may be because total C was not a limiting factor in the rhizosphere. Alternatively, the C may not have been easily degradable, as both the microbial and tree communities were still in dynamic development and years away from attaining a climax situation (Garcia et al., 2005; Sinha et al., 2009).

4.6. Global multivariate analysis

In the four sampling periods, C, N, and P concentrations in litter and microbiological soil attributes performed better at discriminating between the treatments than soil chemistry. The CDA also indicated that dehydrogenase activity and microbial biomass (C_{mic} and N_{mic}) were good indicators for separating treatments (Fig. 3 and Table 4). After 20 months the microbial parameters in $A_{(A+E)}$ and $E_{(A+E)}$ were more similar to those in EN.

At two months $A_{(A+E)}$ and $E_{(A+E)}$ were separated from the monocultures, and dehydrogenase activity, available P, litter N, and litter P were responsible for this discrimination. The lack of difference between E and EN at two months is due to weak effectiveness of the N-fertilizer for improving soil attributes after clear cutting and fallow. Seven months after planting all treatments showed high discrimination by microbial attributes, probably because of rhizosphere effects, which may regulate microbial biomass and its activity (Sinha et al., 2009). The similarity between $A_{(A+E)}$ and $E_{(A+E)}$ might suggest that there was a cumulative effect in soil attributes due to the first rotation that benefited the whole intercropped plantation.

However, the distribution of treatments in the 14-month data changed dramatically in the CDA, showing the highest similarity between $A_{(A+E)}$, EN and E. At this period the discrimination was mostly due to changes in pH, and in litter and soil N (Table 4). *A. mangium* causes soil acidification (Yamashita et al., 2008), which may have been the main feature during this period, suppressing other relevant attributes. After 20 months the plantations evolved in the direction of a better microbial adaptation to pH change, and the relevance of pH in distinguishing between treatments decreased. At this point we observed a greater discrimination between treatments by tree species (CDF1 – Fig. 3D) and plantation type (mixed or pure) (CDF2 – Fig. 3D). This shows that intercropping plays an important role in changing chemical and biological

attributes, given the differences found between monoculture and mixed plantation treatments. Coelho et al. (2007) reported that *A. mangium* has a good competitive capacity in relation to *E. grandis* since the species' root systems explore different niches in the soil profile, although suppression of the initial growth of *A. mangium* by seedlings of *E. grandis* may occur (Laclau et al., 2008). In addition, the N transfer of legumes to *E. grandis* is low or inexistent until 2.5 years after planting (Coelho et al., 2007; Bouillet et al., 2008). In this case, the role of the soil microbes is restricted to the C and nutrient cycles through litter or humus decomposition, because biological N-fixation still does not promote better conditions for *E. grandis*.

Thus, greater metabolic and functional diversity could be important requirements for the sustainability of intercropped plantations. Because of the high dynamism of forest plantations, plantation age, the rhizosphere effect, leaf deposition, and site history are very important for understanding changes in microbial activity. The synergism between the two tree species in the intercropped plantation established a new equilibrium in the soil microbiota, maintaining and stimulating biogeochemical cycling. The stronger relationship between litter contents and microbiological soil attributes demonstrates the important role played by the maintenance and quality of litter in regulating microbial biomass and activity in soils, which also suggests the benefits of mixed-species plantations. These results from the early stages of plantations, and especially in the intercropped treatment, suggest that mixed-species plantations between *E. grandis* and *A. mangium* may have beneficial effects on certain soil chemistry and soil biology indicators, despite the high variation within treatments in all periods. However, continued monitoring of these plantations over several years will be necessary to document other differences not apparent in the early planting stages.

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