

Soil microbial properties in *Eucalyptus grandis* plantations of different ages

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Abstract

Eucalyptus plantations are increasingly used in Brazil to produce wood and energy, although the long-term consequences for ecosystem processes have rarely been evaluated. We investigated the soil microbial properties (soil microbial C, N, respiration, carbon use efficiency, and microbial C-to-N ratio) among *Eucalyptus grandis* plantations of differing ages (1 to 4 years) in Northeast Brazil. An adjacent native forest was used as a reference. In general, soil microbial properties decreased in initial years of land-use change, but recovered to conditions comparable to an adjacent native forest after four years in the rainy season, but not in the dry season. The varying effects of *Eucalyptus* plantations and native forests between seasons may reflect differences in soil microbial communities with different responses to environmental conditions. Our results highlight the importance of long-term monitoring and microbial community analysis in order to adequately assess and understand the impacts of exotic forest plantations on soil microbial properties.

Keywords: Forest, land use, soil microbial biomass, soil quality, tropical soil

1. Introduction

Forest plantations occupy an area of about 200 million hectares worldwide, and they are increasing globally due to growing demands for wood and also to sequester carbon dioxide from the atmosphere (Zhang *et al.*, 2012). In Brazil, *Eucalyptus* plantations have been used in different regions and soil types in order to produce wood and energy (Barreto *et al.*, 2008), and currently 4.7 million hectares are covered with *Eucalyptus* forest (CICB, 2012). The main species used is *Eucalyptus grandis* due to its high adaptability to climate fluctuations, soil types, and water regimes (Araujo *et al.*, 2010).

However, several studies suggested that the afforestation of *Eucalyptus* may have negative ecological effects, for example on soil microbial properties (Zhang *et al.*, 2012; Araujo *et al.*, 2010; Barreto *et al.*, 2008; Cordova *et al.*, 2012). According to Zhang *et al.* (2012), these effects are resulting from soil management (fertilization, liming, and ploughing) and the direct effect of the *Eucalyptus* trees. Previous studies have shown both negative and positive effects of *Eucalyptus* plantations on soil microbial biomass and activity; however, these inconsistent findings stem mainly from short-term studies of one (Behera and

of one (Behera and Sahani, 2003; Sicardi *et al.*, 2004) and two years (Araújo *et al.*, 2010; Cordova *et al.*, 2012); so longer-term approaches are needed to adequately appreciate the belowground consequences of *Eucalyptus* plantations (Zhang *et al.*, 2012; Chen *et al.*, 2013).

Soil microbial biomass is the living component of soil organic matter (SOM) (Jenkinson and Ladd, 1981) and has already been used successfully to assess soil conditions under *Eucalyptus* stands in tropical soils, i.e., evaluating the effect of land-use change from grassland to *E. grandis* plantations (Sicardi *et al.*, 2004), the difference between native forest and *Eucalyptus* forest (Behera and Sahani, 2003), and short-term effects of afforestation with *Eucalyptus* (Araújo *et al.*, 2010). Araújo *et al.* (2010) evaluated the effect of *Eucalyptus* plantation on soil microbial biomass after two years and found a significant decrease in soil microbial biomass probably due to the lack of adaptation of soil microorganisms to the exotic plant litter and rhizosphere inputs from *E. grandis*. However, it is unclear how *Eucalyptus* afforestation decreases soil microbial biomass and activity and, also, how much time is necessary to restore the soil microbial status given that soil microbial communities will recover from the disturbance of land-use change and may adapt to the new conditions. Therefore, we hypothesized that soil microbial properties will recover from the initial decrease in soil microbial biomass following deforestation and early *Eucalyptus* establishment (Araújo *et al.*, 2010). In order to test the longer-term effects of *Eucalyptus grandis* plantations on soil microbial properties, we determined soil microbial biomass C and N, carbon use efficiency and microbial C-to-N ratio under 1, 2, 3 and 4 year old *Eucalyptus* plantations in Northeast Brazil.

2. Material and Methods

The study was conducted at Real Farm located at

Piauí state (09° 49' 55" S and 45° 20' 38" W) in Northeast Brazil. The climate is tropical dry with a mean precipitation of 1,350 mm yr⁻¹ (with rainfall from January through May) and an annual mean temperature of 32°C, with minimum and maximum temperatures of 16°C and 36°C, respectively. According to the Brazilian Soil Survey the soil is classified as an Oxisol (sand: 8%; silt: 41%; clay: 51%).

We selected sites (1 ha) with *Eucalyptus grandis* plantations differing in age since establishment (1 to 4 years). All plantations were established in the same area at Real Farm with largely uniform soil and environmental conditions. We established large sites with a size of 1 ha covering minor local heterogeneity in soil conditions and allowing us to differentiate four plots of 20 x 20 m, which were spaced 10 m from each other. The *E. grandis* plantations differed in age from 1 to 4 years and were established in 2008, 2009, 2010, and 2011, respectively. An adjacent native forest (NF), classified as "cerrado" (savannah), was used as reference with trees of 10-15 m height and a density of 2000-3000 individuals per hectare. All sites (1-4 years) with *E. grandis* presented the same management practices after afforestation, which allowed us the direct comparison of sites differing in age. Each site was deforested, and dolomite lime (4 Mg ha⁻¹) and 400 kg of single super phosphate ha⁻¹ were applied. Two months later, *E. grandis* was planted in a density of 400 plants per ha and fertilized with 150 g per plant of NPK (10:10:10). The main characteristic of *E. grandis* trees are shown in Table 1 (Nunes *et al.*, 2010).

Each site was sampled in the rainy season (March) and in the dry season (September) in 2011. At each plot, the vegetation cover was carefully removed from the soil surface before soil cores (2.5 cm diameter) were taken randomly. Ten soil samples were taken at random locations from each plot from the upper 0-10 cm. The plant litter was evaluated according to Van Soest (1963), and the results are shown in Table 2.

Table 1. Characteristics of *Eucalyptus grandis* trees in plantations differing in age (Araujo et al., 2010)

<i>Eucalyptus</i> age	Canopy closure ratio	Diameter (cm)	Height (m)
1y	0.50	7.0	4.5
2y	0.55	7.6	7.2
3y	0.70	9.4	10.1
4y	0.80	12.7	14.8

Table 2. Content of plant litter, cellulose and lignin from *Eucalyptus grandis* plantations of different age.

<i>Eucalyptus</i> age	Plant litter (t ha ⁻¹)	Cellulose (g kg ⁻¹)	Lignin (g kg ⁻¹)
1y	2.01	136.2	127.5
2y	2.85	193.6	201.4
3y	3.72	242.9	224.4
4y	5.20	248.5	232.5
NF	5.91	312.6	291.3

Ten soil cores from each plot were combined to form one composite sample. All samples were immediately stored in sealed plastic bags in a cooler and transported to the laboratory. The field-moist samples were sieved (2-mm mesh) and stored in sealed plastic bags at 4°C for microbial analyses.

Soil chemical properties are shown in Table 3. Soil pH was determined in a 1:2.5 soil/water extract. Exchangeable Ca was determined using extraction with 1 M KCl. Available P and exchangeable K were extracted using Mehlich-1 extraction method and determined by colorimetry and photometry,

respectively (Tedesco et al., 1995). Total organic C (TOC) was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans and Bremner, 1998).

Soil microbial biomass C (MBC) and N (MBN) were determined according to Vance et al. (1987) with extraction of C and N from fumigated and non-fumigated soils by K₂SO₄. Extraction efficiency coefficients of 0.38 and 0.45 were used to convert the difference in C and N between fumigated and non-fumigated soil in MBC and MBN, respectively.

Table 3. Chemical properties of soil under *Eucalyptus grandis* plantations of different ages.

Site	pH	Al ⁺³	H ⁺ + Al ⁺³	Ca ⁺²	Mg ⁺²	K ⁺	P
	(H ₂ O)	—cmol _c dm ⁻³ —			—mg dm ⁻³ —		
1y	7.8	0.03	2.76	3.9	1.9	256.7	29.8
2y	8.1	0.04	2.16	3.8	1.9	170.0	27.2
3y	6.9	0.05	2.42	2.9	2.2	241.7	12.2
4y	7.5	0.04	2.78	3.4	2.1	193.3	16.3
NF	4.2	1.2	2.91	0.5	0.2	32.1	1.5

Soil respiration was measured according to Alef (1995). Soil samples (100 g) were placed in 300-ml glass containers closed with rubber stoppers, moistened at 60% of the maximum water holding capacity and incubated for 3 days at 25 °C. Glass vials holding 10 ml of 0.5 N NaOH to trap the evolved CO₂ were placed in the above containers. On day 3 after incubation, the glass vial was removed and the CO₂ trapped in NaOH was determined titrimetrically. The $q\text{CO}_2$ was calculated as the ratio of basal respiration to microbial biomass C. The $q\text{CO}_2$ results were expressed as g CO₂-C d⁻¹ g⁻¹ MBC. Moreover, we calculated the ratio between MBC and MBN as well as between MBC and TOC, with the latter being a common measure for carbon availability (Santos *et al.*, 2012).

The results are expressed on the basis of oven-dry soil. The data were checked if they met the requirements for parametric statistical tests (normality and homogeneity of variances) and subsequently subjected to analysis of variance (ANOVA) to detect significant differences among the sites differing in age. When significant site effects were detected, the means were compared using the least significant difference (LSD) test ($p < 0.05$). All data were analyzed using the SAS 9.3 (SAS Institute, Cary, NC).

3. Results

Total organic carbon (TOC) contents were similar among *E. grandis* ages (2y, 3y and 4y), except for 1y *E. grandis* plantation, which showed the lowest TOC content in both seasons (Table 4). Also, the TOC content found in 2y, 3y and 4y were similar to the TOC content in NF.

The microbial properties differed between the rainy season and the dry season (Table 4). Soil microbial biomass C (MBC) and N (MBN) contents increased significantly under 4y *Eucalyptus* as compared to the other *Eucalyptus* plantations in both seasons (Table 4). In the rainy season, soil MBC and MBN in the 4y *Eucalyptus* plantation were similar to that in native forest. However, in the dry season, the values of soil MBC and MBN were lower than in the native forest. In addition, we found lower soil MBC and MBN content during the first three years as compared to the native forest in both seasons.

In the rainy season, soil respiration values were similar in all *Eucalyptus* plantations, and they showed higher values than native forest soil (Table 5). However, in the dry season, the values of soil respiration were similar for all evaluated sites. The metabolic quotient ($q\text{CO}_2$) was significantly greater

in 1y to 3y *Eucalyptus* plantations than in 4y *Eucalyptus* plantation and in native forest in both seasons (Table 5).

The MBC-to-MBN and MBC-to-TOC ratios did not differ significantly between evaluated sites, including native forest, in the rainy season (Table 5). However, in the dry season, MBC-to-MBN ratio was significantly

higher in 4y *Eucalyptus* plantation and in native forest than in 1y, 2y, and 3y plantations.

MBC-to-TOC ratio did not vary significantly between the different sites in the dry season, but it increased from 1y, 2y, and 3y to 4y plantations as well as from 4y plantation to native forest in the rainy season.

Table 4. Total organic C (TOC), microbial biomass C (MBC) and N (MBN) under *Eucalyptus grandis* plantations of different ages and under native forest in the rainy and dry season.

Site	TOC		MBC		MBN	
	(g kg ⁻¹)		(mg C kg ⁻¹)		(mg N kg ⁻¹)	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
1 y	25 ± 2.1 ^b	24 ± 1.7 ^b	409 ± 14 ^b	115 ± 22 ^c	24 ± 1.3 ^c	14 ± 1.1 ^c
2 y	31 ± 3.7 ^a	30 ± 0.6 ^a	466 ± 59 ^b	163 ± 28 ^c	35 ± 2.5 ^b	15 ± 2.5 ^c
3 y	30 ± 3.0 ^a	29 ± 3.6 ^a	468 ± 63 ^b	155 ± 35 ^c	35 ± 1.3 ^b	15 ± 1.3 ^c
4 y	31 ± 1.2 ^a	32 ± 0.8 ^a	673 ± 101 ^a	274 ± 36 ^b	51 ± 5.8 ^a	23 ± 1.9 ^b
NF	32 ± 1.8 ^a	32 ± 1.4 ^a	660 ± 117 ^a	439 ± 17 ^a	50 ± 3.6 ^a	37 ± 1.4 ^a

Means ± standard deviation. Means followed by the same letter within each column are not significantly different at 5% level (LSD test).

Table 5. Soil respiration (CO₂), respiratory quotient (qCO₂), ratio between soil microbial biomass C and soil microbial biomass N (MBC-to-MBN), and ratio between soil microbial biomass C and total organic carbon content (MBC-to-TOC) under *Eucalyptus grandis* plantations of different ages and under native forest in the rainy and dry season.

Site	CO ₂		qCO ₂		MBC-to-MBN		MBC-to-TOC	
	(mg kg ⁻¹ d ⁻¹)						(%)	
	rainy	dry	rainy	dry	rainy	dry	rainy	dry
1y	497 ± 51 ^{ab}	190 ± 29 ^a	1.2 ± 0.8 ^a	1.6 ± 0.2 ^a	1.61 ± 0.08 ^a	0.73 ± 1.3 ^c	1.65 ± 0.1 ^a	0.45 ± 0.1 ^c
2y	502 ± 93 ^a	180 ± 25 ^a	1.1 ± 0.3 ^{ab}	1.1 ± 0.1 ^b	1.41 ± 0.31 ^a	1.07 ± 1.7 ^b	1.32 ± 0.2 ^a	0.52 ± 0.1 ^c
3y	568 ± 42 ^a	172 ± 17 ^a	1.2 ± 0.1 ^a	1.1 ± 0.3 ^b	1.65 ± 0.38 ^a	0.97 ± 1.1 ^b	1.30 ± 0.1 ^a	0.53 ± 0.1 ^c
4y	523 ± 118 ^a	169 ± 40 ^a	0.7 ± 0.1 ^{bc}	0.6 ± 0.1 ^c	2.13 ± 0.27 ^a	1.55 ± 0.6 ^a	1.32 ± 0.1 ^a	0.86 ± 0.1 ^b
NF	331 ± 103 ^b	155 ± 37 ^a	0.5 ± 0.1 ^c	0.3 ± 0.8 ^c	2.03 ± 0.36 ^a	1.67 ± 1.1 ^a	1.30 ± 0.1 ^a	1.30 ± 0.2 ^a

Means ± standard deviation. Means followed by the same letter within each column are not significantly different at 5% level

4. Discussion

Eucalyptus plantations are increasingly used in Brazil to produce wood and fuel with heretofore unknown long-term consequences for soil microbial properties. The results of the present study show that gross soil microbial properties in *Eucalyptus* plantations approach conditions of a reference native forest in the rainy season four years after plantation. Although we found distinct differences between the rainy and dry season, the direction of age effects of *Eucalyptus* plantations was largely consistent across seasons. However, differences between the native forest and the 4y *Eucalyptus* plantation were more pronounced in the dry season, indicating different soil microbial communities and responses to dry soil conditions.

The results for TOC indicate that organic C decreased in the first year of the conversion of native forest to *Eucalyptus* plantations. Probably, there are two main reasons for this decrease: a) due to lower organic matter inputs to the soil since young *Eucalyptus* stands may provide a low amount of inputs of plant residues to the soil (Zhang *et al.*, 2012); and b) during the deforestation of native vegetation and implantation of *E. grandis* soil macro-aggregates were disrupted and exposed to microbial breakdown, with the consequence of organic matter being lost from soil (Chen *et al.*, 2013). On the other hand, according to Hou (2006), *Eucalyptus* has a high growth rate and C fixation potential; therefore, part of the assimilated C is transported to the soil through litter fall and rhizodeposits, thereby increasing soil organic C content with time (Lima *et al.*, 2006). Similar results in tropical soils were found by Barreto *et al.* (2008), who observed elevated TOC after 3 years of *Eucalyptus* plantation.

Soil microbial properties differed between seasons, and this pattern is in agreement with Silva *et al.* (2012) for tropical soil. Such effects of season may be mainly due to variations in soil humidity and temperature (Araujo *et al.*, 2013). The decrease in soil microbial biomass during the first three years after the conversion indicates that the deforestation and establishment of

Eucalyptus negatively affected soil microorganisms. Soil microbial biomass acts as driver for a wide range of soil and ecosystem processes (Bardgett and Straalen 2008; Bardgett and Wardle 2010). However, plant diversity and soil microbial biomass should be tightly coupled and changes in these interactions may influence ecosystem functioning (Grigulis *et al.*, 2013; Yang *et al.*, 2013). Thus, the deforestation of native vegetation decreased the plant diversity and impacted soil microbial biomass. This impact may be attributed to differences in the amount and quality of resources entering the below-ground compartment in the form of litter and root exudates. The decrease in soil microbial biomass has consequences for ecosystem services, such as carbon storage and retention of nutrients in soil (Steinbeiss *et al.* 2008; De Deyn *et al.* 2009, Malik *et al.*, 2013).

We suggest three possible factors that may have caused the negative effect on soil microbial biomass: a) during conversion of native vegetation to *E. grandis*, clear-cutting and plowing destroyed vegetation cover; b) the native microorganisms from tropical soils were probably not adapted to the rhizosphere conditions of the exotic species *E. grandis*; and c) the limited C and N inputs from young *Eucalyptus* stands did not support high soil microbial biomass. Similar reports on the decrease of soil microbial biomass after conversion of native forest to *Eucalyptus* forest have been published before (Behera and Sahani, 2003; Araujo *et al.*, 2010; Zhang *et al.* 2012). These studies suggested a possible toxic compound from the leaves of *Eucalyptus* that caused a harmful effect on soil microbial biomass (Behera and Sahani, 2003), and the slow adaptation of soil microorganisms to the *Eucalyptus* rhizosphere (Araujo *et al.*, 2010). As soil microbial biomass in the 4 y *Eucalyptus* plantation was similar to that of native forest only in the rainy season and not at dry season, soil microbial community structure is likely to differ between those sites and to respond differently to dry condition as reported by Araujo *et al.* (2013, 2014) in degraded and restored lands from Northeast, Brazil.

Some previous studies and our study suggest that effects of *E. grandis* plantations on soil microbial biomass strongly depend on the amount of C inputs to soil (Table 3; Zhang *et al.*, 2012). Moreover, according to Chen *et al.* (2013), the effects of disturbance during the conversion of native vegetation to *Eucalyptus* plantations decreased with plantation age, accompanied by an increase in the size and functional activity of the soil microbial communities (Wu *et al.*, 2011). Other studies showed a recovery of soil microbial communities after seven to nineteen years of *Eucalyptus* establishment (Gama-Rodrigues *et al.*, 2008; Zhang *et al.*, 2012; Chen *et al.*, 2013). Zhang *et al.* (2012) and Chen *et al.* (2013) found that soil MBC and MBN decreased initially following afforestation by *E. grandis*, and afterwards both properties increased with stand age. However, these previous studies performed only one sampling per year and could not investigate seasonal effects, and both studies reported a much longer recovery time of soil microbial properties than we did in our study. Our results suggest that although soil microbial biomass did not differ significantly between 4y *Eucalyptus* plantations and native forest in the rainy season, differences were still apparent in the dry season. These results suggest different soil microbial communities between the sites with varying responses to dry soil conditions. While *E. grandis* may support high soil microbial biomass only during wet soil conditions through high carbon inputs to the soil, soil under native forest may be more buffered against climatic variations like in the dry season (Araujo *et al.*, 2013).

The values for soil respiration showed that soil microorganisms from *Eucalyptus* plantations may be more active than in native forest. It is likely that higher soil respiration indicates an ecological stress and lower C use efficiency (Islan and Weil, 2000). To test this, we evaluated the metabolic quotient ($q\text{CO}_2$), which is a useful indicator of soil microbial C use efficiency and stress (Anderson and Domsch, 1990; Fernandes *et al.*, 2005). Indeed, the metabolic quotient was significantly lower in 4y and native forest than in younger plantations. Therefore, our

results suggest that microbial efficiency seems to recover with time due to an increase in organic matter and the establishment of a more autochthonous soil microbial community.

The conversion of native forest to *Eucalyptus* forest caused different responses of microbial indices, such as MBC-to-MBN and MBC-to-TOC ratios, which varied between seasons. For the MBC-to-TOC ratio, an index indicating the availability of organic matter in soils (Santos *et al.*, 2012), the results showed that, in the rainy season, soil water content probably contributed to an increase in MBC-to-TOC ratio under *Eucalyptus* plantations, and the sites did not differ significantly. On the other hand, in the dry season, the ratio was significantly higher in native forest and 4y *E. grandis* plantation. This may indicate lower soil organic matter availability to soil microorganisms in younger plantations and higher conservation of soil moisture in 4y *E. grandis* plantation permitting the usage of C sources by soil microorganisms and thereby increased MBC-to-TOC ratio.

5. Conclusions

The time since the establishment of *Eucalyptus* plantation had significant impacts on the soil microbial biomass C, N, respiration, and C use efficiency, with all those properties recovering after four years of *Eucalyptus grandis* plantation in the rainy season. The responses of soil microbial biomass seem to be regulated by the availability of soil organic C, which accumulated after land conversion. Soil microbial properties recovered four years after land conversion to a level comparable to a reference native forest in the rainy season, but differed significantly from native forest in the dry season indicating different soil microbial community composition and functioning. Our results highlight the importance of long-term monitoring and soil microbial community analysis in order to adequately assess the impacts of exotic forest plantations on soil microbial properties.

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