CHANGES IN SOIL PH, POLYPHENOL CONTENT AND MICROBIAL COMMUNITY MEDIATED BY *EUCALYPTUS CAMALDULENSIS*

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Abstract. *Eucalyptus camaldulensis* has been the main exotic species planted in reforestation programs in the tropics due to its fast growth and adaptability to climate variations. Based on the premise that the conversion from natural grazed pastures to commercial *Eucalyptus* plantations generates significant changes in soil properties, we assessed the impact of this exotic plantation on soil chemical and biological indicators. The study was conducted in 6 plantations across Senegal following a decreasing rainfall gradient from south to north. The plantations were divided in three lots according to their age: young plantations (established in 2003, 6 years old); intermediate plantations (established in 1998, 11 years old) and old plantations (established in 1982 and 1983, 26 years old). Our results clearly showed that *E. camaldulensis* plants significantly modified soil pH and soil bacterial community at all sites regardless of the age of the plantation. Microbial biomass (assessed by substrate-induced respiration), community structure (assessed by denaturing gradient gel electrophoresis profiles) and function (assessed by Catabolic Response Profile using different substrates) were all significantly decreased. The acidifying effect of *E. camaldulensis*, the effect of high level of polyphenols and their impact on microbial communities and ecosystem functioning were discussed.

Keywords: *eucalyptus, allelopathy, microbial diversity, catabolic diversity, enzyme activities*

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Introduction

Plantations of fast-growing trees have been extended in Sahelian zones because of their ecological plasticity (Bernhard-Reversat and Loumeto, 2002). Expansion of *Eucalyptus* plantations has been the focus of several studies throughout the world, usually related to their economic importance. *Eucalyptus* is commonly used in reforestation because of its high cellulose production, high fiber quality, and resistance to environmental stresses and diseases (Turnbull, 1999; Berthelot et al., 2000; Cossalter and Pye-Smith, 2003). Several environmental impact studies on carbon flux, water balance and soil fertility have been conducted in industrial plantations in Congo and Brazil, in response to criticisms by opponents of *Eucalyptus* plantations (Bouillet et al., 1997; Bouillet and Bernhard-Reversat, 2001; Laclau et al., 2004). It has been shown that, in addition to their acidifying affects (Cannell, 1999; Rhoades and Binkley, 1996; Farley et al., 2008), exotic species have mineral and water needs significantly higher than native species (Benhard-Reversat, 1987; Laclau, et al., 2005). It is noteworthy that, despite being very informative, these studies did not take into account the microbiological characteristics and more specifically, microbial genetic and functional diversities in soils with *Eucalyptus* plantations.

In Senegal, many *Eucalyptus* plantations have been planted in different regions by the Senegalese Institute of Agricultural Research (DRFP/ISRA), in its tree improvement program. The extent of studies conducted in the past encouraged researchers to select *E. camaldulensis* as the species which is best adapted to soil and climatic conditions of the experimental stations throughout Senegal (Giffard, 1969; Hamel, 1981). But little attention has been paid to diversity and functionalities of microbial communities under *Eucalyptus* soil. Yet, microbial communities are the most sensitive and most affected by the replacement of native vegetation by exogenous plant cover (Yu, 2005).

In fact, soil microorganisms are extremely diverse and play an important role in ecosystems. In soils, they mediate nutrient cycling, organic matter decomposition, soil aggregate formation, soil carbon storage (Zinn et al., 2002), and have an impact on composition of plant communities (Hooper et al., 2000; Wardle, 2002) and plant disease prevention and bio-control (Kennedy, 1998; Biró et al., 2000; Artursonn, et al., 2006). The microbial community composition and functioning can be influenced by exudates from roots and litter quality and quantity. According to Powlson et al. (1987), soil microbial biomass measurement can give an early indication of soil health before changes in total organic C and N can be reliably detected. In order to draw meaningful conclusions we assessed a set of parameters (microbial biomass, enzyme activities, functional diversity, etc.) as early indicators of stress and disturbance (Dick et al., 1996; Nannipieri and Eldor, 2009).

So far in Senegal, studies on impact of *Eucalyptus* plantations on soil health were limited to greenhouse or site scale. This is the first large scale study conducted in *Eucalyptus* plantations in Senegal (across several sites and different ages of plantations).

The aim of this study was to determine the impact of *E. camaldulensis* plantations on microbial genetic and functional diversities, including microbial biomass. Importantly, studies have indicated that *Eucalyptus* leaves contain toxic organic compounds (high quantities of lignin, polyphenol compounds, allelochemicals, etc.) which may have a deleterious impact on soil microorganisms. We hypothesized that *E. camaldulensis* will lead to changes in soil chemical and microbiological properties that will ultimately limit the catabolic capabilities of native microbial communities.

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Materials and Methods

Study sites and soil sampling

The sampling sites were selected in 6-, 11- and 26-year-old *Eucalyptus* plantations considered respectively as young, intermediate and old. Young plantations correspond to Sinthiou, Kolda and Nioro. As shown on the map (*Fig 1*), Nioro is located in south-west of Senegal (15°19N; 04°17W), Sinthiou is in the North (17° 15N; 06° 82W while Kolda is in South Senegal (14° 27 N; 05°77W). Intermediate plantation corresponds to Matam (North) collection (17°30N; 06° 87W) and old plantations correspond to Lompoul (17°07 N; 03°69 W) and Tamba sites (15°23 N; 06°40W) respectively in the center and in the North of Senegal. The rainfall distribution patterns across Senegal are presented in *Fig 1*. From each site, five soil samples were collected under five *Eucalyptus* trees from 0 to 30 cm soil depth and mixed to form one composite sample (noted SC). And three composite samples were collected per plantation. This soil was considered to be influenced by *Eucalyptus*. At each site, three other composite soil sample was formed with soil collected in areas free of *Eucalyptus* and located at a distance of 30 m from *Eucalyptus* plantings (control sample or HC). These soil samples were supposedly uninfluenced by *Eucalyptus.*

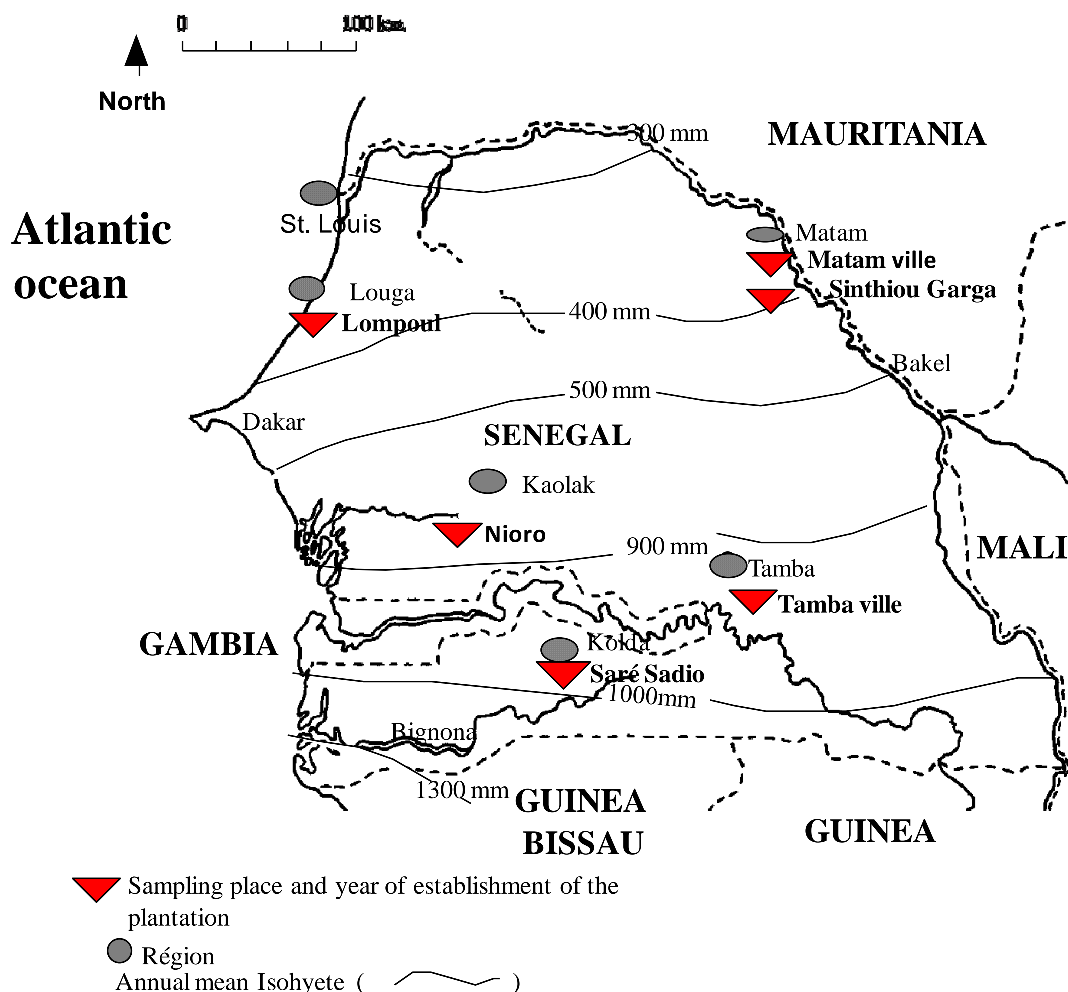

Figure 1. *Study site and the exact locations of sampling*

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Soil analyses

Chemical analysis of soil

All soil samples were characterized by measuring pH, total soil C and N after dry combustion in Elemental Analyzer (*LECO Corporation, St. Joseph, MI, USA*). Total and available P were analyzed by Olsen-Dabin method (Olsen et al., 1954; Aubert, 1978). Analyses were performed at LAMA (Laboratoire des Moyens Analytiques IRD/ISRA, laboratory certified by International Organization for Standardization ISO 9001 version 2008).

Total polyphenol content

The method described by Macheix et al. (1990) was adapted to determine total polyphenol content. One gram of each soil sample was shaken in 20 ml cold methanol (80% v/v) during 15 minutes and the mixture was centrifuged for 3 minutes at 5000 rpm at 4°C. This step was repeated three times before the supernatants were evaporated to remove methanol. A solution of ammonium sulphate (40% v/v) was added to the aqueous extract in order to increase the ionic strength of the solution and metaphosphoric acid solution 20% (1/10 v/v) was also added to prevent oxidation of phenolic compounds.

This phase was followed by depigmentation and defatting of aqueous extract with petroleum ether (v / 2). The extract was purified by ethylene acetate (v/v) and evaporated to dryness at 35°C with a rotary evaporator and the residue was recovered in 2 ml of pure methanol. Extraction was repeated 3 times for each soil sample.

Total polyphenol content was determined by spectrophotometry, using (+/-)-catechin as standard range (Singleton and Rossi, 1965). Briefly, 50 µl of the diluted sample extract was mixed with 1.35 ml of distilled water and 200 µl of Folin-Ciocalteu's reagent. Then, 400 µl of sodium carbonate solution (20% w/v) was added. The test tubes were placed in a water bath for 20 minutes at 40 ° C before absorbance was measured at 760 nm. The concentration of polyphenols in samples was derived from a standard curve (+/-) - catechin ranging from 5 to 30 µg ml^{-1}.

Total microbial biomass

Microbial biomass (MB) was estimated using substrate induced respiration method (Anderson and Domsch, 1978). The soil microbial content was estimated from the maximum rate of glucose-induced respiration by applying the formula below:

$$x = 40.04y + 0.37 \quad \text{(Eq. 1)}$$

where y = the maximum initial rate of respiration (in ml CO_2 100 g $soil^{-1}$ h^{-1}), and x = mg microbial-C 100 g $soil^{-1}$. Microbial CO_2 respiration was determined by direct injection into a micro GC Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA.) equipped with a TCD detector using helium as the carrier gas.

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Soil enzyme activity

Acid and alkaline phosphatases and dehydogenase activity were determined on *Eucalyptus* rhizospheric soils and compared to control soil samples.

These phosphatase activities (alkaline and acid) were determined on 0.5 g of dry soil according to the method of Eivazi and Tabatabai (1977).

For each soil sample, soil were mixed with 400 µL of modified universal sterile buffer (at pH 5.8 for acid phosphatase and pH 11 for alkaline phosphatase) and 100 µl of p-nitrophenyl phosphate solution (pNPP) before being incubated for 1 hour at 37°C on a rotary shaker. The reaction was stopped by adding 100 µl of $CaCl_2$ and 400 µl of NaOH, and the soil suspensions were centrifuged at 12000 rpm for 10 minutes. Then, the absorbance readings were taken at 400 nm. Analyses were conducted in triplicate and one non-substrate control and results are expressed as µg p-nitrophenol release.g^{-1} h^{-1} at 37°C.

Dehydrogenase activity was assayed by a method from Casida et al. (1964) with few modifications. One g soil sample was mixed with 1 ml of tris buffer, 1 ml 4% (w/v) of 2, 3, 5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 37 °C. Dehydrogenase enzymes convert TTC to 2, 3, 5-triphenylformazan (TPF). 1 ml of 4 % TTC and 2.5 ml of H_2O were added to each tube before they were inverted a few times and incubated at 37°C for 24 hours. A control sample contained all the chemicals mentioned above except the TTC. Each soil sample was extracted with 5 ml of acetone after incubation. The extract was centrifuged and the optical density (O.D.) of the supernatant was read at 546 nm in a spectrophotometer. Results were expressed as µg TPF g^{-1} h^{-1}.

Catabolic Response Profiles

The functional diversity of heterotrophic microbial communities was determined by measuring the patterns of *in situ* catabolic potential (ISCP, CRP). The ISCP is a physiological approach developed by Degens and Harris (1997). This method is based in a serial measure of CO_2 production in a short term after adding different organic substrates. Thirty three substrates (33) belonging to various chemical groups: eleven amino acids (arginine, aspargine, L-cysteine, L-glutamic acid, L-hisdidine, L-serine, L-tyrosine, L-lysine, L-leucine, L-proline, L-glutamine), six carbohydrates (amidon, D-mannose, D-glucose, sucrose, threalose, maltose), twelve organic acids (gallic acid, ascorbic acid, citric acid, fumaric acid, malic acid, quinic acid, succinic acid, tartaric acid, malonic acid, α-ketoglutaric acid, oxalic acid, panthothenic acid), one amine (D-glucosamine) and three alcohols (meso inositol, sorbitol, mannitol) were used on three replicate soil samples to evaluate soil microflora capacity to catabolize different C sources. Each substrate was added at 0.5 mg C.g $soil^{-1}$ concentration in 70% water-holding capacity of each soil in vacutainers bottles. Solutions were adjusted to pH 5.8-6.2 before addition to soil, using NaOH or HCl to increase or decrease the pH at the time of preparation in order to avoid any substrate-pH effects on microbial communities. A control without substrate, with only water added was included. Bottles were incubated for 2 h at ambient temperature (25°C) and CO_2 fluxes from the soils were measured by µCPG (Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA.) equipped with a TCD detector using Helium as the carrier gas). Results were expressed as µgCO_2 g^{-1} soil h^{-1}.

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Catabolic evenness (E), defined as the variability in substrate utilization, was evaluated by $E=1/\Sigma p_i^2$ where pi = ratio of respiration response on the ith substrate to the sum of the respiration responses on all substrates.

Genetic diversity

Whole-community DNA was directly extracted from 0.5 g of sample using a bead-beating method (Fast DNA SPIN Kit for soil, Bio 101 Inc., USA) following manufacturer's instructions.

The 16S rDNA (V3 sequence) fragments of the bacterial soil community were amplified with 338f-GC and 518r primers (Muyzer et al., 1993). The PCR and DGGE were performed as described by Fall et al. (2004). The number of bands and their positions on the DGGE gel were analyzed using the Bio-Profil Biogene program (Vilber Lourmat) and dendrograms were created based on Dice coefficient of similarity.

The species richness on DGGE gels (R) was calculated as the mean number of bands present (Vivas et al., 2008, 2009). The structural diversity of the microbial community was examined by the Shannon index of general diversity and Simpson index of dominance D (Simpson, 1949). For these analyses, each band was presumed to represent the ability of that bacterial species to be amplified (Vivas et al., 2008, 2009). The intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon H' and Simpson D indexes were calculated from the following equations: $H' = -\Sigma\,(P_i \log P_i^2)$ $D = \Sigma P_i^2$; P_i = ni/N; ni = height of peak and N = sum of all peak heights in the curve.

Statistical analyses

Co-inertia analysis (CIA) was used to analyze the relationship between chemical and microbial properties and sample type in different sites. Additionally, Student-Newman Keuls test was done to separate the means when the ANOVA revealed significant differences at 5% level. The relationship of Karl Pearson is used to correlate the different variables measured in this experiment. Between-group analysis (BGA) was used for the SIR responses because number of cases is lower than the number of variables (12 soil samples and 33 SIR substrates). The free ADE4 software (Thioulouse et al., 1997) was used to perform BGA computations.

Results

ACP analysis showed clear separation between plantations according to age. Young and old plantations (young SC and old SC) correspond to axis 1 (38.44% of the total contribution rate) and were associated with low pH, low MB, and low diversity index (*Fig. 2, Table 1*). While intermediate plantation (med SC) was associated to axis 2 (31.41% of the total contribution rate) and is mainly characterized by very high levels of phenols and phosphatase (*Fig. 2; Table 1*).

Table 1. *Change in chemical and biological variables according to plantation age*

	Desy	Ac. phos	Al. Phos	H	E	MB	Total poly	pH_2O
med	8,48 a	764,12a	364,28a	1,47ab	28,81ab	4276,14a	18,1a	6,67a
old	6,91 b	382,93b	95,99b	1,45b	30,83a	1015,40b	6,44b	5,49b

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young	3,07 c	300,71c	51,93c	1,49a	26,49b	737,79b	6,06b	5,47b
	med> old> young			med= old< young	med= old> young	med> old= young		

Data in the same column followed by the same letter are not significantly different according to one-way ANOVA (P < 0.05). Abbreviations: Shannon (H), Simpson (D), Species richness (R), total microbial biomass (MB); alkaline phosphatase (Al. phos), acid phosphatase (Ac. Phos), dehydrogenase (Desy), total polyphenol content (Total poly)

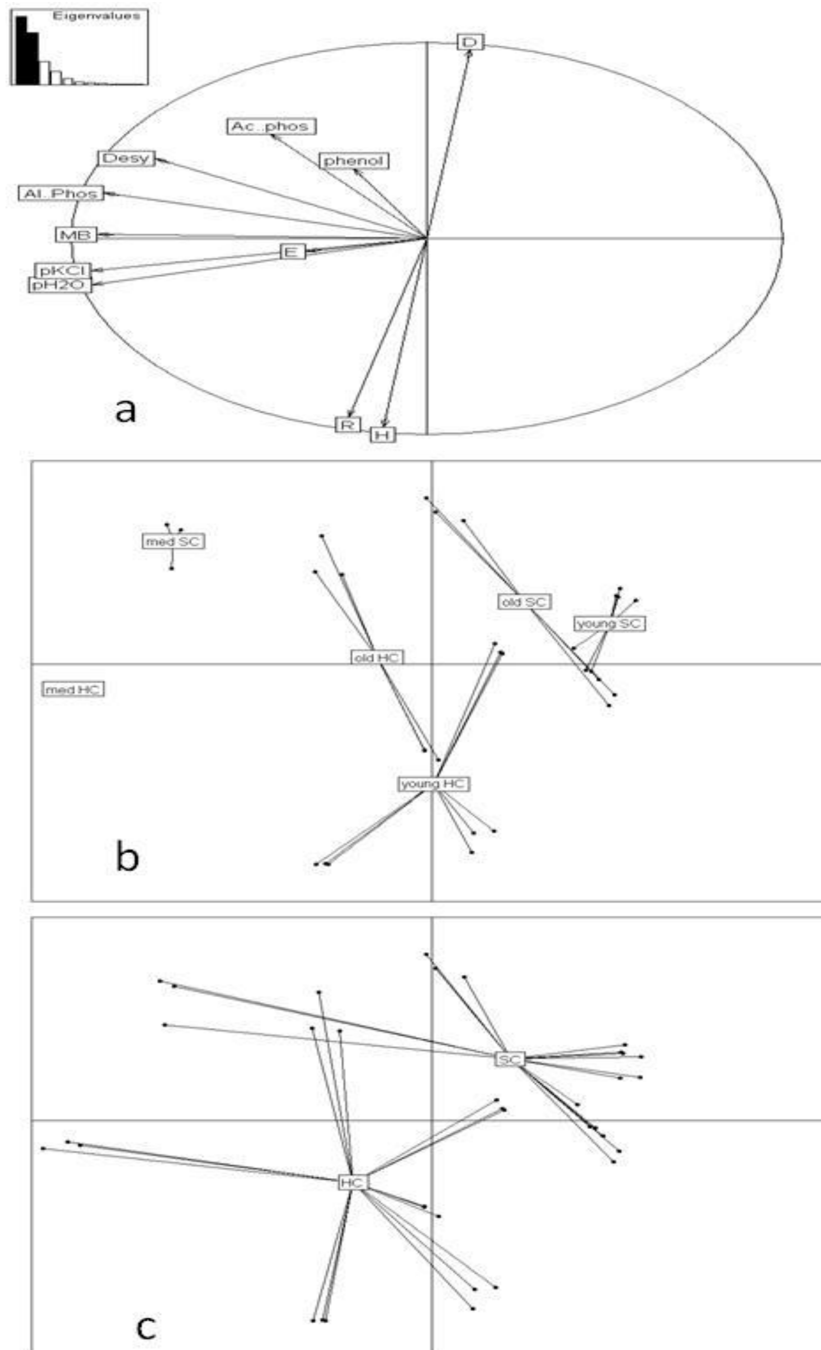

Figure 2. *Principal Component Analysis showing the relationship between the chemical and microbiological properties(a) of Eucalyptus plantations based on age (b) and sample type (HCvsSC) (c); SC: under Eucalyptus samples and HC : control samples*

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Canonical correlation analysis (*Fig. 2c*) showed a sharp contrast between HC and SC samples on basis of different variables measured. Irrespective of the age, the control samples (HC) were associated with low total polyphenol, high pH, high microbial biomass, high alkaline phosphatase, high dehydogenase and greater diversity index (H' and R). ANOVA between separate means is presented in *Table 2* and *Table 3*.

Differences in pH between HC and SC were significantly lower in soils under *Eucalyptus* compared to soils without *Eucalyptus* in all age groups (*Table 2*). However, this acidity was not associated with age of plantation. For example, old plantation of Lompoul and young plantation of Nioro showed the same Δ_{pH} (HC-SC) (1.2 and 1.3 respectively). Also, acidification effect on microbial activities was different. In the old plantation of Lompoul, microbial biomass was not changed but its activity and diversity were strongly modified (dehydogenase, alkaline phosphatase, E and H'). In contrast, in the old planting of Tamba, BM was significantly reduced as well microbial activities although, catabolic diversity remained intact. Within the group of young plantations, the effect of planting *Eucalyptus* on biological variables appeared similar through reduced BM, enzymatic activity and microbial diversity. ANOVA analysis showed that the difference in polyphenol content between HC and SC were significantly higher in SC irrespective of the age group (*Fig. 2*).

Functional diversity

In terms of functional diversity in the different age groups, Acid phosphatase activity did not present any significant difference between HC under SC (except in the intermediate plantation at Matam). While alkaline phosphatase activity was significantly highest in *Eucalyptus*-free soil (HC) (*Table 2*, *Fig. 2a* and *2c*). In most of the case, microbial biomass and dehydrogenase activity showed the same trend in different age groups.

The ISCP patterns highlight a strong site effect mainly due to the opposition of Matam/Lompoul, Sinthiou and Kolda. The soil of Matam was characterized by high levels of P, N, and high pH compared to other samples (*Table 4* and *Fig. 3a*).

If the site effect is removed, the difference between HC and SC is very clear (*Fig. 3a*). All groups of substrates induced significantly higher CO_2 emissions in control soils (HC) compared to SC samples except for the old plantation of Lompoul (*Fig. 3a*). The higher catabolic evenness E found in HC samples (*Table 2*) confirmed the functional difference. This difference in ISCP response between HC and SC samples was more marked in younger plantations (Sinthiou Garba, Nioro and Kolda sites) (data not shown) than old and intermediate plantations. However the permutation test of BGA between HC/SC was not significant (*Fig. 3b*).

Genetic diversity

Table 3 showed the effect of *Eucalyptus* plantations on bacterial communities. Importantly, the Shannon index showed that the microbial community diversity, characterized by the number and intensity of DGGE bands, was significantly higher in sites free of *Eucalyptus* (HC) compared to *Eucalyptus*-covered sites (SC) for each couple of soil samples. Specific richness (R) showed the same trend although the differences were not significant in all site. Contrarily, Simpson's dominance (D) was higher in SC samples compared to the controls for all groups (old, young and med). Dominance measures the relative abundances and decreases progressively as the relative abundance of microbes become more equitable. The maximum dominance (value 1) corresponds to a site that contains one dominant species.

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Table 2. *pH and biological characteristic of soils (means of three replicates)*

	old				med		young					
	Lompoul		Tamba		Matam		Sinthiou		Kolda		Nioro	
	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC
pH_{H2O}	6.34 a	5.12 c	6.41 b	5.87 a	7.44 a	6.87 b	6.17 b	5.35 e	5.28 c	5.55 d	6.83 a	5.53 d
pH_{KCl}	5.37 b	4.46 c	5.62 b	5.30 a	6.86 a	6.00 b	5.04 c	4.28 e	5.28 b	4.95 c	6.32 a	4.66 d
Total poly. *mg. g soil*$^{-1}$	4.81 bc	10.58 a	2.37 c	6.52 b	7.78 b	28.41 a	2.78 c	17.09 a	0.617 c	10.40 b	3.10 c	4.67 c
MB (µg*C-CO2 .100g soil*$^{-1}$*. h*$^{-1}$*)*	49,16 c	49,07 c	187,36 a	120,57 b	541,25 a	313,98 b	58,67 b	21,51 d	45,28 b	30,13 c	242,86 a	44,23 b
Simpson-Yule index (*E*)	30.10 b	28.73 c	32.2 a	32.3 a	30.20 a	27 ,0 b	31.70 a	30 ,0 b	24.1 d	18.2 e	29.6 b	25.2 c
Ac. phos. *(µg* pNPP g^{-1} .h^{-1})	206.1 c	346.7 cd	415.7 ab	509.2 a	333.6 b	1194.6 a	262.5 b	264.5 b	370.7 a	429.0 a	221.2 b	256.1 b
Al. phos (*µg* pNPP g^{-1}.h^{-1})	89.0 b	25.0 c	188.0 a	81.7 b	388.8 a	339.6 b	73.8 b	6.4 e	119.0 a	33.9 d	54.9 c	23.4 d
Desy (µg TPF g^{-1}. h^{-1})	6.51 b	1.22 c	11.02 a	8.56 b	8.765 a	8.194 a	2.11 b	1.24 c	5.09 a	2.978 b	4.64 a	2.392 b

Data in the same line followed by the same letter in each age range are not significantly different according to one-way ANOVA ($P < 0.05$). HC and SC as in *Figure 2.* MB: total microbial biomass, Al. phos: alkaline phosphatase, Ac. Phos: acid phosphatase, Desy: Dehydrogenase.

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Table 3. *Shannon (H), Simpson (D), Species richness (R) diversity indexes values for DGGE profiles under and out cover of E. camaldulensis*

Age	old				med		young					
	LHC	LSC	THC	TSC	MHC	MSC	SHC	SSC	KHC	KSC	NHC	NSC
H	1,523 a	1,480 b	1,426 c	1,397 d	1.484 a	1.460 a	1.574 b	1.452 e	1,470 d	1,486 c	1,586 a	1,426 f
D	0,031 e	0,034 d	0,03 a	0,04 a	0,034 b	0,04 a	0,03 d	0,04 a	0,035 b	0,038 c	0,04 d	0,03 a
R	37 b	36 b	31 a	27 c	35,6 a	34 ,6 a	40 a	40 a	33 b	32,6 b	41 a	29 c

Data in the same line followed by the same letter in each age range are not significantly different according to one-way ANOVA ($P < 0.05$). Abbreviations for sampling site L: Lompoul, M: Matam, S: Sinthiou, T: Tamba, K: Kolda, and N: Nioro; HC : control samples and SC : Under *Eucalyptus* samples.

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Table 4. *Soil chemical characteristics*

age	old				med		young					
site	Lompoul		Tamba		Matam		Sinthiou		Kolda		Nioro	
sample	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC
N total (%)	0.028	0.056	0.069	0.056	0.093	0.062	0.024	0.022	0.045	0.049	0.050	0.044
Carbone total (%)	0,295	0,688	0.992	0.807	0,95	0,699	0,24	0,224	0.599	0.671	0,629	0,54
P. total (mg/kg)	28	44	82	58	351	237	70	48	49	53	98	56
P. available (mg/kg)	3.49	11.35	6.11	8.73	141.40	47.13	4.36	6.11	3.93	4.80	20.08	9.16

HC: control samples, SC: Under *Eucalyptus* samples

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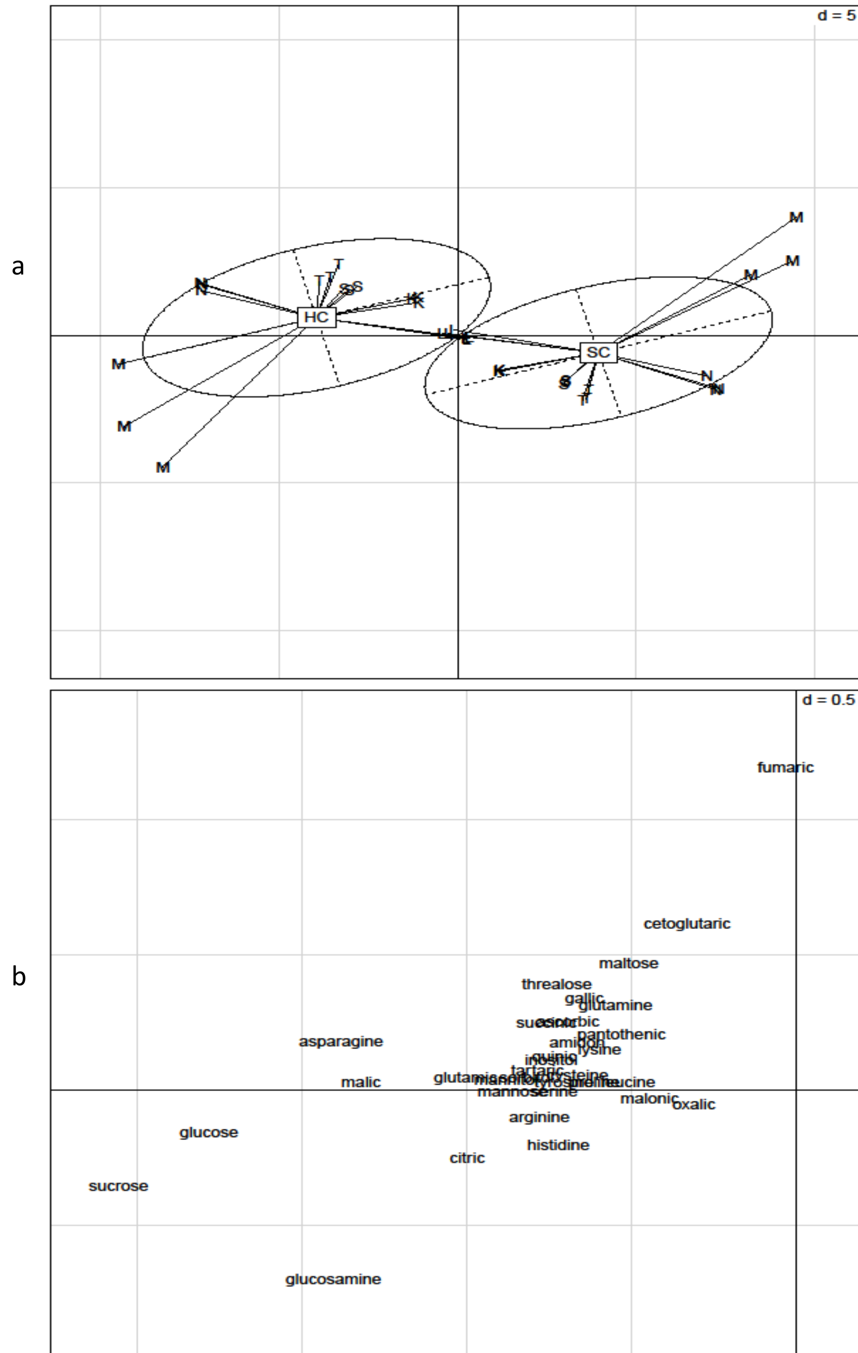

Figure 3. *Between-group analysis (BGA) of in situ catabolic potential (ISCP) responses of soils under Eucalyptus compared with control soils. M: Matam, N: Nioro, L: Lompoul, S: Sinthiou Garba, K: Kolda , T: Tamba, HC : control samples and SC: Under Eucalyptus samples. a) Factor map of subtracts b) Factor map of SIR responses of soils.*

Comparison of the bacterial community from *Eucalyptus* sites and control displayed distinct profiles on the DGGE gel (*Fig. 4*). The UPGMA dendrograms revealed that the structures of the bacterial communities from the young plantations were similar (similarity coefficient between 70 and 85%) to that from the old plantations (similarity coefficient 60%) (*Fig. 4a* and *4b*).

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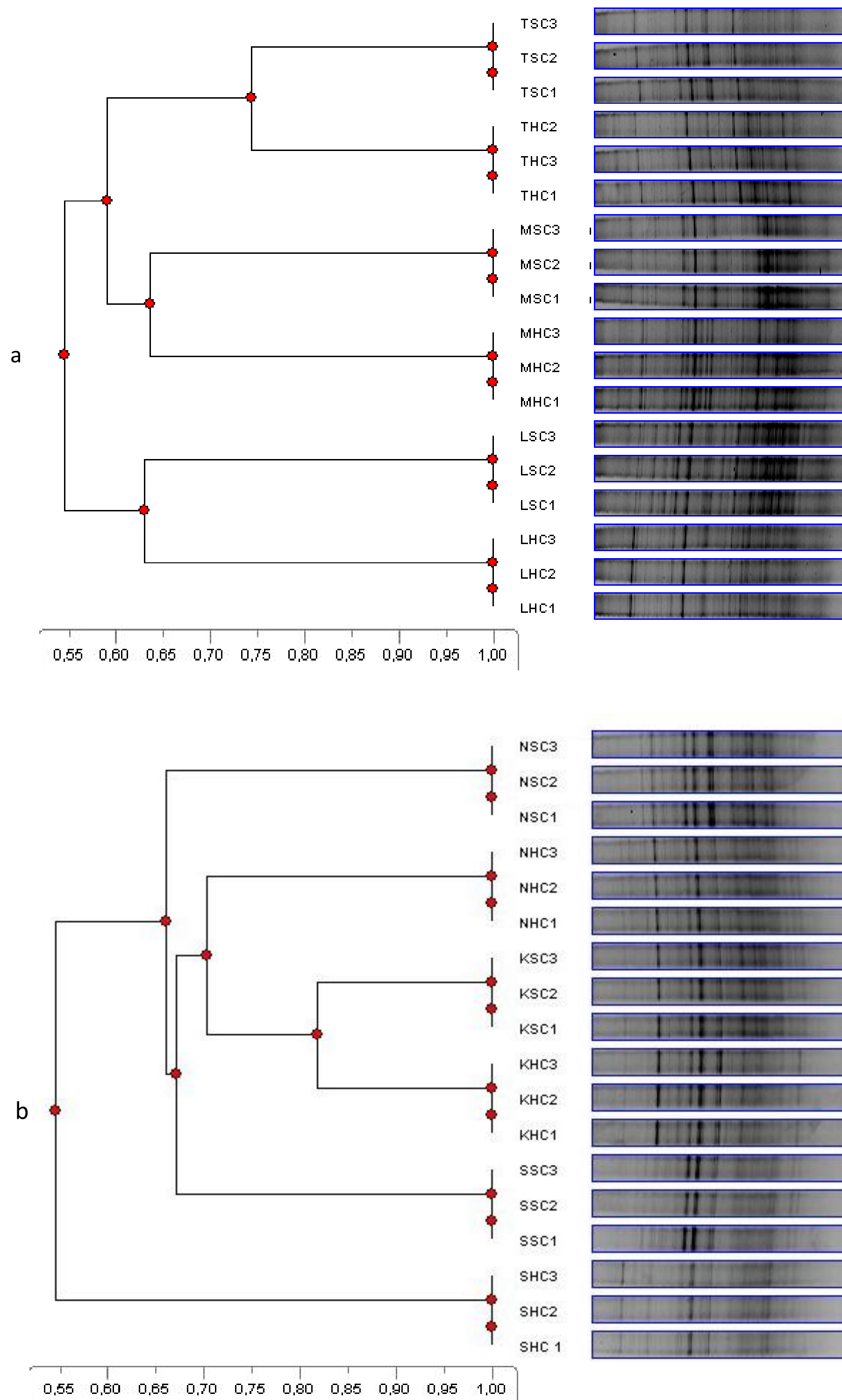

Figure 4. *Dendrograms comparing the DGGE profiles of bacterial communities of soil sampling in HC and SC of E. camaldulensis plantations. L, M, S, T, K, N; HC: and SC are the same as in figure 3. Number 1, 2 and 3 correspond to three replications of each sample*

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There were positive and significant correlations between microbial biomass and soil pH (R^2=0.87, p<0.05) and between MB and alkaline phosphatase (R^2=0.88, p<0.05). Also, positive correlations were found between soil total polyphenols content and acid phosphatase (R^2=0.65, p<0.05). In contrast, significant negative correlation existed between catabolic evenness (E) and total polyphenols content (R^2 = - 0.618, p<0.05) (*Table 5*).

***Table5.** Correlation analysis between different variables measured in soils*

Variables	Desy	Ac. phos.	Al. phos.	BM	pH	Total poly	E	H
Déshy	**1**							
Ac. phos.	**0,477**	**1**						
Al. phos.	**0,710**	0,563	**1**					
BM	**0,616**	0,329	**0,881**	**1**				
pH	0,393	0,202	**0,792**	**0,868**	**1**			
poly	0,208	**0,654**	0,377	0,342	0,054	**1**		
E	-0,391	**-0,824**	**0,697**	-0,517	-0,351	**-0,618**	**1**	
H	-0,301	-0,171	-0,278	-0,083	0,139	0,387	-0,061	**1**

Data values are different from 0 at significance level alpha = 0.05. Abbreviations: Al. phos: alkaline phosphatase, Ac. phos: acid phosphatase, Desy: dehydrogenase, Total poly: total polyphenol content.

Discussion

Ours results showed that *Eucalyptus* modified soil properties by acidification and /or accumulation of antimicrobial compounds such as phenols. In intermediate plantations, high levels of polyphenol in soils resulted from litter accumulation because plants were at the shorter spacing (observed during the sampling). In old and young plantations acidification seemed most obvious. This acidification of soils under *Eucalyptus* could be caused by an accelerated extraction of basic cations by this fast-growing tree species, especially when extraction was not offset by fertilization. Indeed, a recent study reported an acidification of surface water due to soil depletion of cations when watershed and pasture were replaced by *Eucalyptus* plantations (Farley et al., 2008). *Eucalyptus* litter can also release many acidic compounds. According to Swift et al. (1979), decreasing pH in decaying litter was mainly due to the leaching of acidic material from the vacuoles. This acidifying effect was also reported by a set of studies conducted in Uruguayon an experimental site, ten years after the original vegetation was replaced by *Eucalyptus* plantation (Durán et al., 2001; Pérez Bidegain et al., 2001; Sicardi et al., 2004).

The consequences of these soil chemical modifications were the decline in microbial biomass, activity and diversity. In fact, the results obtained from the current study showed low microbial biomass and low catabolic capacity and low diversity (Shannon index and Evenness) under *Eucalyptus* soil. Similar results were obtained on *Gmelina arborea* Roxb and *E. camaldulensis*, two exotic species, by Sanon et al. (2006)

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and Kisa et al. (2007) respectively. These authors have shown that the exotic plants considerably alter soil properties by modifying both microbial community structure and functional diversity.

Ours results showed that acidification was not proportional to plantation age and its effect on microbial activity and biomass could be different even within the same age plantation. This difference could be related to soil texture which unfortunately was not measured in this study. But according to early studies, the soil of Matam (intermediate plantation) was a loam clay soil (Maymard, 1962) and was closed to a river, thus releasing few cations so little subject to acidification. Thus soil pH was highest (pH 6.87) in Matam. Whereas Nioro is a ferruginous soil (Pieri, 1969), Sinthiou and Lompoul are sandy (Maymard, 1962). These soils were more susceptible to acidification. Highest pH in Matam site compared to other plantations could explain highest enzymatic activities and microbial biomass.

Low microbial biomass and activity found under *Eucalyptus* plantation could be due to the toxic impact of harmful allelochemical compounds (including polyphenols) released from the *Eucalyptus* leaf litter. Negative correlation between catabolic Evenness (E) and total polyphenol contents suggests that phenols reduce microbial activity. Previously, Dellacassa et al. (1989) have reported antimicrobial activity of *Eucalyptus* leaf extract toward pure culture of soil bacteria. Consistently, our study also reported high rates of total polyphenols under *Eucalyptus* soil samples compared to adjacent soils. Polyphenols are known for their negative role in the mineralization process of organic matter either by complexing the protein nitrogen which would make it inaccessible to microorganisms or by the inhibition of microorganisms or enzymes responsible for the transformation of ammonium nitrate (Mangelot and Toutet, 1980; Duponnois et al., 2001; Diallo et al., 2006). The results of Blum and Shafer (1988) and Diallo et al. (2006) showed that the effect of phenols depend on the phenolic acid composition rather than concentration. According to these authors, acidic phenolic compounds have more negative effects alkaline than phenolic. The determination of soil phosphatase activities indicated less bacteria activity under *Eucalyptus* (evidenced by alkaline phosphatase) than in adjacent samplings. Furthermore, high fungal activity (evidenced by acid phosphatase) was found in samples under *Eucalyptus*. This change in soil microorganism activities could be related to changes in pH induced by *E. camaldulensis*. Similar results have previously been documented by Bradget et al. (1993) who showed that acidification increased abundance of fungal communities in the Boreal forest, whereas bacteria increase in alkaline soils. Our findings thus support previous observations suggesting that soil microbial communities composition is greatly controlled by pH as shown by the strong and positive correlation between pH and MB.

Changes (acidifying effects and/or high polyphenol content) mediated by *E. camaldulensis* in soils can reduce catabolic groups and/or soil catabolic ability. Indeed, *E. camaldulensis*, by its root exudates and litter fall select microorganisms able to use its carbon residues and support its acidifying effect. Meyer (1994) and Kourtev et al. (2003) demonstrated that exotic species promote changes in functional groups within the microbiota. Similar results were reported by Saetre and Bååth (2000) and Yao et al. (2000) which showed that changes in the ratio of Gram-negative to Gram-positive bacteria were related to quality of organic matter in the soil under exotic species. Recent studies that have used BIOLOG (Yu et al., 2005), noted great modifications in catabolic activity forms of soil bacterial community with 31 carbon resources in soils treated by *Eupatorium adenophorum* (an exotic invasive species) root water extract. In our study,

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the combined effect of low pH and litter accumulation could explain the lack of herbaceous layer noted under *E. camaldulensis*.

Conclusion

The results showed that *Eucalyptus* cultivation negatively impacted on soils at all sites regardless of the age of the plantation. Intermediate plantation appeared to be characterized by high levels of phenols, while in young and old plantations pH effect was dominant. In both situations, this had a negative effect on microbial biomass, activity and diversity.

Among all the measured indicators, acidification, phosphatases activities and the accumulation of phenols were the most common observations in all sites. The effect of *E. camadulensis* would start with changes in these parameters, which will in turn affect microbial communities by changing their biomass, structure and/or catabolic diversity. The synergistic effect of these changes in soil might resulted in a novel ecological niche in soil which is less favorable to herbaceous plants growth and might probably induce the lack of herbaceous layer under *Eucalyptus* plants. Our study thus supports the existence of environmental hazards that may result from the use of this exotic plant for reforestation purposes. The present findings should therefore be of great importance for reforestation programs challenging plant biodiversity preservation.

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