Review Article

Effect of soil collected under *Eucalyptus camaldulensis* dehn on peanut (*Arachis hypogaea* L.) development and soil microbial community structure

ABSTRACT

Aims: The objective of this study was to determine the effect of soil sampled under *E. camaldulensis* on peanut development and microbial community.
Place and Duration of Study: Soil sampling at the 0-10 cm horizon was conducted at different distances: 1.5 m, 3 m, 4.5 m, 6 m and 30 m from 3 randomly selected E. camaldulensis plants in four sites: Karamba, Nicia, Rokout and Dioncome
Methodology: A two-factor randomized complete block design was set up in a greenhouse experiment and parameters such as chlorophyll content, acetylene reducing activity, mycorrhization rate, number of nodules, mass of dry matter and microbial community structure were studied.
Results: No significant difference was noted among the parameters regardless of the sampling distance for the Karamba, Nicia and Dioncome sites ($P>0.05$). In the case of Rokout site, however, the chlorophyll content measured at 4.5 m was significantly different from the chlorophyll content measured at 1.5 m from the tree. For aboveground dry biomass, no significant difference was noted regardless of sampling distance at Karamba and Rokout sites except for samples collected at 4.5 m ($Pa\leq0$, 045; $Pc\leq0.029$). No significant difference was also noted for root biomass regardless of sampling distance at the Karamba, Rokout, and Dioncome sites ($P>0.05$). However, at Nicia, the biomass of the control treatment was significantly higher compared to others ($P=0.021$). Nodulation did not vary according to sampling distance ($P>0.05$). The amount of nitrogen fixed is higher at 6 m from *Eucalyptus* compared to other distances at the Karamba and Nicia sites ($P<0.02$). In contrast, at Boucotte it is higher at 1.5 m from the tree ($P<0.03$). Mycorrhization intensity was significantly higher at 6 m and 30 m from the tree compared to other distances.
Conclusion: Microbial community structure differed between soil collected under and outside of the canopy of *E. camaldulensis*.

Keywords: Eucalyptus camaldulensis, peanut, microbial community

1. INTRODUCTION

In arid and semi-arid zones, the major constraints to crop production are poor soils, low and irregular rainfall. In addition, overexploitation of the land (expansion of the area under crops, overgrazing) and the advance of salinity lead to desertification and soil degradation [1]. Since colonial times, African forests have been subjected to irreversible management policies resulting in the plantation of large areas with exotic trees, such as those belonging to the genera Pinus and Eucalyptus. The mass introduction of these exotic species in Africa

and particularly in Casamance has consequences on plant biodiversity but also on soil microbial communities [2]. Indeed, the microclimate due to the presence of these trees, particularly *E. camaldulensis*, can have effects on crops depending on the degree of root radiation. It has been shown that certain species of Eucalyptus do have an inhibiting effect on the associated vegetation. (1970) [3] noted that *E. camaldulensis* inhibited some improved forage grasses such as *Bromus mollis* and *Lolium multiflorum*. Under *E. camaldulensis* canopy, soil moisture is higher underneath than outside. This may be due in part to the amount of shade that helps reduce transpiration from the underlying crops and evaporation of water [4]. *E. camaldulensis* plants are demanding in terms of water and mineral elements. Leaf biomass can be quite large and difficult to decompose; which, in some cases, leads to an increase in soil acidity, an increase in exchangeable aluminum and a decrease in water retention capacity, as well as a decrease in enzyme activities [5]. As a consequence of this decrease in soil fertility, physiological parameters and crop yields are directly affected. The introduction of legumes in an agroforestry system in association with *E. camaldulensis* could be an alternative to solve this problem. The effect of *E. camaldulensis* on the associated peanut crops could be positive if Eucalyptus lines and crop lines were sufficiently distant. Indeed, legumes in association with rhizobiaceae are able to fix atmospheric nitrogen, thus enriching the soil in nitrogen. This nitrogen enrichment promotes the activity of microorganisms and improves nutrient cycling, while positively influencing tree growth [6]. However, even though the legume positively influences tree growth, little research has been conducted to evaluate the actual effects of the tree on the legume and soil microbial activity. It is in this perspective that this work was conducted to determine the effect of soil sampled under *E. camaldulensis* on peanut production and on some growth parameters, but also on the soil microbial community. The assumption is that the presence of *E. camaldulensis* through its litter would have positive effects on the development of legumes and on the structure of the soil microbial community.

2. MATERIAL AND METHODS

2.1 Soil sampling

Soil was collected at different distances from the collar 3 randomly selected *E. camaldulensis* plants at each site. Four distances from the tree were determined following the East-West, North-South orientations: 1.5m, 3m, 4.5m and 6m (Fig. 1). For each tree and for each distance from the tree, 4 samples were taken with an auger at the 0-10 cm horizon and mixed to form one (1) composite sample per distance. At each site, 3 control samples off-cover (HC), were taken in an east-west, north-south orientation. These control samples are assumed to be uninfluenced by *E. camaldulensis*. A total of 60 composite soil samples were collected from the four (4) sites. These soil samples were then used as the base substrate for the greenhouse trial.

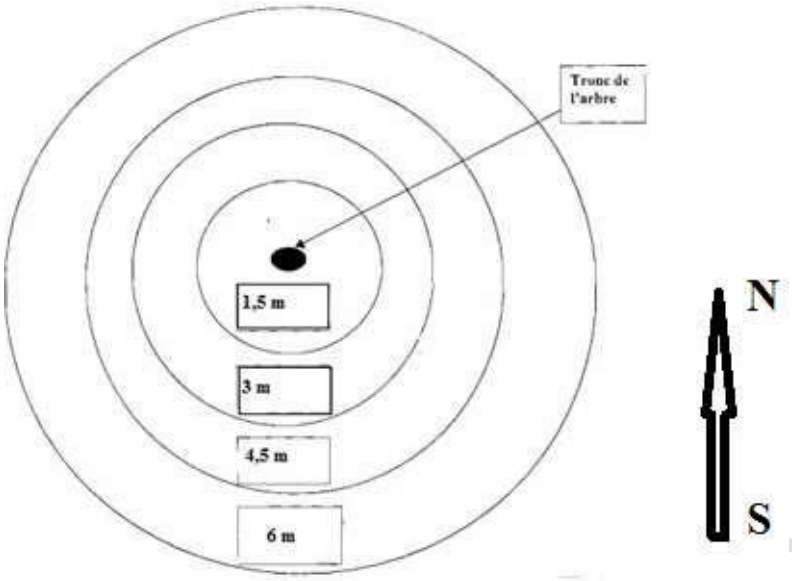


Fig. 1. Soil sampling design

2.2 Experimental design

A randomized complete block design with two factors (site and distance to tree) was used for this greenhouse experiment. Pots with a capacity of 2 L were filled with 1.5 kg of dry soil from the 60 samples taken from the different sites. The pots were grouped into blocks by site of origin. Each block consisted of 15 pots including 3 HC controls and 12 samples taken under the canopy of *E. camaldulensis* plants at D 1.5m, D 3m, D 4.5m, D 6m from the 3 trees.

The variety of peanut sown in the pots is 'Boulkousse'. Two peanut seeds were sown per pot and then after a week, only one plant was kept per pot. Plants were watered every other day with the same amount of tap water. After 60 days of growth, they were harvested (Fig. 2).

Fig. 2. Peanut plants grown in greenhouse

2.3 Aboveground and root biomass sampling

Aboveground and root biomass of peanut was harvested at 60 days after sowing (DAS). The plants were first separated from the soil, cleaned with tap water and then aboveground biomass was manually separated from the root part.

A portion of the aboveground biomass was collected for measurement of chlorophyll content. To determine the acetylene reducing activity and the mycorrhization rate, a mass equal to 10 g of roots was isolated. The remaining aboveground and root biomass were oven-dried at 65°C for 72 h and then weighed with a precision balance 10-2 (Sartorius TE 124S, Germany).

2.4 Extraction and determination of chlorophyll

Extraction of chlorophyll was done following the method of [7]. A 100 mg mass of freshly harvested leaves was ground in 80% acetone buffer (80 ml acetone in 20 ml 2.5 mM sodium phosphate buffer at pH 7.8) and then incubated overnight at 4°C in smoked corex tubes, to avoid photo-oxidation of chlorophyll. The supernatant was centrifuged at 10000 g (Rotor Nr 12154, Sigma 3K15, USA) for 10 min at 4°C. Optical density (OD) was determined for wavelengths 663 nm and 645 nm with a spectrophotometer (Ultrospec 3000). A blank control was prepared using 80% acetone (Fig. 3).

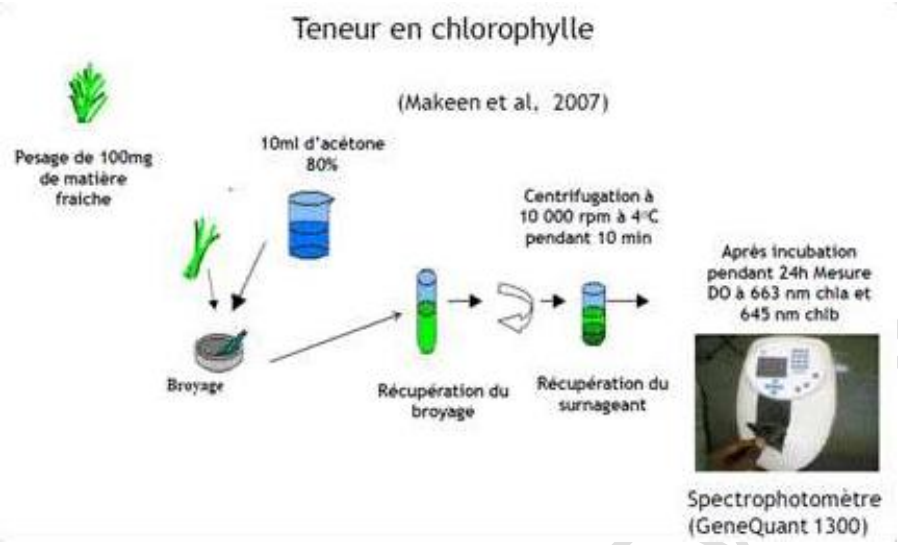


Fig. 3. Different phases of chlorophyll content measurement [8]

The total chlorophyll content was determined by the following formula: Total Chlorophyll= [(8,02* OD 663+ (20,2* OD 645)] * (V/M)

With OD= Optical density, V=Volume, M= Total mass of leaves

2.5 Measurement of acetylene reducing activity "ARA

Biological fixation of atmospheric nitrogen is an activity specific to a class of plants of which legumes are a part. This activity is facilitated by an enzyme called nitrogenase. This non-specific enzyme is able to catalyze other substrates characterized by the presence of a triple bond, such as acetylene (C H22), which it reduces to ethylene (C H24). To measure acetylene reducing activity, nodulated peanut roots were rinsed and placed in 500 ml vials and resealed (Fig. 4). A volume of 1 ml is taken from these 500 ml flasks containing the roots and then released to the air corresponding to 10% of the incubation volume. An equivalent volume (1 ml) of acetylene is injected into these root vials. These flasks are then incubated at 36°C for 1 hour. The measurement of acetylene reducing activity was then done using a chromatograph. The ethylene peak is observed at 0.4, while the acetylene peak is observed at 0.48 on the chromatograph.

Fig. 4. Nodulated peanut roots and 500 ml flasks containing nodulated roots

The calculation of the SCBA expression is performed according to the following equations:

Number of moles of C H24 = (X *4.287.10-8) moles of C H24 / l of air

Number of mol of N2 = 3 n C H24 mol of N2 / l of air

2.6 Study of the microbial community

2.6.1. Determination of mycorrhization and nodulation rates

In order to determine the mycorrhization intensity, root staining was performed following the method of Phillips and Hayman (1970) [9]. Fresh root fragments were soaked in a KOH solution (10%) and then boiled in a water bath for 1 h at 90°C to clarify them. The KOH was removed by rinsing with water and then Trypan blue as a dye was added to the tubes containing the roots and boiled for 20 min. A final rinse was performed to remove excess dye. Root fragments of about 1.5 cm were placed between slides and coverslips at a rate of 10 fragments/ slide and 5 slides per treatment. The fragments were then observed under a microscope (× 250) and the mycorrhization intensity of each fragment was determined following the scoring system of [10] (Fig. 5).

For the nodulation rate, the nodules formed on the roots of each plant were harvested, counted, dried and then weighed using a precision balance 10-2 (Sartorius TE 124S)

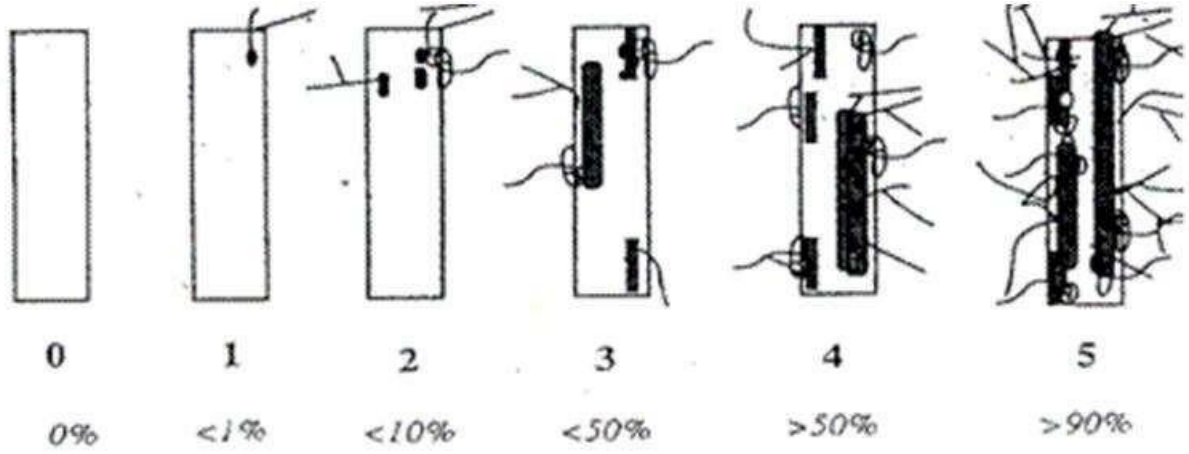


Fig. 5. Mycorrhizal infection scoring (class 0 to class 5) [10]

2.6.2. Characterization of the structure of the bacterial community

The structure of the bacterial community was determined by PCR-DGGE (Polymerase Chain Reaction - Denaturing gradient gel electrophoresis), after soil DNA extraction.

2.6.2.1 Extraction of total DNA from soil

Total DNA from each sample was extracted from 0.5g of soil using a FastDNA Spin kit for soil (MP biomedicals, Qbiogene,). For all four (4) sites, one (1) composite sample per treatment was taken. Soil samples contained in a buffer solution (Sodium Phosphate Buffer and MT Buffer) were vigorously agitated using the Fast REP FP120 Bead-beater (SAVANT Instrument Inc. Holbrook NY) for 30 seconds at 5500 beats/min and then centrifuged for 30 seconds (1400g Ependorf centrifuge 5415R). DNA is recovered after protein precipitation (PPS, Qbiogene). The DNA is then purified. Its concentration is estimated by agarose gel electrophoresis and after comparison of the fluorescence intensity of the extracted DNA with a range of thymus DNA of known concentration (6.25 to 200 ng/µl).

2.6.2.2 PCR amplification of the DNA of the total bacterial community

The study of the total bacterial community was performed by targeting the V3 region, which is a variable region of the 16S rDNA of total bacteria. Amplification of the V3 region was performed using a model 9700 thermal cycler (Perking-Elmer, France). The universal primer pair EUB338f (5'- ACTCCTACGGGAGGCAGCAG-3') with GC clamp [11] and UNIV518r (5'-ATTACCGCGGCTGCTGG-3') (1997) [12] was used. The obtained rDNA fragments of about 200 bp were checked on a 0.8% agarose gel (Amersham Biosciences, USA) after staining with ethidium bromide (10 mg ml-1).

2.6.2.3 DGGE (Denaturing Gradient Gel Electrophoresis)

The rDNA fragments obtained by PCR are separated on an 8% denaturing polyacrylamide gel by electrophoresis (DGGE). Migration is performed at 100 volts for 18 h in 1X TAE buffer at 60°C. This procedure allows the DNA fragments of the microorganisms to migrate according to their nucleotide composition (A, T, G and C) and thus determine the structure of

the bacterial communities. The revelation of the rDNA fragment profiles is done under UV light (254 nm), after staining the gel with BET (1 mg/ml) for 30 min and washing with demineralized water for 10 min. The acquisition of the images of the rDNA profiles obtained was done with the Bio-Capt software (Vilber Lourmat). V 99.04. The profiles obtained were analyzed with Phoretix 1D. Presence-absence matrices were used to determine the difference between the profiles of each treatment of DGGE gels. The same software was used to construct the similarity dendrogram with the Dice coefficient (5% confidence interval) using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) algorithm.

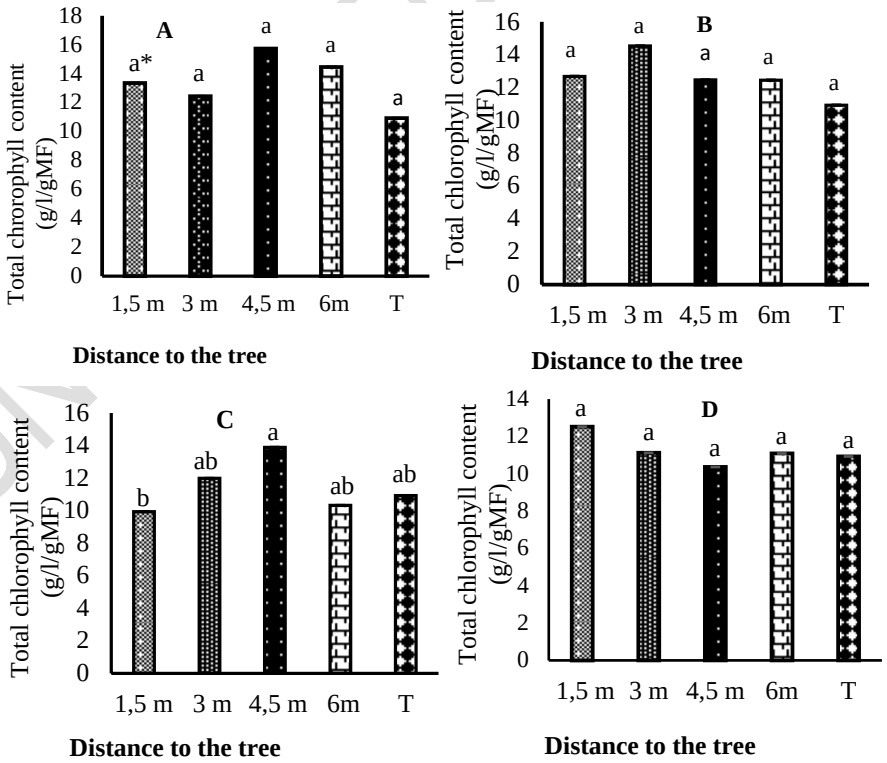
2.7 Statistical analysis

The analyses of variance (ANOVA) were performed with XLSTAT 2014 version 16.5.03. The Fisher LSD test was used to compare the means when the ANOVA revealed significant differences at the 5% level.

3. RESULTS AND DISCUSSION

3.1 Effect of soil collected at different distances from Eucalyptus camaldulensis on total chlorophyll content

Chlorophyll contents of peanut plants grown in soils sampled at different distances from *E. camaldulensis* were not significantly different regardless of sampling distance at the Karamba, Nicia, and Dioncome sites (PA = 0.442; PB = 0.697; PD =0.802, Fisher's 5% threshold) (Fig. 6).



*Bars with the same letters are not significantly different at the 5% threshold

Fig. 6. Variation in chlorophyll contents of peanut plants as a function of distance from *E. camaldulensis* canopy

Site A = Karamba, Site B = Nicia, Site C = Rokout, Site D = Dioncome

However, no significant difference was noted at Rokout sites for distances D 1.5 m and D 4.5 m from *E. camaldulensis* (PC =0.035, Fisher, LSD at 5% threshold) (Fig. 6).

3.2 Effect of soil taken at different distances from *E. camaldulensis* on above-ground and dry root biomass

The dry aboveground biomass measured was not significantly different regardless of the distance from the tree, with the exception of the Karamba site (P>0.05, Table 1). In this site, the biomass obtained at D 4.5 m from *E. camaldulensis* is higher than at D 1.5m, D 3m and D 6m from the tree (P=0.045).

Table 1. Variation in aboveground and root biomass by site and distance from *E. camaldulensis*

Distance to the tree	BA (g)				BR (g)			
	Site A	**Site B**	**Site C**	**Site D**	**Site A**	**Site B**	**Site C**	**Site D**
At 1.5 m	1,72 b*	2,15 a	2.49 ab	2,53 a	0,35 a	0.23 ab	0,26 a	0,36 a
At 3 m	1,78 b	1,96 a	2.84 ab	2,83 a	0,28 a	0,19 b	0,26 a	0,41 a
At 4.5 m	2,61 a	2,75 a	3,39 a	3,02 a	0,27 a	0.21 ab	0,32 a	049 a
At 6 m	1,93 b	2,19 a	2.42 ab	2,79 a	0,27 a	0.24 ab	0,26 a	0,35 a
T	2.22 ab	2,31 a	2,2 b	2,7 a	0,37 a	0,39 a	0,34 a	0,33 a
Pr > F	-	ns	ns	ns	ns	-	ns	ns

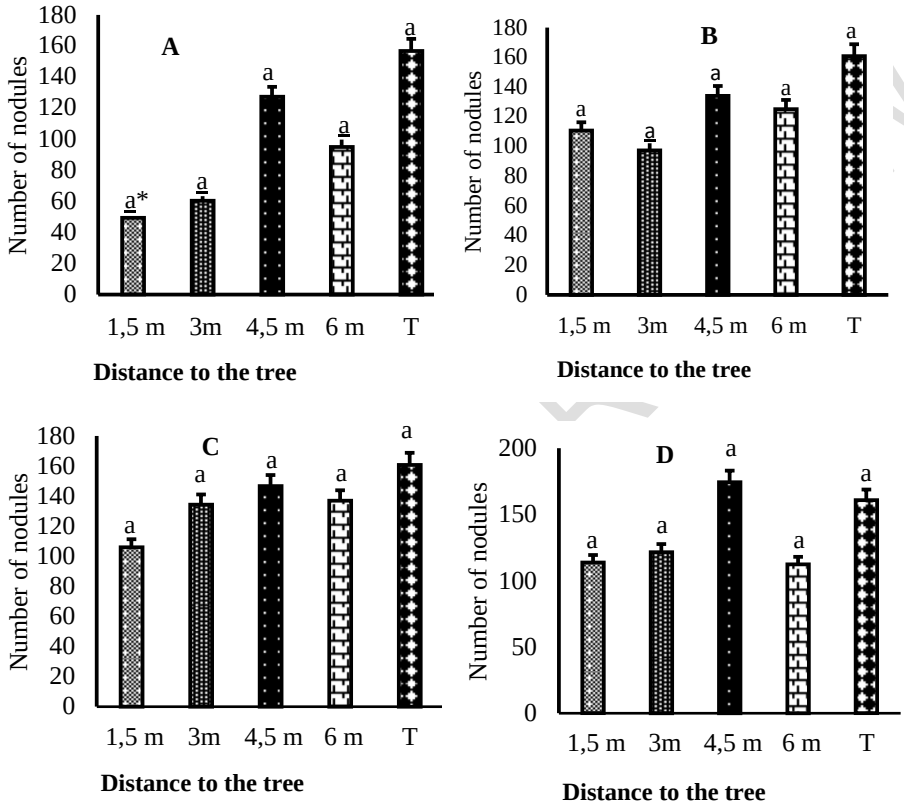
*At each site, treatments with the same letters per column are not statistically different (Fisher test, P<0.05). BA= Aboveground biomass, BR= Root biomass

Site A = Karamba, Site B = Nicia, Site C = Rokout, Site D = Dioncome

The same trend was observed for root biomass. In fact, the analysis of root biomass data showed no significant difference whatever the distance from *E. camaldulensis* at the Karamba, Rokout and Dioncome sites (P>0.05). In contrast, at Nicia, there was a significant difference between the biomass obtained from the control and that measured at 3 m from E.camaldulensis (P =0.022). Indeed, the root biomass obtained at the control level is 0.39 g while that measured at 3 m from the tree is 0.19 g. In sum, these analyses show that the sampling distance from *E. camaldulensis* has no significant effect on the aerial and root biomass of groundnut (P>0.05).

3.3 Effect of soil collected at different distances from Eucalyptus camaldulensis on peanut nodulation

Statistical analysis (ANOVA) showed no significant difference between the number of nodules obtained with soil collected at 1.5 m; 3 m; 4.5 m; 6 m from *E. camaldulensis* compared to the control at the Nicia, Rokout and Dioncome sites, with respectively PB =0.434, PC = 0.434, PD = 0.363 (Fisher's test at the 5% threshold) (Fig. 7).



*Bars with the same letters are not significantly different at the 5% threshold

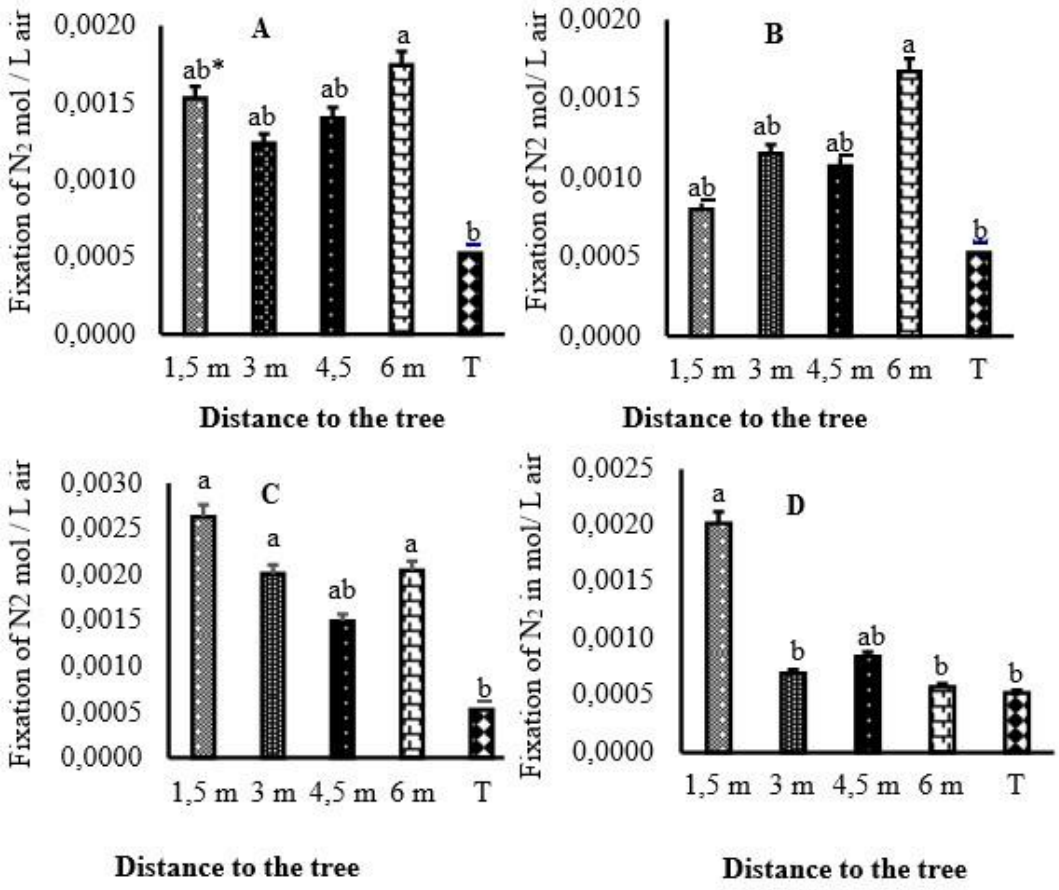
Fig. 7. Variation in the number of nodules produced by peanut as a function of distance from *E. camaldulensis*

Site A = Karamba, Site B = Nicia, Site C = Rokout, Site D = Dioncome

In contrast, at Karamba, the average number of nodules counted on groundnut plants grown in soil sampled at the control level was significantly higher compared to that obtained at 1.5 m and 3 m from *E. camaldulensis* (P=0.05, Fisher, LSD, at the 5% threshold). Overall, the results of the analysis (ANOVA) showed that distance from *E. camaldulensis* had no significant effect on peanut nodulation (P>0.05, Fisher, at 5% threshold) (Fig. 7). Sampling distance does not negatively influence the number of nodules

3.4 Effect of soil taken at different distances from Eucalyptus camaldulensis on biological nitrogen fixation

The amount of nitrogen fixed by peanut plants grown on soil sampled at 6 m from *E. camaldulensis* was significantly higher than that of plants grown in control soils at Karamba and Nicia (Pk =0.022, Pn =0.046, Fisher, LSD at 5% threshold) (Fig. 8).



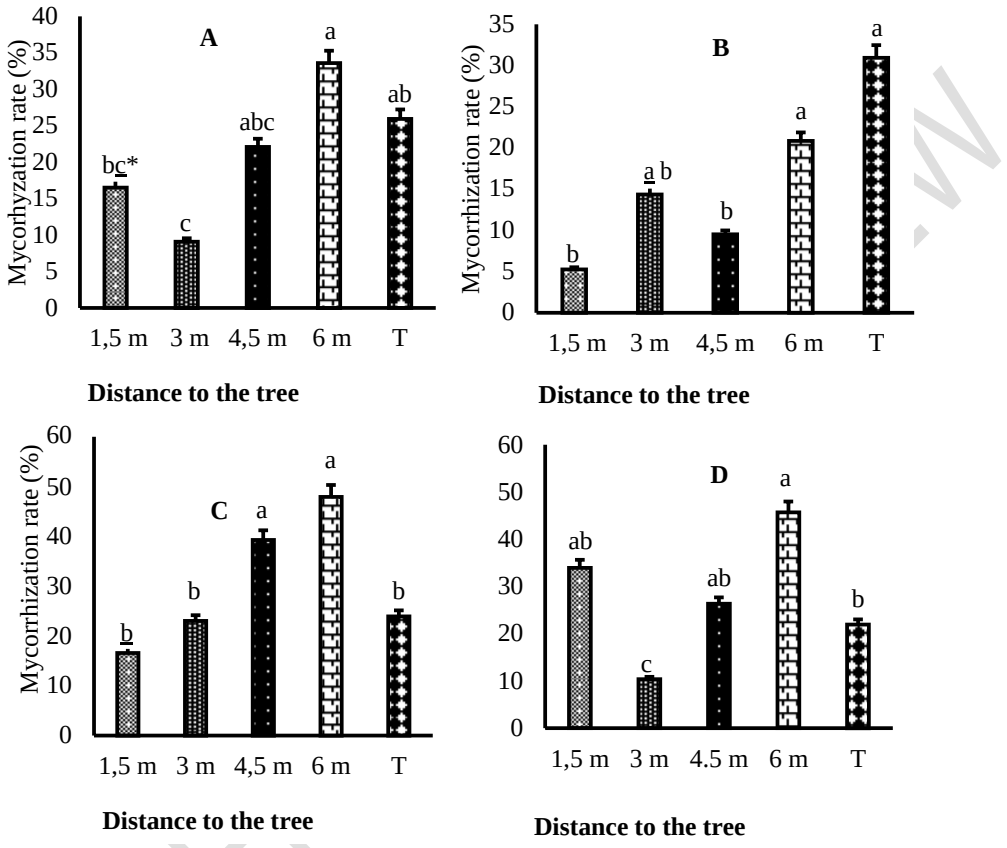
Bars with the same letters are not significantly different at the 5% threshold

Fig. 8. Variation in biological nitrogen fixation with soil removal distance from *E. camaldulensis*. Site A = Karamba, Site B = Nicia, Site C = Rokout, Site D = Dioncome

In contrast, no significant difference was noted between the amount of nitrogen fixed at 6 m from *E. camaldulensis* compared to other distances. This same trend was observed at the Rokout and Dioncome sites. Indeed the amount of nitrogen fixed by plants grown on soil taken at 1.5 m from *E. camaldulensis* was significantly different from that fixed by plants grown at the control at the Rokout and Dioncome sites (Pr ≤0.03, PD ≤0.043, Fisher, at the 5% threshold). However, no significant difference was noted between N fixed at 1.5 m from *E. camaldulensis* and that at other distances (P>0.05, Fisher, at 5% threshold). Overall, soil collected at different distances from the collar of *E. camaldulensis* had statistically significant effects on biological nitrogen fixation (P=0.00013, Fisher, at the 5% threshold) (Fig. 8). Biological nitrogen fixation varied with sampling distance from *E. camaldulensis*.

3.5 Effect of soil taken at different distances from Eucalyptus camaldulensis on mycorrhization

The rate of mycorrhization is significantly higher for plants grown on soil collected at 6 m from *E. camaldulensis* in the Karamba, and Dioncome sites (Fig. 9) (PK =0.021, PD <0.0001, Fisher, LSD at 5% threshold) (Fig. 9).



Bars with the same letters are not significantly different at the 5% threshold

Fig. 9. Variation in mycorrhization intensity with distance from *E. camaldulensis*

Site A = Karamba, Site B = Nicia, Site C = Rokout, Site D = Dioncome

At Nicia, mycorrhization intensity is significantly higher at D 6m and at the control compared to the other sampling distances (Pn =0.026, Fisher, 5% threshold). Similarly, at the Rokout site, the mycorrhization intensity measured at D 4.5m and D 6m of *E. camaldulensis* was significantly higher compared to that of the plants grown at the other sampling distances (Pr =0.002, Fisher, LSD at 5% threshold). In sum, the analysis revealed that mycorrhization intensity varied with site and sampling distance (P=0.022, Fisher, LSD at 5% threshold) (Fig. 9). Distance from *E. camaldulensis* had positive effects on mycorrhization intensity.

3. 6 Correlation between measured variables

Table 2 presents the Pearson correlation coefficients between the physiological parameters of peanut and the biological parameters of the soil.

Table 2: Correlation between the different measured parameters

Variables	**B A**	**B R**	**Total Chl**	**Mycorh**	**nodules**	**N2 Fixing**	**Microbial community**
B A	**1**						
B R	0,25	**1**					
Total Chl	**0,769**	-0,379	**1**				
Mycorh	0,170	0,066	-0,092	**1**			
nodules	**0,3404**	**0,9385**	-0,331	0,269	**1**		
Fixing N2	0,279	**-0,705**	**0,661**	**0,376**	**0,647**	1	
Microbial community	**0,386**	-0,523	0,527	**0,701**	-0,288	**0,828**	**1**

A positive and significant correlation exists between chlorophyll content and aboveground biomass (r = 0.77; p = 0.05). Similarly, there is a strong correlation between the number of nodules and root biomass (r = 0.94; p = 0.05). Biological nitrogen fixation is also correlated with chlorophyll content (r=0.66; P=0.05), between biological fixation of atmospheric nitrogen and the number of nodules (r=0.65; P=0.05). This correlation is also high between microbial communities and the biological fixation of atmospheric nitrogen (r= 0.83, P=0.05) and between total microbial communities and mycorrhization (r=0.70; P=0.05). On the other hand, the correlation is negative between biological fixation of atmospheric nitrogen and root biomass of peanut (r =-0.70; p=0.05). Between mycorrhization and aboveground biomass, there is no correlation (r=0.006; P=0.05). Figure 10 showed a clear separation of the studied parameters.

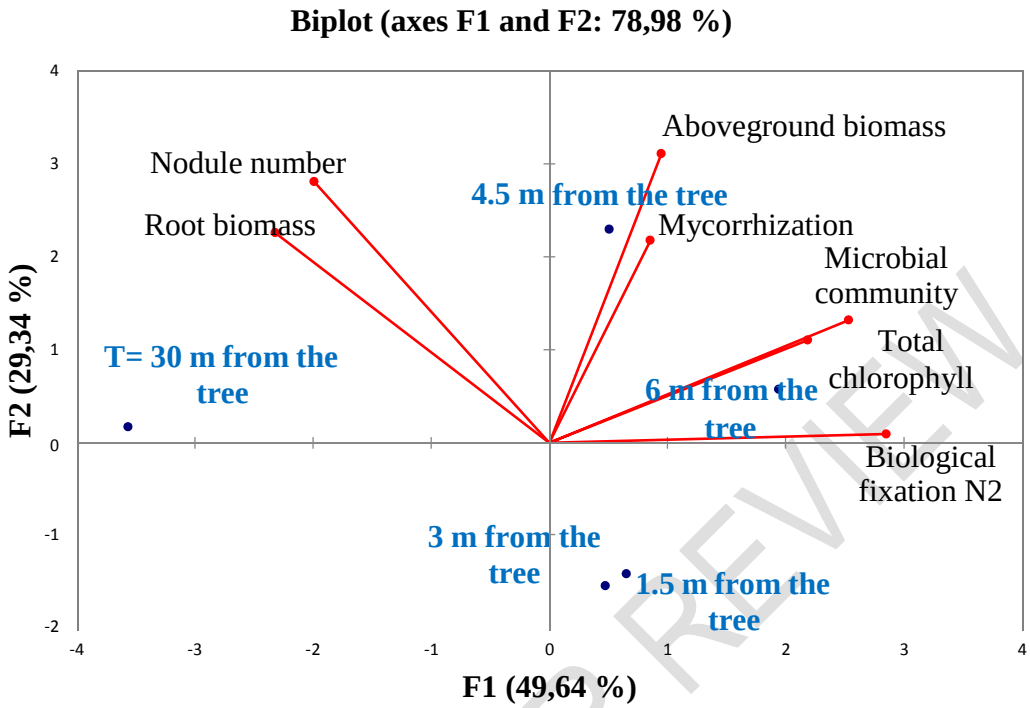


Fig. 10. Correlation between measured variables and distance to *E. camaldulensis*

Along the F1 axis, on the positive abscissa side, the parameters aerial biomass, mycorrhization, microbial community, total chlorophyll and biological nitrogen fixation have high values for D 4.5m and D 6 m. Along the same axis, these parameters are opposed to the root biomass and nodule number parameters, which have high values for the control. This axis 1 explains 49.64 % of the variations observed in the distribution and correlation between parameters such as aerial biomass, mycorrhization, microbial community, total chlorophyll and biological nitrogen fixation.

Along the F2 axis, on the positive ordinate side, it can be seen that the above-ground and root biomass parameters, mycorrhization intensity, microbial community, chlorophyll content, and biological nitrogen fixation have the highest values at 4.5 m, 6 m and at the control level for *E. camaldulensis*. These different parameters measured at these distances are opposed to those obtained at 1.5 m and 3 m for *E. camaldulensis*.

3.7 Effect of soil collected at different distances from Eucalyptus camaldulensis on the microbial community

Examination of the DGGE profiles from the different treatments showed the presence of dominant and similar microbial bands or communities across sampling distances to *E. camaldulensis* (Fig. 11). This result shows the existence of stable microbial communities in both habitats (the area globally influenced by the tree and the control area), which were not impacted by *E. camaldulensis* regardless of the sampling distance to Eucalyptus camaldulensis.

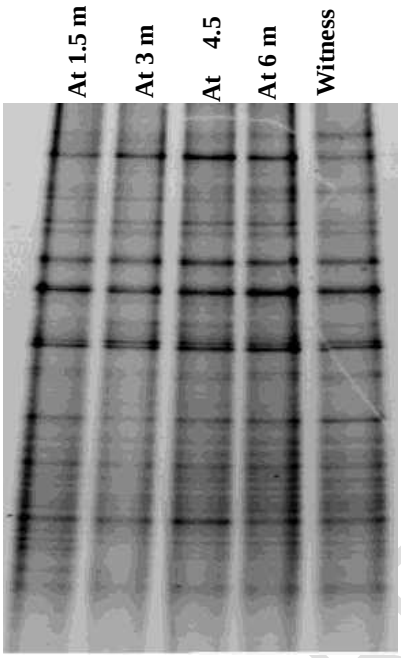


Fig. 11. DGGE profiles of 16S rDNA of total bacterial communities in soils collected at different distances under *E. camaldulensis*

The analysis of the similarity dendrogram (Fig. 12) showed a separation of the profiles of the soils collected at different distances into 2 large clusters or groups. A first cluster D1, which is formed by the bacterial community profiles of the soils under direct influence of *E. camaldulensis*, and a second cluster D2, which is formed only by the bacterial community profiles of the control soils, are distinguished. The structure of the microbial community is different depending on whether the soil is sampled under or without *E. camaldulensis*.

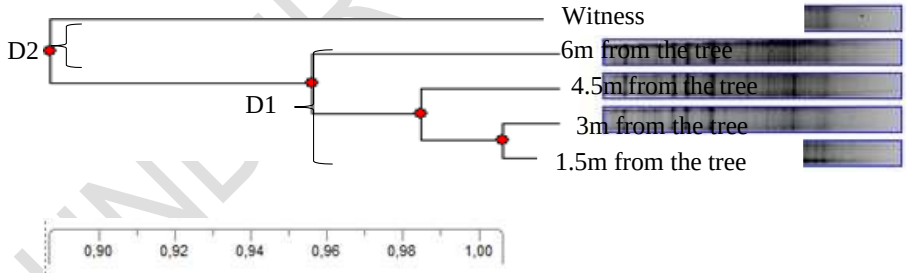


Fig. 12. Similarity dendrogram

4. DISCUSSION

The results of the study showed no significant difference in the chlorophyll content of peanuts obtained from all soils collected at different distances from the foot of *E. camaldulensis* except for field C. Thus, the chlorophyll content does not depend on the distance from *E. camaldulensis*. This result confirms that of [12], who showed that chlorophyll contents depend on the polyphenol content of the soil litter. Indeed, they found that the chlorophyll content of peanut plants was slightly reduced for plants grown on soil at 1% and significantly reduced at 5% amendment with Eucalyptus litter compared to plants

grown on control treatments. In this study, above-ground and root biomass of plants grown on soils sampled under and without *E. camaldulensis* at different distances were not significantly different for most sites. Indeed, the distance of soil sampling from *E. camaldulensis* does not have a significant effect on the above-ground biomass and root biomass of groundnut. This could be due to the abundant rainfall in the area. In their greenhouse study [13], they found that the root biomass of Acacia senegal, measured in the uncovered area, was significantly higher than that measured in the covered area. However, they found no significant difference in root biomass measured in the uncovered and covered areas. The same study conducted on peanut showed that there was no significant effect between aboveground and root biomass measured under and outside the canopy [14].

The results of the study showed that the number of nodules did not depend on the distance of sampling from *E. camaldulensis*. Indeed, no significant difference was noted in the number of nodules counted in peanut plants grown at all soil sampling levels under *E. camaldulensis* compared to control soils. This result may be due to the way *E. camaldulensis* trees were planted in the fields. The *E. camaldulensis* trees were planted in rows 8 m apart and 2 m apart within the row. This result disagrees with that of [14], which showed that the number of nodules was greater in HC compared to SC for the groundnut species.

Mycorrhization intensity is higher at D 6 m and at the controls compared to the other sampling distances. Mycorrhization intensity is dependent on the presence and decomposition of litter that falls below the Eucalyptus. This may also be due to the low density of *E. camaldulensis* plants coupled with the good rainfall in the area. This would favor litter decomposition. The study [14] showed that the addition of 1% litter had no significant effect on either the intensity of mycorrhization or on the weight and number of nodules. But the high dose of Eucalyptus litter (5%) would significantly reduce mycorrhization and nodulation of A. hypogeae plants. [15] agrees, showing that the introduction of exotic species would modify the microbial functions of the soil as well as the structure of the soil mycorrhizal fungi.

Examination of the DGGE profiles from the different treatments showed similar dominant microbial bands or communities across soil sampling levels for *E. camaldulensis*. This evolutionary structure of microbial communities in soils sampled between 4.5 m and 6 m from *E. camaldulensis*, compared to soils sampled at 1.5 m and 3 m from the tree and control soils, would be due to the presence of a microclimate favorable to these communities for these distances. This result confirms that of [16] and [17], who showed that Eucalyptus does not always change the microbial community structure of the soil. [14] showed the opposite by demonstrating the dominance of fungi under Eucalyptus while outside the *E. camaldulensis* crown bacterial activity was dominant. According to him, fungi are less sensitive to the Eucalyptus effect than microbial communities. The structure of the microbial communities depends on the distance from *E. camaldulensis*. This could be due to the effect of exudates produced by *E. camaldulensis* which would inhibit the development of microorganisms.

5. CONCLUSION

The results of this study showed that the distance of soil sampling from *E. camaldulensis* had no significant effects on variables such as chlorophyll content, aerial and root biomass, and number of nodules. In contrast, mycorrhization intensity was higher for peanut plants grown on soil sampled 6 m from *E. camaldulensis* than for peanut plants grown on control soils. Regarding the genetic structure of the microbial communities, examination of the

DGGE profiles of the different treatments showed the presence of similar dominant microbial communities at different soil sampling levels in relation to *E. camaldulensis*. This result demonstrates the existence of stable microbial communities in both habitats, which were not impacted by *E. camaldulensis*, regardless of the sampling distance for *E. camaldulensis*.

It would be important to specifically identify the different communities according to the distance of soil removal from *E. camaldulensis*.

It should be noted, however, that it would be very interesting to test this study in the field to evaluate the yield of peanuts grown in association with *E. camaldulensis* plants or amended with *E. camaldulensis* biochar.

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