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Effect of distance and depth on microbial biomass and mineral nitrogen content under *Acacia senegal* (L.) Willd. trees

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ABSTRACT

The relations between plants and soil biota involve positive and negative feedbacks between soil organisms, their chemical environment, and plants. Then, characterization of microbial community functioning is important to understand these relations. An experiment was conducted in a field system in the north of Senegal for two years (2005 and 2006) in order to investigate the effect of depth and distance from *Acacia senegal* tree stem on soil microbial biomass and inorganic-N content. Soils were sampled during dry season (April, T_0) and wet season (August, T_1) along transects (R_0, foot tree; $R_{/2}$, approximately 0.50 m distance from the stem; and R, approximately 1 m distance from the stem) and at different layers: 0–25 cm, 25–50 cm and 50–75 cm of *A. senegal* trees rhizosphere. Total microbial biomass and inorganic-N content were negatively correlated to the distance from tree stem and the depth. The highest values of microbial biomass and mineral nitrogen were found at the foot tree (R_0) and at 0–25 cm layer. Inorganic-N was mostly in nitrate form (NO_3^-) during the dry season. In contrast, during the wet season, inorganic-N was dominated by ammoniac form (NH_4^+). Soil total microbial biomass and inorganic-N ($NH_4^+ + NO_3^-$) were negatively correlated. Our results suggest a positive influence of *A. senegal* rhizosphere on soil microbial biomass and inorganic-N content.

1. Introduction

Acacia senegal (L.) Willd. is widely distributed through arid and semiarid areas of Africa and the Middle East. *A. senegal* produces the internationally-traded commodity 'gum-arabic'. This leguminous tree improves soil fertility of degraded areas through its ability to fix atmospheric nitrogen in symbiosis with rhizobia. Several authors showed a high genetic diversity of rhizobia associated to *A. senegal* (Nick et al., 1999; Sarr et al., 2005; Fall et al., 2008).

Relations between plants and soil biota involve positive and negative feedbacks. The rhizosphere, defined as the volume of soil adjacent to and influenced by the plant roots (Sørensen, 1997), is of great importance to plant health and soil fertility. Roots are known to excrete several forms of organic compounds. Microbial population in the vicinity of the roots is influenced by root exudates (Brant et al., 2006) and could be different in composition and density (Bowen and Rovira, 1991).

Soil microbial biomass is an essential component of most terrestrial ecosystems because it regulates nutrient cycling, and acts as a highly labile source of plant-available nutrients (Singh et al., 1989). Soil microbial biomass is most sensitive to changes in organic matter status than the total amount of organic C (Sparling, 1992). The microbial biomass has been used as an index of soil fertility (Staddon et al., 1999), which depends primarily on rates of nutrient fluxes. Environmental factors such as geographical location, vegetation, land use, land cover, soil type can influence soil microbial biomass (Black et al., 2003). An increase in the size of soil microbial biomass is essential for the improvement of soil fertility. Plant roots have been shown to affect the microbial growth by reducing soil available N or soil moisture, or by providing C substrates for microbial growth (Jackson et al., 1989).

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Nitrogen (N) mineralization is of a crucial importance in natural forest ecosystems where N has been reported to be a limiting nutrient for plant growth (Clein and Schimel, 1995). The initial product of organic-N heterotrophic mineralization is ammonium, which is further oxidized by autotrophic microbes to form nitrate through nitrification. N mineralization is influence by soil microclimate (Wang et al., 2006) and the amount and quality of organic matter (Sall et al., 2003). Water availability controlled soil microbial activity and thus the rates of N net mineralization (Nicolardot et al., 2001). However, Zaman and Chang (2004) reported that soil temperature and soil moisture were most important than substrate quality in controlling mineral N in agroforestry systems.

Soil microbial biomass and mineral nitrogen content are the most important indicators of soil fertility (Staddon et al., 1999; Adrover et al., 2012). Thus the main objective of this work was to evaluate the effect of legumes rhizosphere in particular *A. senegal* on these soil components. We assessed the spatial and seasonal variations of soil total microbial biomass and inorganic-N content in natural conditions under mature *A. senegal* trees during dry and wet seasons of two years 2005 and 2006 in the north part of Senegal.

2. Material and methods

2.1. Soils and samplings

Soil samples were collected at Kamb (an arid savannah with 400–500 mm of annual rainfall), 300 km north of Dakar (Senegal). In this part of Sahel, temperatures values rank between 25 °C and 39 °C with an annual average temperature of approximately 29 °C. Soils were sampled at two periods: T_0 (dry season, April), T_1 (wet season, August) during two years 2005 and 2006 in a plantation of *A. senegal*, 13 years old. The plantation was separated into two blocks with 48 plants each block. For each sampling period, soils were collected from three trees of each block. For each tree, soil samples were taken along transects from the stem up to 1 m distance (R_0, foot tree; $R_{/2}$, 0.50 m distance from the foot tree and R, 1 m distance from the foot tree). Soil samplings were replicated in four directions around the tree stem (East, West, North and South), at depths of 0–25 cm, 25–50 cm, and 50–75 cm. For each tree, the four soils samples collected at the same distance to the tree stem and at the same depth were pooled to obtain a homogenous soil sample around the tree. Then, for each tree, nine soils samples were collected. Thus for each block, we have 27 soils samples. During the second year (2006), soils samples were taken only at 0–25 cm depth because in the first season (2005), we found that most of the mineral nitrogen content and microbial biomass were found in layer 0–25 cm. The soil characteristics for each soil depth and distance from tree stem are presented in Table 1. For each sampling period, soil moisture was determined.

2.2. Determination of gravimetric soil moisture

For each sampling period, 10 g from soil samples were dried in an air-forced oven at 105 °C during 72 h for determining soil moisture content.

2.3. Soil microbial biomass

Microbial biomass N was determined by the fumigation-extraction method (Amato and Ladd, 1988) by measuring ninhydrin reactive N compounds extracted from soils with 1 M KCl after a 10-days fumigation period. Fumigated and unfumigated soil samples were suspended in KCl solution, shaken at 25 °C for 1 h and then filtered (Whatman 0.45 μm) and stored frozen for further analysis. Ninhydrin reactive N content was determined colorimetrically by flow injection analysis (Evolution II, Alliance-Instruments, France). Microbial biomass C was estimated from the gain in ninhydrin reactive N after fumigation, multiplied by 21 (Amato and Ladd, 1988). Microbial biomass was expressed in μg C g^{-1} dry soil.

2.4. Soil inorganic-N content

Soil inorganic-N content was determined colorimetrically in the KCl 1 M extract by flow injection analysis according to the method of Bremner (1965). The results were expressed as μg N (NH_4^+ or NO_3^-) g^{-1} dry soil.

3. Results

3.1. Soils chemical characteristics

Results showed that soils were acids with pH ranging between 5.3 and 6.6. A weak variation of pH in relation to distance from tree stem and the depth was noted (Table 1). Regarding the nutrient elements, we observed that mineral nitrogen, organic carbon and soluble phosphorus decreased with distance from tree stem. Mineral nitrogen content decreased with depth whatever the distance. However, no variation in relation to depth was observed at R for the organic carbon and soluble phosphorus.

3.2. Soils moisture

Soils moisture was determined for the two sampling periods. We presented only soil moisture recorded only at R_0 and 0–25 cm layer. Results showed that soil moisture was higher during the wet season (August) for the two years. The soil moisture was 0.41% and 9.40% during respectively the dry season and the wet season of the year 2005 against 0.35% and 7.45% respectively in dry and wet seasons of the year 2006. A decrease of soil moisture was observed during the second year 2006 comparatively to the first year (2005) of the experiment.

Table 1
Chemical characteristics of soils collected in dry season of April 2005 (T_0, 2005) at different layers (0–25 cm; 25–50 cm and 50–75 cm) and different distances to tree stem (R_0; $R_{/2}$ and R).

Layers (cm)	[a]R_0				$R_{/2}$				R			
	pH_{H2O}	N	C	P	pH_{H2O}	N	C	P	pH_{H2O}	N	C	P
0–25	5.3	14.4	0.23	29.8	6.4	8.2	0.17	8.3	6.6	4.9	0.13	5.2
25–50	5.9	11.6	0.14	11.8	6.5	7.0	0.11	7.4	6.5	3.7	0.10	5.8
50–75	6.0	10.3	0.12	9.2	6.0	5.8	0.11	4.8	6.3	3.2	0.10	5.6

[a] R_0, foot tree; $R_{/2}$, 0.50 m distance from the stem and R, 1 m distance from the stem. The six soils sampled at the same distances and depths were pooled to get one composite soil sample. N, NH_4^++NO_3^- (mg/kg of soil); C, organic carbon (%) and P, soluble phosphorus (mg/kg of soil).

Table 2
Microbial biomass (in μg C g^{-1} dry soil) of soils collected at different layers (0–25 cm; 25–50 cm and 50–75 cm) and different distances to *A. senegal* tree stem (R_0; $R_{/2}$ and R) during the different sampling periods of the two years (2005 and 2006) of the experiment. In 2006, only layer 0–25 cm was sampled.

Layers (cm)	2005						2006					
	Dry season April (T_0)			Wet season August (T_1)			Dry season April (T_0)			Wet season August (T_1)		
	[a]R_0	$R_{/2}$	R	R_0	$R_{/2}$	R	R_0	$R_{/2}$	R	R_0	$R_{/2}$	R
0–25	[b]^{A}29a[c]	^{A}16a	^{A}12a	^{A}39b	^{A}33b	^{A}37b	^{A}15	^{A}7	^{A}3	^{A}60	^{A}59	^{A}61
25–50	^{B}22a	^{A}7a	^{A}4a	^{B}21a	^{B}19 ab	^{A}9a	–	–	–	–	–	–
50–75	^{A}10a	^{A}4a	^{A}3a	^{A}16a	^{A}13a	^{A}7a	–	–	–	–	–	–

[a] R_0, foot tree; $R_{/2}$, 0.50 m distance from the stem and R, 1 m distance from the stem.
[b] For each season, values within of line followed by same upper case letter comparing distance effect are not significantly different at $P < 0.05$ (Student–Newman and Keuls test).
[c] For each season, value within of column followed by same lower case letter comparing depth effect are not significantly different at $P < 0.05$ (Student–Newman and Keuls test).

3.3. Spatial and seasonal variations of soil microbial biomass

Soil microbial biomass decreased with increasing depth and distance from tree stem, whatever the soil sampling period and year except the wet season (T_1) of the second year (Table 2). During the dry season of 2005, the highest value of microbial biomass was found at the foot tree (R_0) and at 0–25 cm soil layer (29 μg C g^{-1} dry soil) and the lowest value was obtained in the R position and at 50–75 cm soil layer (3 μg C g^{-1} dry soil). No significant difference ($P > 0.05$) was noted on the effect of distance on microbial biomass of soils collected at 0–25 cm and 50–75 cm layers for all the sampling periods. However, a significant effect of distance ($P < 0.05$) was observed on microbial biomass of soils sampled at 25–50 cm layer. Considering the depth effect, results showed no significant difference ($P > 0.05$) was observed in soil microbial biomass during the dry season of 2005. Nevertheless, in the wet season, microbial biomass of soils collected at 0–25 cm layer was significantly greater ($P < 0.05$) than the one of soils coming from 25 to 50 cm and 50–75 cm layers except for $R_{/2}$.

Microbial biomass increased during the wet season compared to the dry season (Table 2). For 2005 and at 0–25 cm layer, soil microbial biomass content was 39 and 29 μg C g^{-1} dry soil, respectively for the wet season and the dry season. This increase was more marked during the second year of the experiment. Indeed, soil microbial biomass was multiplied by 4, 8 and 20 respectively at R_0, $R_{/2}$ and R distances at 0–25 cm layer.

3.4. Inorganic-N content

As soil microbial biomass, inorganic-N content decreased with the depth and the distance from tree stem (Table 3). Whatever the sampling period, the highest amount of mineral nitrogen content was recorded in layer 0–25 cm and at foot tree (R_0). During the first year (2005), the amount of mineral N measured around the foot tree (R_0) was significantly ($P < 0.05$) higher than that measured at R at 0–25 cm layer. No significant difference ($P > 0.05$) was noted on the effect of distance on inorganic-N content of soils collected at 0–25 cm and 50–75 cm layers for all the sampling periods of 2005 and the wet season of 2006.

Soil mineral nitrogen content was reduced during the wet season (T_1) by comparison to the dry season (T_0) (Table 3) for the two years of experience. For the soil layer 0–25 cm, mineral N content was 9.1 μg N g^{-1} dry soil at T_0 against 3.7 μg N g^{-1} dry soil at T_1 during the first year (2005). Hence, influence of the tree stem was less evident.

3.5. Seasonal variations of ammonium (NH_4^+) and nitrate (NO_3^-)

Table 4 showed that during the dry season (T_0), the highest amount of mineral nitrogen was in nitrate form (NO_3^-) in contrast during the wet season (T_1) it was mainly in ammoniac form (NH_4^+) for the two years. Significant difference ($P < 0.05$) was observed between ammoniac form and nitrate form for the most of soil samples. For example in 2006, mineral nitrogen content amount of 6.7 μg N–NO_3^- g^{-1} dry soil and 3.0 μg N–NH_4^+ g^{-1} dry soil were recorded at T_0 against 1.2 μg N–NO_3^- g^{-1} dry soil and 3.9 μg N–NH_4^+ g^{-1} dry soil at T_1 (Table 4).

3.6. Comparative evolution of soil microbial biomass and inorganic-N content

Fig. 1 showed that soil microbial biomass and mineral nitrogen content (NH_4^++NO_3^-) were negatively correlated. For the two years of the experiment, soil microbial biomass was higher during the rainy season (T_1) while the lowest mineral nitrogen contents were recorded at this same period. Inorganic-N content was higher during the dry season (T_0) at the lowest microbial biomass value.

4. Discussion

The decrease in soil microbial biomass in relation to the depth and the distance from tree stem could be attributed to the amount of nutrient elements such as carbon, nitrogen. Hence, this decrease in microbial biomass could also correlate to root biomass. *A. senegal* root biomass was highest at R_0 and 0–25 cm layer (data not shown). As reported in several studies, the diversity and numbers of microorganisms in rhizosphere are, to a large extent, determined by the composition and concentration of root exudates excreted by plants (Lynch, 1990; Yang et al., 2001; Marschner et al., 2004). However, the most pronounced aspect of this 'rhizosphere effect' is quantitative with microbial population sizes and activities increasing closer to the root (Castro-Sowinski et al., 2007). Root

Table 3
Inorganic-N content (in μg NH_4^+ + μg NO_3^- g^{-1} dry soil) of soils collected at different layers (0–25 cm; 25–50 cm and 50–75 cm) and different distances to *A. senegal* tree stem (R_0; $R_{/2}$ and R) during the different sampling periods of the two years (2005 and 2006) of the experiment. In 2006, only layer 0–25 cm was sampled.

Years	Layers (cm)	Dry season (T_0)			Wet season (T_1)		
		[a]R_0	$R_{/2}$	R	R_0	$R_{/2}$	R
2005	0–25	[b]^{B}9.1b[c]	^{A}4.3b	^{A}4.1a	^{B}3.7b	^{A}1.6a	^{AB}2.6b
	25–50	^{A}4.4a	^{A}4.0b	^{A}4.5a	^{A}2.1ab	^{A}1.4a	^{A}1.3a
	50–75	^{B}4.1a	^{A}1.7a	^{B}3.7a	^{A}1.5a	^{A}1.3a	^{A}1.3a
2006	0–25	^{B}9.7	^{B}8.1	^{A}4.7	^{A}5.1	^{A}3.5	^{A}4.1

[a] R_0, foot tree; $R_{/2}$, 0.50 m distance from the stem and R, 1 m distance from the stem.
[b] For each season, values within of line followed by same upper case letter comparing distance effect are not significantly different at $P < 0.05$ (Student–Newman and Keuls test).
[c] For each season, value within of column followed by same lower case letter comparing depth effect are not significantly different at $P < 0.05$ (Student–Newman and Keuls test).

Table 4
Different forms of inorganic-N (in μg NH_4^+ and μg NO_3^- g^{-1} dry soil) of soils collected at different layers (0–25 cm; 25–50 cm and 50–75 cm) and different distances to *A. senegal* tree stem (R_0; $R_{/2}$ and R) during the different sampling periods of the two years (2005 and 2006) of the experiment. In 2006, only layer 0–25 cm was sampled.

Years (cm)	Layers	Dry season (April, T_0)						Wet season (August, T_1)					
		[a]R_0		$R_{/2}$		R		R_0		$R_{/2}$		R	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
2005	0–25	2.1a[b]	7.0b	1.8a	2.5a	1.0a	3.1b	3.1b	0.6a	1.3b	0.3a	1.9b	0.7a
	25–50	0.8a	3.6b	1.1a	2.9b	1.5a	4.0b	1.4b	0.7a	1.4b	0.0a	1.3b	0.0a
	50–75	0.7a	3.4b	0.7a	1.0a	1.6a	2.1a	1.1b	0.4a	1.3b	0.0a	1.3b	0.0a
2006	0–25	3.0a	6.7b	1.2a	6.9b	2.3a	2.4a	3.9b	1.2a	2.8b	0.7a	3.4b	0.7a

[a] R_0, foot tree; $R_{/2}$, 0.50 m distance from the stem and R, 1 m distance from the stem.
[b] For each depth, values within of line followed by same letter comparing ammonium (NH_4^+) and nitrate (NO_3^-) content in the same distance are not significantly different at $P < 0.05$ (Student–Newman and Keuls test).

exudates mainly serve as nutrient sources for microorganisms (De Troch and Vanderleyden, 1996). Legume species rhizosphere stimulated the growth of microorganisms by their rhizodeposition, rich in amino acids (Jones, 1999) and soluble sugars (Jensen, 1996). Similar results were obtained by Raubuch and Beese (2005).

The reduction in microbial biomass of soils sampled in dry season (T_0) could be explained by the decrease of water availability. Several studies suggest that seasonal variation in microbial biomass is related to variation in soil water potential (Piao et al., 2000; Ford et al., 2007) and substrate (i.e., C) availability (Srivastava, 1992). Changes in soil moisture status can markedly affect the magnitude of the soil microbial biomass (Schnürer et al., 1986) because many soil microorganisms are known to be intolerant of low soil moisture contents (Harris, 1981). In our study, soil moisture was weak during the dry season and high during the wet season.

Our results showed that mineral nitrogen content decreased with the depth and distance from tree stem. Inorganic-N content was higher at R_0 (foot tree) and 0–25 cm soil layer. Similar results were obtained by Maithani et al. (1998) and Iyyemperumala et al. (2007). Inorganic-N pools variation could be attributed to variation in mineralization rates, to the plants and microbes uptake, and losses through soil erosion, leaching, run-off and denitrification. The highest value of inorganic-N content obtained at R_0 and at 0–25 cm layer could be explained by the fact that nitrogen mineralization in the rhizosphere are strongly influenced by plant root exudates, which consist of easily degradable organic carbon compounds (Lynch, 1990).

Comparing the inorganic-N pool between seasons, our results showed that inorganic-N content decreased during the wet season. This phenomenon was mainly due to the immobilization of inorganic-N by the microorganisms (Sall et al., 2007) and the demand for this nutrient by weeds which grow vigorously during the wet season (Arunachalam et al., 1996). Conversely, the increase in inorganic-N content during dry season may be attributed to the mineralization of the dead microorganisms by the microbial community that survived desiccation (Kieft et al., 1987) and a decrease in demand by plants owing to slow growth. During dry season, plant uptake was reduced and the microbial activity was lower due to the lack of humidity resulting in an increase in pool of mineral N during these periods in comparison with wet periods.

Considering the seasonal variation of the different forms of mineral nitrogen, we observed that inorganic-N was mostly in nitrate form (NO_3^-) during the dry season and in ammoniac form (NH_4^+) during the wet season. The decrease of NO_3^- form during the wet season could be mainly due to high soil moisture content, plant uptake (Jackson et al., 1989; Schimel et al., 1989) or reduction in ammonium-N (NH_4^+) availability (Montangnini et al., 1986).

Results showed that soil microbial biomass and mineral nitrogen content were negatively correlated. Soil inorganic-N decreased in wet season when microbial biomass was high. The

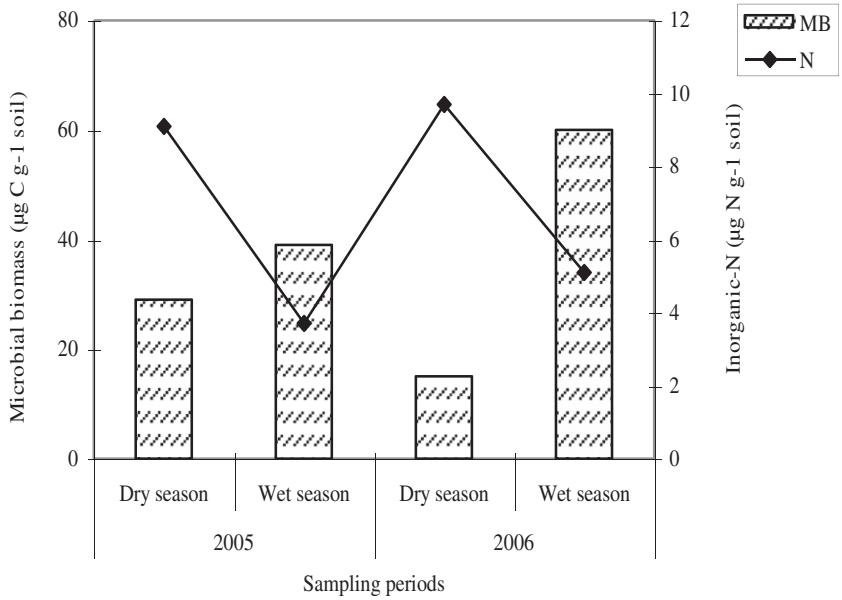

Fig. 1. Comparative evolution between soil microbial biomass and inorganic-N content at R_0 and 0–25 cm layer during the two years of the experiment. MB = microbial biomass, N = inorganic nitrogen.

increase of soil microbial biomass induced an increase of inorganic-N immobilization by microorganisms and then provokes a decrease in soil mineral nitrogen content (Sall et al., 2007).

5. Conclusion

This study showed that soil microbial biomass and inorganic-N were higher near *A. senegal* foot tree. Our results suggest a positive influence of *A. senegal* rhizosphere on soil microbial biomass and inorganic-N content. The significance of these findings is that due to mineral enrichment of the soil, the association with *A. senegal* might be beneficial for crops in agroforestry systems in N-deficient soils of the Sahelian zone. Soil total microbial biomass and inorganic-N content were negatively correlated. Soil microbial biomass increased during the rainy season while mineral N content decreased. Inorganic-N was mainly in nitrate form during the dry season and as ammonium during the wet season. In further investigations, it will be important to study the functional diversity of microorganisms in the rhizosphere of *A. senegal*.

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