Total microbial activity of soils on natural gum groves of Acacia senegal in Niger

ALZOUMA MAYAKI Zoubeirou¹, ABDOU Maman Manssour², ALOU Abdourahamane², ASSOUMANE Aichatou¹, and ELHADJI SEYBOU Djibo¹

¹Département de Biologie, Faculté des Sciences et Techniques, Université Abdou Moumouni, BP : 10662 Niamey, Niger

²Faculté des Sciences Agronomiques et de l'Environnement, Département Sciences de L'Environnement et Adaptation au Changement Climatique, Université de Tillabéri, BP : 175 Tillabéri, Niger

Copyright © 2016 ISSR Journals. This is an open access article distributed under the *Creative Commons Attribution License*, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT: This research showed positive effect of *A. senegal* on soils total microbial activity on different sites in Niger. Soils were sampled under and outside *A. senegal* crown. The depth of soil sampling is 0-25 cm. sites that samples take out are: Azzai, Bader, Malam Maimari, N'Guel kolo, Kokoye and Kiki. Activity was greater on soils under *A. senegal* crown, than outside crown. Results showed strong correlation between total microbial activity and soils physico-chemical parameters. Correlation was positive on soils with higher clay content, and negative on those with higher sand content. Thus, under *A. senegal* crown, soils total microbial activity was significantly different between studied sites. Activity on Kiki's site with value of 5,9 µg/g/h, was twice that obtained at N'Guel kolo. On all sites, total microbial activities on soils outside of *A. senegal* crown, was either a third lower (Kokoye and N'Guel kolo), or half lower (Kiki, Malam Maimari, Bader and Azzai), than under the crown. *A. senegal* is legume plant, that can contribute to fertilize and stabilize poor soils. A better valorisation of *A. senegal* would allow development of agroforestry system in nitrogen deficient soils of the Sahelian zone. Agroforestry practices could increase plant diversity, control soil erosion and sequester organic carbon.

KEYWORDS: Acacia senegal, crown, soil, microbial activity, Niger.

1 INTRODUCTION

In arid and semi-arid regions of Africa, land degradation is mainly due to extensive agriculture, deforestation, overgrazing and declining soil fertility. This degradation is the main threat to nature conservation and Food security [1].

In Africa, arid and semi-arid surfaces cover over 55% of the land, and dryness is a consequence low annual rainfall ranging from 100 to 600 mm, and precipitations occur only over a short 2-4 months rainy season [2].

Parks in the Sahelian zone are mainly made up of acacia species, which play decisive role in its ecosystem balance. Among these acacia species, *A. senegal* tree's that has been identified as having great potential for restoring degraded and vulnerable agro ecosystems [3]. The tree is particularly used in fallow land, and found on degraded soils to restore soil fertility [4]. The tree is capable of establishing symbiotic relationships with soil endomycorrhizal fungi, and such relationships promote better development of the tree through improved mineral nutrition [5].

A. senegal trees provide various economical goods such as production of gum Arabic, fodder supply and fuel. It is furthermore used as pharmaceutical product in traditional practices.

Research showed positive effects of *A. senegal* tree on soil fertility through its symbiotic association with nitrogen-fixing bacteria *Rhizobia* sp. ([6] & [7]. Debris from these plant species are important means of transferring plant elements to soils [8]. Mineralization of such debris is one of the main sources of nutrients for plant growth [9]. Also, quality and quantity of debris influences chemical and biological fertility of soil through interactions with microorganisms.

In all ecosystems, soil micro-organisms play an important role in maintaining soil structure, facilitate organic matter degradation, nutrient recycling, and carbon sequestration [10].

Microorganisms have crucial role in nutrient availability, plant growth and health. They are key factor influencing functioning of ecosystems, and sustainability of soil resources [11]. Soil microorganisms secrete extracellular enzymes that increase decomposition of organic matter, and nitrogen compounds transformation [12]. Metabolic activities of soil microbial communities reflect vigor and vitality of the microbial processes associated with nutriment recycling. These activities are sensitive indicators of environmental stresses responsible for soil quality degradation.

Thus, environmental variability and heterogeneity of resources associated with arid ecosystems may increase microbial functional diversity [13]. In relation this observation, microbial parameters can be used as soil quality indicators [14]. Soil microorganisms are sensitive to land usage and management ([11], [15], [16], [17], [18]).

This research is aimed at assessing total microbial activity of soils under and off various gum groves crown in Niger.

2 MATERIALS AND METHODS

2.1 GEOLOCATION AND DESCRIPTION OF SITES

Study was carried out in different gum basins of Niger (Figure 1). In the Western basin, sites used were Kiki and Kokoiye, in the Central Basin selected sites were Bader Goula and Azzai, and the eastern sites used were Malam Mainari and N'guel Kolo.

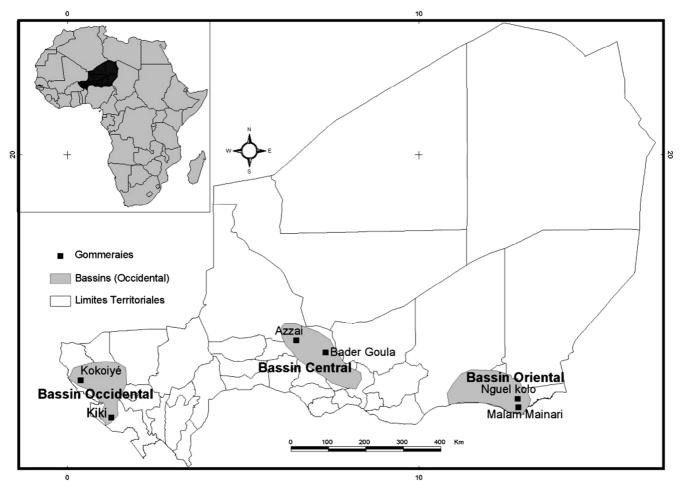


Fig. 1. Studied gum groves sites of Niger

Climatic data of each site is presented in Table 1.

Sites	Climat	Dry Season	Rainy Season	Rain fall (mm) (1995-2015)	Geographical coordinates
Kiki	Soudano-sahelian	october-april	may-september	622	N 12°59'36,4" and E 01°32'57"
Kokoiye	Sahelian	october-april	may-september	425	N 13°59'06,1" and E 0°44'32,3"
Bader Goula	Sahelian	october-may	june-september	376	N 14°43'28,7" and E 7°14'27,10"
Azzai	Sahelian	october-may	june-september	350	N 15°02'22,8" and E 06°24'46,2"
Malam Mainari	Sahelian	october-may	june-september	309	N 13°17'39,8" and E 12°18'27,5"
N'guel Kolo	Sahelian	october-may	june-september	300	N 13°29'28,9" and E 12°20'38,9"

Table 1. Climatic data of studied gum groves regions

2.2 SOILS SAMPLING

Soils were sampled at the end of rainy season at 0-25 cm depth. Soils were sampled as follow method:

- under the crown of *A. senegal*
- outside the crown of *A. senegal* (control).

Soils were collected at different points under *A. senegal*'s crown in radiating scheme following opposites directions away from the trunk (East / West / North / South).

For selected tree, sampled soils were mixed up to make composite soil, which was thereafter subsampled for laboratory analysis. Soil total microbial activity was measured using hydrolysis test of Fluorescein Diacetate (FDA) according to method described [19].

For each control sample, sampling and mixing up of soils for subsampling was done by taking up off crown, a number of soil sample equivalent to that taken under crown.

The test measures on spectrophotometer, the amount of fluorescein released by hydrolysis. The technique allows assessment of soil overall microbial activity.

For each soil subsample, total microbial activity was performed on three (3) enzyme containing assays, and a control assay without enzyme, referred to as substrate control.

Enzyme control was made up of 15 ml of phosphate buffer (8,7 g 11 K₂HPO₄ and 1,3 g 11 KH₂PO₄, pH 7,6), 200 µl of sterile demineralized water, and 1 g of soil. Each assay was performed on 15 ml of phosphate buffer, 200 µl of FDA (1 mg ml⁻¹), and 1 g of soil. Substrate control was carried out only once, on 15 ml of phosphate buffer associated with 200 µl of FDA.

Samples were slightly vortexed then incubated with stirring at 30 °C for one hour. After incubation, reaction was stopped with 1 ml pure acetone (100%) per tube. Tube's contents were vortexed then centrifuged for 5 min at 10,000 rpm. Optical density was read on spectrophotometer set at 490 nm wavelength over 1 ml supernatant. A standard 6 point-ranges was prepared to calculate FDA concentration in fluorescein per μ g of soil and per hour.

3 RESULTS

On all studied sites, results show that FDA values are significantly higher for soil sampled under *A. senegal* crown than on control soil samples (Fig. 2). Data also furthermore suggest that total microbial activity level is significantly different amongst the sites (Fig. 2). Thus, highest level of activity under crown is obtained at Kiki (5.9 μ g. g⁻¹.h⁻¹) and lowest at Bader is observed (2.8 μ g. g⁻¹.h⁻¹). Similar analysis of control data shows no significant difference among control soils (Fig. 2). Relative difference of 3,1 μ g. g⁻¹.h⁻¹ is found between values at the two extreme under crown. There is also a significate difference in total microbial activity of soils outside *A. senegal* crown between Malam Mainari site and N'Guel Kolo, Azzai and Bader Goula. However, the difference was not significant between sites of N'Guel Kolo, Azzai and Bader Goula. Kiki and Kokoiyé sites have higher total microbial activity

(3 µg. g^{-1} . h^{-1}) outside *A. senegal* crown compared to other sites.

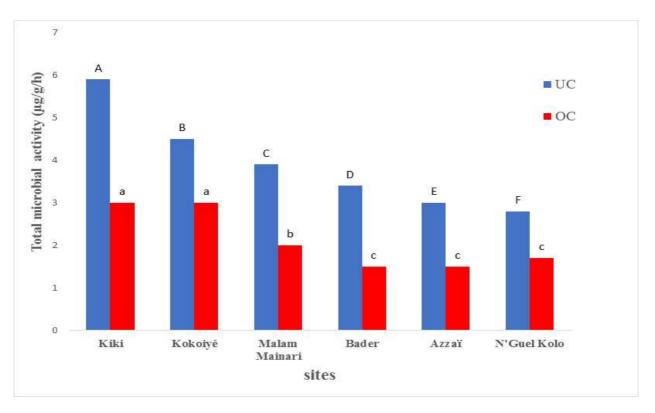


Fig. 2. Total microbial activity measured in the soil under and outside crown of A. senegal trees in different provenances.

UC: under the crown OC: outside the crown

Values followed by the same letter are not statistically different.

PRINCIPAL COMPONENT ANALYZES BETWEEN TOTAL MICROBIAL ACTIVITY, PHYSICO-CHEMICAL PARAMETERS AND SITE RAINFALL

Principal Component Analysis of soils under and outside *A. senegal* crown has been realized on parameters of each site. Total microbial activity (FDA), chemical content (total carbon C, total nitrogen N, assimilated phosphorus P ass and pH), physical content (silt, clay, sand) and rainfall have been projected on axes of principal component analysis (Fig. 2). Total variance of data from soils under and off crown of trees is comprehensively explained by Axe F1 (89.98%), and axe F2 (86.34%) (Fig. 3).

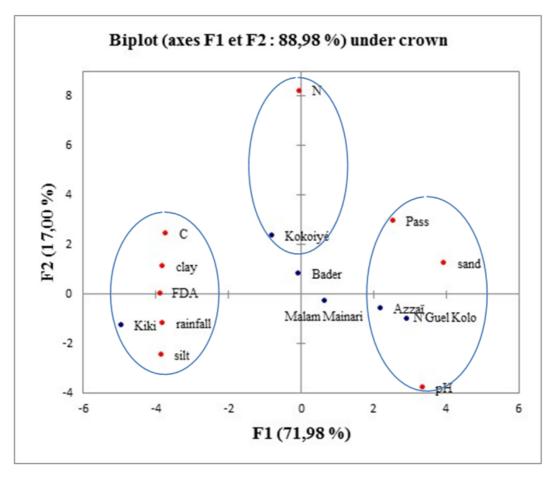


Fig. 3. Principal component analysis of soils under A. senegal crown on axes F1 and F2 carried out on different parameters of sites

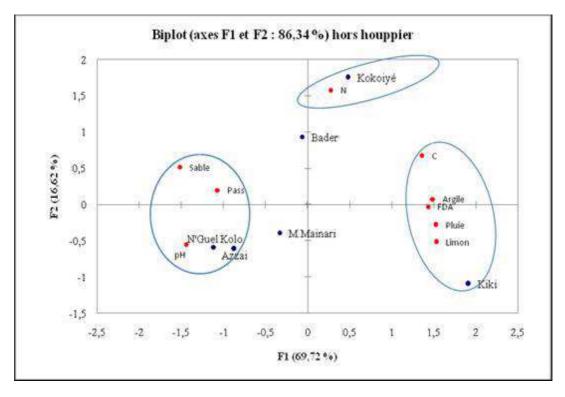


Fig. 4. Principal component analysis of soils outside A. senegal crown on axes F1 and F2

Results further show that total microbial activity is also influenced by soil physico-chemical parameters and rainfall (Figs. 3 and 4). Thus, three groups were deducted from both components of the factor under study:

- first group is represented by Kiki site where FDA is positively related to soil content of clay, silt, carbon and rainfall,
- second group is formed by N'Guel Kolo and Azzai sites, where total microbial activity is negatively related to soil content in sand, pH and assimilated phosphorus,
- third group is made up of Kokoiyé, where total microbial activity is negatively related to nitrogen content of the soils.

CORRELATIONS BETWEEN TOTAL MICROBIAL ACTIVITY, PHYSICO-CHEMICAL PARAMETERS AND SITE RAINFALL

Variables relationships are studied using Pearson correlation (Table 2 and Table 3). Results from this analysis show strong positive correlation between clayey and silty soil, to total microbial activity. Similarly, positive correlation exists between silty soil, carbon, and rainfall, to total microbial activity. Correlation is negative r between sandy soil, and pH, to total microbial activity (Table 2 and Table 3).

Table 2: FDA Pearson correlation matrix to key soil variables measured under tree crown.

Variables	FDA	рН	С	N	P ass	Clay	Silt	Sand	Rainfall
FDA	1								
рН	-0,748	1							
С	0,868	-0,834	1						
N	-0,037	-0,479	0,304	1					
P ass	-0,404	0,468	-0,514	0,219	1				
Clay	0,979	-0,757	0,929	0,100	-0,375	1			
Silt	0,922	-0,657	0,775	-0,285	-0,678	0,867	1		
Sand	-0,971	0,714	-0,854	0,154	0,592	-0,942	-0,984	1	
Rainfall	0,878	-0,791	0,779	-0,133	-0,664	0,803	0,922	-0,908	1

Table 3: FDA Pearson correlation matrix to key soil variables measured outside tree crown.

Variables	FDA	рН	С	N	P ass	Clay	Silt	Sand	Rainfall
FDA	1								
рН	-0,884	1							
С	0,626	-0,795	1						
Ν	0,093	-0,488	0,545	1					
P ass	-0,279	0,451	-0,599	-0,081	1				
Clay	0,900	-0,813	0,835	0,149	-0,396	1			
Silt	0,826	-0,732	0,665	-0,147	-0,692	0,844	1		
Sand	-0,831	0,710	-0,688	0,159	0,648	-0,884	-0,993	1	
Rainfall	0,807	-0,824	0,667	0,024	-0,737	0,758	0,956	-0,919	1

4 DISCUSSION

Results show increase in soil total microbial activity under *A. senegal* crown, relative to control samples. Our results confirmed previous report that *A. senegal* tree improves microbiological characteristics of rhizospheric soil, through rhizodeposition and rhizodecomposition of organic matter [20]. Other researcher showed that *A. Senegal* rhizosphere plays positive influence on microbial biomass, and influence is greatest near tree foot [21].

According to [22], enzymatic activity of soils under *A. senegal* crown is higher, perhaps as a result of active microbial biomass (intracellular enzymes), or to increased enzyme production by microbial biomass (extracellular enzymes).

Researchers also expressed hypothesis, that activity under tree crown is higher, as a result of combined action of both intracellular, and extracellular enzymes ([22] & [23]). Such production of enzyme could explain that the greatest amount of organic matter is observed under tree crown for it creates microclimate favorable to soil's microorganisms development. Increase of total microbial activity might be correlated to rhizodeposition. In fact, diversity and number of microorganisms in

rhizosphere are largely determined by composition and concentration of root exudates excreted by plants ([24]and [25]). Changes in rhizospheric soils by root exudates can influence abundance of telluric microbial populations.

Most pronounced aspect of rhizosphere effect on soil is increase in size and activity of microbial population near root [26]. However, decrease of microorganism number is proportional to distance from roots [27]. This explains why total microbial activity is more important under than outside crown. Rhizosphere is considered to be an area of increased microbial activity, where plant roots transfer 17% of photosynthesis products. Majority of transferred products is available for soil microbial community, thus increasing number of microorganisms present in the area ([28]; [29]). [30] estimated that microorganisms present on rhizospheric soil are 19 to 32 times greater than on soil outside influence of plant root system. Thus, organic matter quality and composition of soil microbial community could be factors influencing relative production of soil enzymes ([31], [32], [33]).

Principal component analysis shows positive correlation between total microbial activity, and clay content of soils. This analysis is corroborated by negative relationship between total microbial activity, and soil sand content. These analyses suggest relationships between soil physico-chemical composition, and enzymatic activity. Such relationships explain why total microbial activity is greatest at Kiki and KoKoiyé, sites that are located in Niger River basin, and lower on sandy sites at N'Guel Kolo and Azzai (Fig. 2, and Table 1).

Activities are greater on clayey soils, for clayey soils tend to form clay-humic complexes, that can more easily associate with soils chemical elements.

Soils rich in clay form micro-aggregates that are niches for microorganisms [20]. As a matter of fact, soil enzymatic activities increase proportional to its clay content, for clayey soils are rich in carbon and nutrients [34].

Microbial activity changes based on soil types, and increase is proportional to soil content of clay. Clay effect on activity is presumed due to higher protection of soil organic matter, and better production of enzyme which in turn influences biological condition of soil [20].

Enzymes can adsorb on clay colloidal particles, or associate with humic substances in organic matter to allow soil stabilization. Researchers also showed that enzyme such β -glucosidase influences soil properties ([35], and [36]). Other workers showed that physico-chemical properties of soil influence, activity of soil enzymes [37].

Some authors consider physical part of soil to be responsible of variation in soil microbial activity [38]. Size of soil particles, not only affects bacterial biomass, but also determines community structure. According to [38] & [39], parameters such as clay percentage and soil texture, influence survival and proliferation of bacteria in the soil.

In our study, Principal Component Analysis revealed positive correlation between total microbial activity and soil carbon. This result confirms finding by [40], which reported that carbonaceous input from plants to rhizosphere microorganisms, stimulates growth of microorganisms and thus is referred to as "Rhizosphere effect ".

Positive correlation is found between total microbial activity and rainfall, and such relationship explains why total microbial activity is more important at Kiki which possesses both high rainfall and high clay content soil. Conversely, microbial activity is low at N'Guel Kolo where both rainfall and clay content of soil are extremely low. These are in line with finding by [41] and [42] whose teams showed that size of soil microbial biomass is affected by changes in soil moisture. Furthermore, [43] and [44] reported that several soil-born microorganisms are sensitive to low water content of soil.

5 CONCLUSION

Research showed that soil total microbial activity is higher under *A. senegal* crown. We suggest that increase of microbial activity is due to positive influence of tree rhizosphere on soil microbial activity. Total microbial activity decreases rapidly moving away from tree trunk. Work also suggests that high concentration of soil microbial biomass is an indication of greater decomposition of organic matter into mineral elements. Consequently, soil microbial biomass may be used as reliable indicator of soil fertility. Overall, it appears that as legume tree, *A. senegal* contributes to fertility of poor soils, depending on local soil structure and rainfall pattern, as found on clayey soils at Kiki and Kokoiyé, and confirmed by low fertility of sandy soils at N'Guel Kolo and Azzai.

Nitrogen deficient Sahel soils could be restored to greater fertility levels, through greater use of *A. senegal* tree in all Sahel Agro-systems, and particularly in those based on agriculture and forestry. Thus, changes in agro-forestry practices are strongly recommended in Sahel, as to include more of *A. senegal* trees for better soil erosion control, improving plant diversity, and increasing crop production.

ACKNOWLEDGEMENT

We express our sincere thanks to ACACIAGUM Project (FP6-INCO-32233) for financing these works and the Laboratory of Pedology to the Faculty of Agronomy of Abdou Moumouni University of Niamey for analyzing soils samples.

REFERENCES

- [1] Garrity D. P., Akinnifesi F. K., Ajayi O. C., Weldesemayat S. G., Mowo J. G., Kalinganire A., Larwanou M., Bayala J., 2010. Evergreen Agriculture: a robust approach to sustainable food security in Africa. *Food Security*, 2: 197-214.
- [2] Wickens GE, Seif El Din AG, Guinko S, Nahal I., 1995. Role of Acacia species in the rural economy of dry Africa and the Near East. In: 13 col. Phot. FRDF ed. Rome, 134.
- [3] Sprent JI, Odee D, Dokota D, 2010. African legumes: a vital but under-utilized resource. J. Exp. Bot., 66: 1257-1265.
- [4] Raddad. EY, Luukkanen. O; Adaptive genetic variation in water-use efficiency and gum yield in *Acacia Senegal* provenances grown on clay soil in the Blue Nile region, Sudan. Forest Ecology and Management; (2006) 226: 219-229.
- [5] Sarr. A, Faye. A, Oihabib. A, Houeibib. MAJO, Neyra. M, Lesueur. D ; Inoculation en station et au champ d'*Acacia senegal* avec des souches selectionnées de *Rhizobium*. Bois et Forêts des Tropiques ; (2005) 283: 5-18.
- [6] Abdou M. M., Zoubeirou A. M., Kadri A., Ambouta J. M. K., Dan Lamso N., 2013. Effet de l'arbre *Acacia senegal* sur la fertilité des sols de gommeraies au Niger. *Int. J. Biol. Chem. Sci.*, 7(6): 2328-2337.
- [7] Faye A, Sall S, Chotte JL, Lesueur D, 2007. Soil bio-functioning under *Acacia nilotica* var. Tomentosa protected along the Senegal River. *Nutr. Cycl. Agroecosyst.*, 79: 35-44.
- [8] Rustad L. E., Cronan C. S., 1989. Cycling of aluminum and nutrients in litterfall of a red spruce (*Picea rubens Sarg.*) stand in Maine. *Canadian Journal of Forest Research* 19, 18-23.
- [9] Switzer G. L., Nelson L. E., 1972. Nutrient accumulation and cycling in loblolly pine (*Pinus taeda L.*) plantation ecosystems: the first twenty years. *Soil Science Society of America Journal* 36, 143-147.
- [10] Groffman PM, Bohlen PJ., 1999. Soil and sediment biodiversity: cross-system comparisons and large scale effects. BioSc 49: 139–148.
- [11] Sparling GP., 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International. 97–119.
- [12] Koch GP., 1916. Diastase activity and invertase activity of bacteria. Soil Sci 1: 179–196.
- [13] Zak JC, Sinsabaugh R, MacKay W., 1995. Windows of opportunity in desert ecosystems: their implications to fungal community development. Can J Bot 73: S1407–S1414.
- [14] Franchini JC, Crispino CC, Souza RA, Torres E, Hungaria M., 2007. Microbiological parameters as indicators of soil quality under various soil management and crop rotation systems in southern Brazil. Soil Till Res 92: 18–29.
- [15] Chen C, Condron LM, Davis M, Sherlock RR., 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. Plant Soil 220: 151–163.
- [16] Gomez E, Bisaro V, Conti M., 2000. Potential C-source utilization patterns of bacterial communities as influenced by clearing and land use in a vertic soil of Argentina. Appl Soil Ecol 152: 273–281.
- [17] Li Q, Lee AH, Wollum AG., 2004. Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control. Soil Biol Biochem 36: 571–579.
- [18] Zhang L, Xu ZH., 2008. Assessing bacterial diversity in soil: a brief review. J Soil Sediment 8: 379–388.
- [19] Alef K. 1998. Estimation of hydrolysis of fluorescein diacatate. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Acacdemic Press, London. pp 232–233.
- [20] Bakhoum N., 2012. Relations entre *Acacia senegal* (L.) Willd. et les communautés microbiennes du sol: effets sur la fertilité des sols et la durabilité de la production de gomme arabique. Thèse de doctorat de l'Université Cheikh Anta Diop, Sénégal, 199P.
- [21] Fall D., Diouf D., Zoubeirou A. M., Bakhoum N., Faye A., Sall S. N., 2012. Effect of distance and depth on microbial biomass and mineral content under *Acacia Senegal (L.) Willd.* trees. *Journal of Environmental Management*, 5260-5264.
- [22] Acosta-Martinez V., Acosta-Mercado D., Sotomayor-Ramirez D., Cruz-Rodriguez L., 2008. Microbial communities and enzymatic activities under different management in semi-arid soils. *Appl Soil Ecol* 38: 249–260.
- [23] Klose S. & Tabatabai M. A., 1999. Urease activity of microbial biomass in soils. Soil Bio. Biochem. 31:205-211.
- [24] Yang CH, Crowley DE. 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Appl. Environ. Microbiol.* 66 (1): 345-351.
- [25] Lynch J. M. & Whipps J. M., 1990. Substrate flow in the rhizosphère. *Plant Soil*, 129 (1): 1-10.

- [26] Castro-Sowinski S., Matan O., Bonafede P., Okon Y., 2007. A Thiredoxin of Sinorhizobium meliloti CE52G is required for melanin production and symbiotic nitrogen fixation. *Mol. Plant. Microb.Interact.* 20:986-993.
- [27] Rouatt J.W. & Katznelson H., 1961. A study of the bacteria on the root surface and in the rhizosphère soil of crop plant. *J. Appl. Bacteriol.*, 24: 164-171.
- [28] Nguyen C., 2003. Rhizodeposition of organic C by plants: mechanisms and control. Agronomie 23: 375–396.
- [29] Salt D.E., Smith R.D., Raskin I., 1998. Phytoremediation. An. Rev. Plant Physiol. Mol. Biol. 49: 643-668.
- [30] Bodelier P.L.E., Wijlhuizen A.G., Blom C.W.P.M., & Laanbroek H.J., 1997. Effect of photoperiod on growth and denitrification by Pseudomonas chlororaphis in the root zone of *Glyceria maxima* studied in a genotobiotic microcosm. *Plant Soil*, 190: 91-103.
- [31] Allison S. D, Vitousek P. M., 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. *Biotropica* 36: 285–296.
- [32] Waldrop M. P., Balser T. C., Firestone M. K., 2000. Linking microbial community composition to function in a tropical soil. *Soil Biol. Biochem.* 32: 1837–1846.
- [33] Sinsabaugh R. L., Antibus R. K., Linkins A. E., 1991. An enzymic approach to the analysis of microbial activity during plant litter decomposition. *Agric Ecosyst Environ* 34: 43–54.
- [34] Dick R. P., Rasmussen D., Turco R., 1996. Soil enzyme activities and biodiversity measurements as integrating biological indicators. In: Doran JW, Jones AJ (eds) Handbook of methods for assessment of soil quality. Soil Science Society of America. Madison, WI, pp 247–272.
- [35] Shi W., E., Bowman D., Iyyemperumal K., 2006. Soil enzyme activities and organic matter composition in a turfgrass chronosequence. *Plant Soil* 288: 285–296.
- [36] Turner B. L., Hopkins D. W., Haygarth P. M., Ostle N., 2002. β-Glucosidase activity in pasture soils. Appl. Soil Ecol. 20: 157–162.
- [37] Wan F., Chen P., 2004. Soil Enzyme Activities under Agroforestry Systems in Northern Jiangsu Province. *Forestry Studies in China* 6: 21–26.
- [38] Bashan Y., Puente M. E., Rodriguez-Mendoza M. N., Toledo G., Holguin G., Ferrera-Cerrato R., Pedrin S., 1995. Survival of *Azospirillum brasilense* in the Bulk Soil and Rhizosphere of 23 Soil Types. *Appl Environ Microbiol* 61(5): 1938-1945.
- [39] Fages J., 1992. An industrial review of Azospirillum inoculant: formulation and application technology. *Symbiosis* 13: 15–26.
- [40] Rovira A. D., 1965. Interaction between plant root and soil microorganism. Ann. Rev. Microbiol., 19: 241-266.
- [41] Schnurer J., Clarholm M., Bostrom S., & Rosswall T., 1986. Effects of moisture on soil microorganisms and nematodes: a field experiment. *Microb. Ecol.*, 12: 217-230.
- [42] Bottner P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ¹⁴C- and ¹⁵N labelled plant material. Soil Boil. Biochem., 17: 329-337.
- [43] Harris R.F., 1981. Effect of water potential on microbial growth and activity. In: Parr, J.F., Gardner, W.R., Elliot, L.F. (Eds.), Water Potential Relations in Soil Microbiology. *Soil Science Society of America*, Madison, pp. 23-95.
- [44] Reid D. S., 1980. Water activity as the criterion of availability. *In*: Ellowood DC, Hedger JN, Latham MJ, Lynch JM, Slater JH (eds) Microbial ecology. Academic Press, London, pp 15-27.