



Effects of Soil Fertility Management on the Diversity of Rhizobacteria in Millet Fields in Niger

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Agriculture in sub-Saharan Africa, and particularly in Niger, is constrained by low productivity, recurrent drought, limited access to fertilisers and environmental degradation, all of which contribute to persistent food insecurity. Rhizobacteria are recognised as important components of the rhizosphere because they can support plant growth and may reduce dependence on chemical fertilisers. However, the response of rhizobacterial communities to farmers' soil fertility management practices in millet fields remains insufficiently documented. This study analysed the effect of farmers' soil fertility management practices on rhizosphere microorganisms. The research was conducted from 2015 to 2017 in three villages (Léléddjé, Moli Haoussa and Alambaré) located near Niger's W National Park. Experimental plots of 16 m² were arranged in

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scattered blocks and subjected to animal penning, mulching, manure application and control treatments. The plant material was a local millet variety, somno (*Pennisetum glaucum* (L.) R. Br.). A total of 36 composite soil samples were analysed over the three years. Bacteria were isolated on five culture media, and colony numbers were expressed as colony-forming units per gram of soil. The highest bacterial counts were recorded on PCA and King A and B media, followed by PCA + GNO, whereas lower counts were observed on EMB and GNO media. Bacterial colony density differed among treatments, with animal penning and manure application producing higher densities than mulching and the control. These findings indicate that traditional soil fertility management practices used by farmers can maintain soils that are densely populated with cultivable rhizobacteria. Such practices may contribute to locally accessible strategies for improving soil biological fertility in millet-based farming systems in Niger.

Keywords: *Soil fertility management; rhizobacteria; millet; Pennisetum glaucum; Niger; rhizosphere; organic manure; mulching; animal penning; bacterial colony density; sustainable agriculture.*

1. Introduction

Agriculture in sub-Saharan African countries is characterised by low productivity (Bado, 2002). The growth in agricultural production observed over the last ten years remains insufficient to meet the needs of an increasing population; in 2010, 239 million of 863 million people were undernourished (FAO et al., 2018). As in other West African countries, Niger's natural resources, which constitute the main basis of production, continue to be affected by the cumulative effects of recurrent droughts, resulting in chronic food insecurity for the population (Gianinazzi et al., 2010; Abdoul Habou et al., 2016). Soil fertility is a critical factor determining the productivity of agricultural systems. It refers to the capacity of soil to supply essential nutrients in adequate amounts and appropriate proportions for optimal plant growth. Maintaining soil fertility is therefore important for achieving crop yields and supporting food security (Bhandari et al., 2025).

Several experiments and approaches have been tested, including water and soil conservation techniques, agroforestry and integrated soil fertility management. However, many of these approaches require high initial investment and often involve substantial changes to agricultural landscapes and practices. Imported fertilisers are difficult to access in rural areas and must often be purchased during the lean season (De Jong, 2013). Commonly recommended solutions, such as chemical fertiliser application, the use of new seed varieties and water management, are not always suitable for this vulnerable region because they are not accessible to all farmers and may contribute to environmental degradation (Becerra, 2012; Dixon & Postgate, 1972; Schenck & Smith, 1982). Although these practices are important for the livelihoods of the populations concerned, they may cause environmental damage that limits sustainable development and can contribute to food insecurity. It is therefore necessary to identify effective technologies that, when applied by producers, can compensate for limited access to fertilisers (Beauchamp et al., 1993; Pérez-Montaña et al., 2014).

Soil microorganisms play a key role in plant nutrient uptake and are involved in a wide range of biological processes. Some microorganisms can mineralise insoluble soil phosphorus, making it available for plant uptake (Beauchamp et al., 1993; Zimmermann, 2011). Apart from chemical fertilisation, microbial phosphate solubilisation and mineralisation appear to be important pathways for increasing the amount of phosphorus available to plants (Timofeeva et al., 2022). Numerous microorganisms in the soil and rhizosphere are known to release phosphorus effectively through solubilisation and mineralisation and are referred to as phosphate-solubilising microorganisms (Bhattacharyya & Jha, 2012; de Andrade et al., 2023).

Rhizobacteria are of considerable agronomic interest because their use could reduce dependence on chemical fertilisers and pesticides (Mosse et al., 1981; Morrissey et al., 2002). These bacteria actively colonise plant roots and can increase plant growth and yield (Wu & Kanamori, 2005). They promote plant growth and are considered an alternative to the use of chemicals in agriculture (Kloepper et al., 1980). They are often used as model rhizobacteria (Vrieze & Sorensen, 2001) and may constitute a substantial proportion, up to 10%, of the cultivable rhizosphere microflora (Robinson et al., 1996).

Despite the recognised importance of rhizobacteria in plant growth, limited information is available on how locally practised soil fertility management strategies influence cultivable rhizobacterial diversity in millet fields

in Niger. In particular, the comparative effects of animal penning, mulching, manure application and untreated soils on bacterial colony density remain insufficiently documented under the conditions of farmers' fields around Niger's W National Park. The overall objective of this study was to analyse the effect of farmers' soil fertility management practices on rhizosphere microorganisms.

2. Materials and methods

2.1 Study Site

The study was conducted in the peripheral zone of W National Park of Niger, in the rural commune of Tamou, located in the extreme south of the Say Department, 75 km from Niamey (Fig. 1). The soil texture is sandy, and the upper horizon, which is low in humus, is very colourful. These soils are fragile, and the upper horizon is sometimes eroded by runoff or wind.

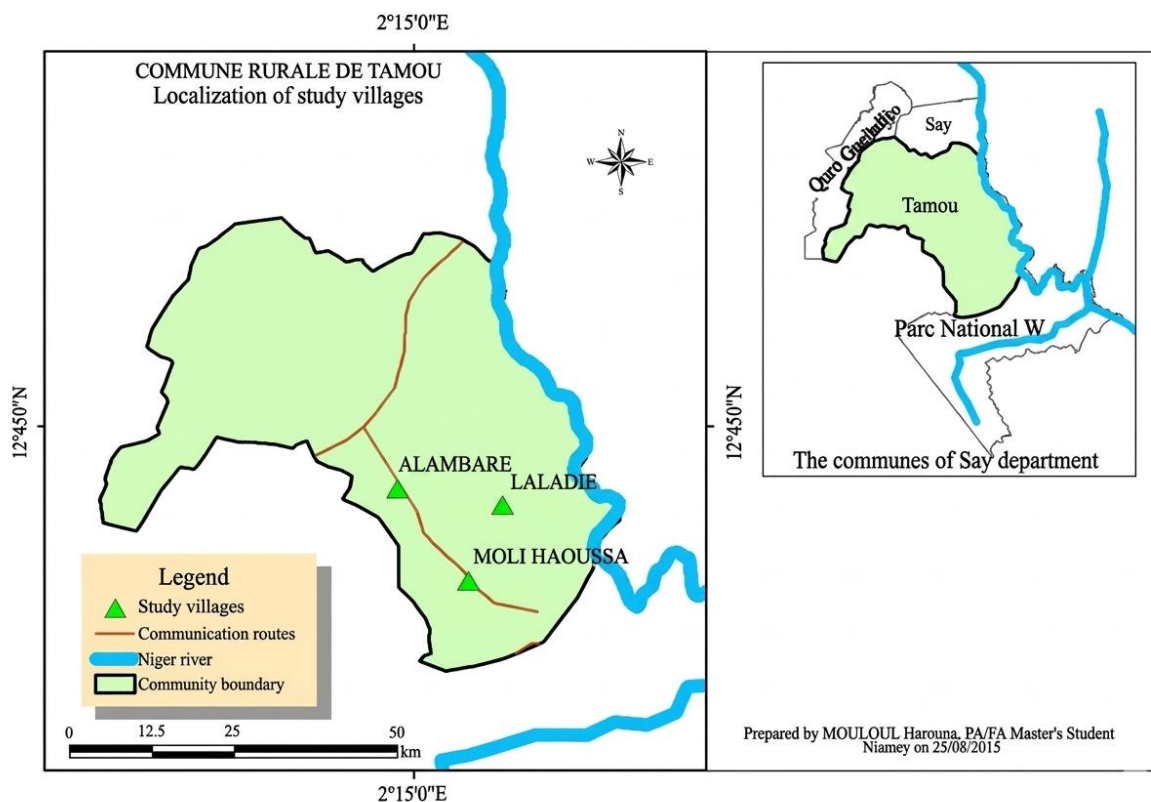


Fig. 1. Map of villages in the study area
(Source: Salissou et al., 2023)

2.2 Method for Studying Rhizobacteria Diversity

To study rhizobacterial diversity, soil samples were collected from an experimental design consisting of 16 m² plots arranged in scattered blocks. The plots were established according to four treatments: animal penning, mulching, organic manure application and an untreated control. The treatments were applied only during the first year of the study (2015), and the plots were monitored over three years, from 2015 to 2017. The plant material used was millet (*Pennisetum glaucum* (L.) R. Br.), a local variety known as somno. The samples analysed covered the three years of the study (2015, 2016 and 2017) and were analysed separately according to fertility management practice. A total of 36 samples were considered, comprising 12 composite samples per year. Dilutions were carried out according to the method described by Curtis and Speck (1976). Bacteria were isolated on five culture media, as summarised in Table 1. The colonies present on the plates were counted to estimate the number of microorganisms per gram of sample using the formula below:

Table 1. Bacteria isolation parameters

Culture medium	Incubation temperature	Incubation time	References
Rhizobacteria			
PCA	37 °C	24 h	Guiraud & Galzy (1980)
GNO	30 °C	24 h	ISO (2003) standard
King A and B	30 °C	48 to 72 h	Aparna & Sarada (2012)
Total bacteria			
PCA+ GN	37 °C	24 h	Guiraud & Galzy (1980)
EMB	37 °C et 44 °C	24 h	ISO (2003) standard

(Source: Salissou et al., 2023) *FAMT*: Total aerobic mesophilic flora; *PCA*: Plate Count Agar; *GNO*: Ordinary Nutrient Agar; *EMB*: Eosin Methylene Blue; *GN*: Nutrient Agar

3. Results and Discussion

3.1 Counting Rhizobacterial Strains

The number of bacteria was estimated and expressed in colony-forming units (CFU) per gram of soil (Table 2). Counting colonies on the different culture media yielded higher numbers of bacteria on PCA (33.5910-10) and King A and B (28.2110-10) media than on the other culture media. These high bacterial numbers were followed by the number of colonies counted on PCA + GNO medium (24.8110-10); the lowest numbers were observed on EMB (17.5510-10) and GNO (17.3910-10) culture media. Davis (2005), in a study on the effect of growth medium and inoculum size on the isolation of soil bacteria, counted between 5.0107 and 6.3109 CFU/g of soil. Bedjaj and El Fatmi (2018), in a study conducted in the Ouargla region on the characterisation of soil microorganisms, counted 94.76×10^6 and 119.2×10^6 microorganisms/g of dry soil. All these results are lower than those obtained in the present study. According to Agbodjato et al. (2015), variability in the number of colonies in soil samples could be due to the physicochemical properties of soil types, which can influence microbial activity in the rhizosphere. Indeed, pH appears to be a parameter that strongly influences the structure and diversity of bacterial communities in soils (Theodorakopoulos, 2013). In general, it is now considered that one gram of soil hosts several thousand species and that the abundance of these species can vary from 10^8 to 10^{11} cells per gram of soil. The high rate of microbial colonies counted in our soil samples confirms that soils resulting from fertility management practices implemented by producers are densely populated with rhizobacteria.

3.2 Morphological Characteristics of Isolated Bacterial Strains

On each culture medium, strains exhibiting different colony morphologies (size, colour, shape and other visible characteristics) (Table 3) were separated. Those with the highest abundance percentages were first purified by successive subcultures, then multiplied and stored at -80 °C until use in the selection tests.

Macroscopic examination (Table 3) of the colonies on the culture media revealed several characteristics, including colour, shape, size, motility, pigmentation and Gram stain. The colour of the different bacterial colonies varied from red, yellow and beige to white. Their shapes were round, oval, regular or irregular, with regular or irregular margins. The surface of the bacterial colonies was either smooth or rough. An earlier study showed that isolated bacterial colonies generally have several macroscopic appearances, including regular, irregular, smooth, mucous and convex forms, and different diameters (≤ 3 mm).

On GNO and King media, the colonies were small, motile and Gram-negative. On King medium, selection was based on fluorescent pigment production. This enabled the observation of fluorescent bacterial strains, some of which were creamy or whitish, with round shapes, regular edges, convex relief and sizes ranging from 0.5 to 2 mm. The same characteristics were reported by Ben Salem and Ayadi (2016) for the identification of these strains on King B medium. However, colonies isolated on PCA medium were large and white. They were Gram-positive. On this culture medium, the colonies were not pigmented. The strains form a genus of Gram-positive, bacillary, aerobic bacteria capable of producing endospores, enabling them to withstand adverse environmental conditions (Logan et al., 1998). On PCA medium supplemented with GNO, the colonies were whitish and mobile, whereas others were immobile. The colonies were mostly Gram-positive and Gram-negative and were mainly rounded, with a creamy, non-pigmented surface. On EMB medium, large, bacillus-shaped colonies were counted. Most colonies had different colours, including blue-black, bright green and greyish forms. Observation of the macroscopic appearance of the colonies allows initial characterisation and may guide interpretation during biochemical and genetic (molecular) identification (Ben Salem & Ayadi, 2016).

Table 2. Count of rhizobacteria resulting from soil fertility management practices

Treatments	Culture media				
	PCA	PCA + GNO	GNO	King A and B	EMB
Animal penning	11,1810 ⁻¹⁰	8,0510 ⁻¹⁰	5,0510 ⁻¹⁰	8,8310 ⁻¹⁰	5,04 10 ⁻¹⁰
Mulching	6,6110 ⁻¹⁰	5,66 10 ⁻¹⁰	5,4810 ⁻¹⁰	6,910 ⁻¹⁰	4,8810 ⁻¹⁰
Manure	9,97 10 ⁻¹⁰	6,42 10 ⁻¹⁰	4,7110 ⁻¹⁰	7,8410 ⁻¹⁰	6,0610 ⁻¹⁰
Control	5,83 10 ⁻¹⁰	4,64 10 ⁻¹⁰	2,1510 ⁻¹⁰	4,6810 ⁻¹⁰	1,5710 ⁻¹⁰
Total	33,59 10 ⁻¹⁰	24,81 10 ⁻¹⁰	17,39 10 ⁻¹⁰	28,2110 ⁻¹⁰	17,5510 ⁻¹⁰

FAMT: Total aerobic mesophilic flora; PCA: Plate Count Agar; GNO: Standard Nutrient Agar; EMB: Eosin Methylene Blue; GN: Nutrient Agar (Salissou et al., 2023)

Table 3. Morphological characterisation of the bacterial colonies observed

Culture media	Size	Mobility	Colour	Gram	Shape	Surface	Pigmentation
Rhizobacteria							
PCA	Large	Mobile	White	G ⁺	Round, oval, bacillary	Smooth	No pigment
GNO	Small	Mobile	Creamy yellow	G ⁻	Rods	Smooth	Red
King A and B	Small	Mobile	yellow and orange	G ⁻	Bacillary	Roughness régular or irrégular	Fluorescent on King B
Total bacteria							
PCA+GNO	Averages Small	Mobile immobile	Whitish	G+/G-	Round, lenticular mass	Creamy	No pigment
EMB	Large size	Mobile immobile	-bleu-noir -incolore -brillant vert -grisâtre	G-	Bacillary	Irregular Convex	pigmented and non-pigmented Colonies

FAMT: Total aerobic mesophilic flora; PCA: Plate Count Agar; GNO: Standard Nutrient Agar; EMB: Eosin Methylene Blue; GN: Nutrient Agar (Salissou et al., 2023)

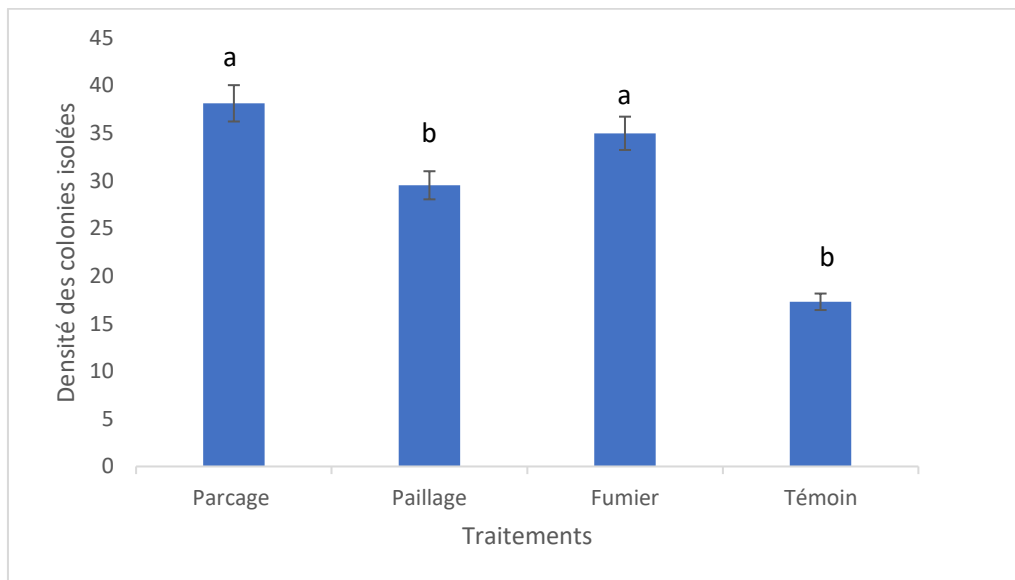


Fig. 2. Number of bacteria by soil fertility management practices

3.3 Variation in Bacterial Colony Density According to Farming Practices

The results of the study revealed a significant difference between treatments. The density of bacterial colonies varied according to soil fertility management practice. The density of bacterial colonies counted in the animal penning and manure treatments was statistically higher than that counted in the mulch and control treatments. Animal penning and manure application were more conducive to bacterial growth. One factor that may promote bacterial growth under animal penning is the higher organic matter content compared with other cultivation practices, including the control. An obstacle to the growth of certain bacteria in the laboratory can be explained by the phenomenon of ‘substrate-accelerated death’ (Calcott & Postgate, 1972). The growth of bacteria initially present in substrate-poor environments may be inhibited in the presence of an excessively high concentration of the same substrate (Calcott & Postgate, 1972). This could explain the low number of microorganisms present in the other samples, particularly those from mulching. The study showed that Petri dishes incubated with soil samples from animal penning and manure treatments produced a high density of bacterial colonies. Low densities were obtained in the mulch and control treatments. Animal penning yielded twice as many bacteria as the control. However, recent studies show that, by modifying certain parameters such as O₂ and CO₂ content or by increasing incubation time, the number of cultivable bacteria can be increased (Stevenson et al., 2004).

4. Conclusion

The study showed that bacterial colony density varied according to the soil fertility management practices applied by farmers in millet fields. Counts obtained from the different culture media indicated that the soils were populated by cultivable rhizobacteria, with higher counts recorded on PCA and King A and B media than on the other media. Differences among treatments were also evident. Animal penning and manure application produced the highest densities of bacterial colonies, whereas mulching and the control had lower densities. Animal penning yielded approximately twice as many bacteria as the control, indicating that this practice, together with manure application, was more favourable to bacterial development under the conditions of the study. The observed response may be related to the organic matter supplied through these practices, which can support microbial activity in the rhizosphere. Overall, the results suggest that traditional soil fertility management practices used by farmers can influence the abundance of cultivable rhizobacteria in millet soils. These practices may therefore represent locally accessible options for maintaining soil biological activity where mineral fertilisers are costly, difficult to obtain or poorly adapted to farming conditions. Further identification and functional evaluation of the isolated strains would help clarify their potential role in supporting millet production.

5. Limitations

This study provides useful information on the variation in cultivable rhizobacteria associated with farmers' soil fertility management practices; however, some limitations should be considered. The analysis was based on bacteria that developed on the selected culture media, and therefore may not represent the full diversity of rhizobacteria present in the soil. Microorganisms that are difficult to culture under the incubation conditions used may have been underestimated or not detected. The study also focused on colony density and morphological characteristics, while biochemical, molecular and functional identification of the isolates was not included. Consequently, the specific taxonomic identity and plant growth-promoting potential of the bacterial strains remain uncertain. The treatments were applied only during the first year, although monitoring continued over three years, which may influence interpretation of temporal effects. In addition, the study was conducted in selected villages near W National Park, and extrapolation to other agroecological zones should be made cautiously.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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