

WATER MANAGEMENT AND NITROGEN RATES EFFECT ON MICROBIAL BIOMASS UNDER LOWLAND RICE

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Abstract: A field experiment was conducted in 2009-2011 rice growing season to determine water management and nitrogen rates effect on soil microbial biomass on dystric gleysol located at Edozighi Southern Guinea Savanna of Nigeria. Treatments were a combination of water management and nitrogen rates arranged in a split plot design with four replications. Soil samples were taken at the 0-20 cm depth during the dough grain stage. Results showed that there were significant differences in fungi count due to water management with values that ranged between 4.00×10^5 cfu/g and 8.00×10^5 cfu/mg. Similarly, significant differences were observed in fungal count due to nitrogen rates with values that ranged between 3.25×10^5 cfu/g and 9.00×10^5 cfu/mg. The same trend was recorded in bacterial count with values that ranged between 2.50×10^6 cfu/g and 5.80×10^6 cfu/mg due water management practices. Additionally, application of different rates of nitrogen also had significant differences in bacterial count with values that ranged between 2.40×10^6 cfu/g and 5.70×10^6 cfu/g. Microbial biomass carbon ranged from 300 mg/kg and 720 mg/kg due to water management and was significantly different. Application of nitrogen also had significant difference in microbial biomass carbon with values that ranged between 300 mg/kg and 700 mg/kg. It also showed a trend to decrease with increase in nitrogen dosage.

Keywords: Water management, nitrogen rates, microbial biomass carbon, lowland rice

1. Introduction

Soil is the habitat of a diverse array of organisms which include both micro flora and fauna. Soil microorganisms play a very important role in soil fertility not only because of their ability to carry out biochemical transformation but also due to their importance as a source and sink of mineral nutrients [1]. Soil microbes, the living part of soil organic matter, function as a transient nutrient sink and are responsible for releasing nutrients from organic matter for use by plants (e.g., N, P and S). An understanding of microbial processes is important for the management of farming systems, particularly those that rely on organic inputs of nutrients [2]. The soil microbial community is involved in numerous crucial roles in the terrestrial carbon cycle [3]. Changes in microbial communities can be used to predict the effects of ecosystem perturbations by organic and conventional management practices [4].

Agricultural activities such as tillage, intercropping, rotations, drainage, irrigation, use of pesticides and fertilizers have significant implications for the microorganisms present in the soil [5]. The soil microorganisms are sensitive to changes in the surrounding soil [6] and have shown that the microbial population changes after fertilization [7]. Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil [8]. The application of chemical fertilizer generally improves crop production; however, concerns have been raised not only about the severe environmental problems posed by such practices but also about the long term sustainability of such systems [9]. On the other hand, use of organic materials (e.g., animal manures, crop residues, green manures, etc.) as an alternative source holds promise. Organic farming has been expanding at an

annual rate of 20% in the last decade [10] and has become a mainstream practice for some crops [11]. Organic applications increased nutrient status, microbial activity and productive potential of soil while the use of only chemical fertilizers in the cropping system resulted in a poor microbial activity and productive potential of soil [12]. In comparison with conventional farming, organic farming has potential benefits in promoting soil structure formation [13]; [14], enhancing soil biodiversity [15]; [16], alleviating environmental stresses [17]; [18], and improving food quality and safety [19]. The use of chemical fertilizer alone was not effective in improving the nutrient status of soil [20].

Changes in soil properties due to cultivation and management and their consequences for production capacity have been a concern of research for many years. Recognition of the importance of soil microorganisms has led to increased interest in measuring the nutrients held in their biomass [21]. Besides living plants roots and organisms, soil microbial biomass is a living portion of soil organic matter. Soil microbial biomass is considered to act both as the agent of biochemical changes in soil and as a repository of plant nutrients such as nitrogen (N) and phosphorus (P) in agricultural ecosystems [22]. The changes in soil organic carbon contents are directly associated with changes in microbial biomass carbon and biological activity in the soil. The response to changes in inputs of organic material is much quicker in soil microbial biomass than in soil organic matter as a whole [23]. Microbial biomass contains labile fraction of organic C and N, which are mineralized rapidly after the death of microbial cells. Soil microbes are typically C-limited [24]; lower microbial biomass in soils from conventional agroecosystems is often caused by reduced organic carbon content in the soil [25]. The quantity and quality of organic inputs are the most important factors

affecting microbial biomass and community structure [26]. Continuous cultivation with frequent tillage results in a rapid loss of OM through increased microbial activity [27]. Recently, microbial biomass and enzyme activities have been recognized as early and indicators of soil stress or productivity changes. Further, there is considerable evidence that they can be used to evaluate the influence of management and land use on soils [28]; [29].

The present investigation was conducted with the aim to assess the impact of inorganic farming practices of lowland rice on the dynamics of soil microbial populations and their activities in paddy fields.

2. Materials and methods, Experimental design

The experiment consisted of eight treatments comprising of four levels of water management (irrigation regimes) as one factor and four levels of nitrogen rates as another factor. The four irrigation regimes include:

- (i) Continuous ponding with 5 cm of standing water from transplanting to hard dough stage (CF).
- (ii) Alternate 30 days ponding with – 7 days drainage – 30 days ponding – 7 days drainage and pond up to hard dough stage (AF30-7-30-7-30-7).
- (iii) Alternate 60 days ponding – 7 days drainage – 30 days ponding – 7 days drainage and pond up to hard dough stage (AF60-7-30-7).
- (iv) Alternate 90 days ponding – 7 days drainage and pond up to hard dough stage (AF90-7).

The four levels of nitrogen rates include 40 kg N ha⁻¹ (control), 60 kg N ha⁻¹, 80 kg N ha⁻¹, and 100 kg N ha⁻¹.

The experiment was laid out in a split plot design with randomized complete block arrangement. Water management was assigned to the main plots and nitrogen rates to the subplots. Each treatment combination was replicated four times. Field observations and measurements were made for the three consecutive seasons using the same experimental design and field layout.

Soil samples were collected from the surface (0-20cm) soil depth in each experimental plot starting from pre-transplanting period and at dough stage period for three years. From each plot, soil samples were collected randomly and mixed thoroughly to get a homogenous mixture. About 250 g of the soil samples collected were stored at 4°C and was used for microbiological analysis.

Isolation and estimation of microbial populations, i.e., fungi using soil plate method [30] and bacteria using dilution plate method [31][32], were carried out using rose Bengal agar media and nutrient agar media for fungi and bacteria, respectively. Media were prepared according to the composition and sterilized in autoclave. Microorganisms were enumerated using soil plate and serial dilution methods on specified media plates and the inoculated plates were incubated at temperatures of 25 and 30°C at duration of 5-7 days and 1-2 days for fungi and bacteria, respectively. After the incubation period, the colony forming units were counted and expressed as cfu/g of soil on a moisture free basis.

Soil microbial biomass carbon (MBC) was determined using the chloroform- fumigation- extraction method given by Anderson and Ingram [33].

3. Results and Discussions

Table 1. shows the effect and interaction of water management and nitrogen rates on fungal count. Statistical analysis has shown that there was significant difference in fungal count due to water management practices. Continuous ponding had the highest fungal count and the lowest was recorded in the alternate ponding treatment (AF30-7-30-7-30-7) with values ranging from 8x10⁵ to 4x10⁵ cfu/g respectively. This is in agreement with the study conducted by Santos [34] who reported higher fungal and bacterial count in rice for continuous irrigation when compared with the alternate flooding.

Table 1. Main effects and interaction of water management and nitrogen rates on fungal count in the lowland soil.

Factor levels/Interactions	cfu/g x 10 ⁵		
	2009	2010	2011
Water management (WM)			
CF	8.00 a	7.79 a	7.50 a
AF30-7-30-7-30-7	4.00 c	4.50 c	4.00 c
AF60-7-30-7	5.00 c	5.25 c	5.75 c
AF90.7	7.25 b	7.00 b	7.10 b
SEM	0.45	0.47	0.35
Nitrogen rates (NR)			
40 kg N ha ⁻¹	7.00 b	6.50 b	6.70 b
60 kg N ha ⁻¹	5.00 c	5.25 c	5.40 c
80 kg N ha ⁻¹	9.00 a	8.87 a	8.90 a
100 kg N ha ⁻¹	3.25 c	4.00 c	5.20 c
SEM	0.40	0.45	0.35
Interaction (WMxNR)	Ns	Ns	Ns

Similarly, with respect to nitrogen rates there were also significant differences in fungal count. Application of 80 kg N ha⁻¹ had the highest fungal count while application of 100 kg N ha⁻¹ had the lowest with values ranging between 9x10⁵ to 3.25x10⁵ cfu/g respectively. There was no interaction between water management and nitrogen rates. Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil [35]. The application of chemical fertilizer generally improves crop production; however, concerns have been raised not only about the severe environmental problems posed by such practices but also about the long term sustainability of such systems.

There was no significant difference in the interaction between water management and nitrogen rates.

3.1 Effect of water management and nitrogen rates on bacterial count

Significant differences in bacterial count were observed due to water management practices (Table 2). Bacterial count ranged between 2.5x10⁶ and 5.80x10⁶ cfu/g.

Continuous flooding had the highest number of bacteria with a value of 5.80×10^6 cfu/g while the lowest number of bacteria was recorded by alternate flooding AF30-7-30-7-30-7 with a value of 2.50×10^6 cfu/g. This is also in agreement with the study conducted by Santos [37] who reported higher fungi and bacteria count in rice for continuous irrigation when compared with the alternate flooding.

Table 2. Main effects and interactions of water management and nitrogen rates on bacterial count in lowland soil.

Factor levels/ Interactions	cfu/g x 10 ⁶		
	2009	2010	2011
Water management (WM)			
CF	5.80 a	5.75 a	5.70 a
AF30-7-30-7-30-7	2.50 c	2.45 c	2.50 c
AF60-7-30-7	3.45 c	3.00 c	3.25 c
AF90-7	4.00 b	4.25 b	4.50 b
SEM	0.25	0.40	0.45
Nitrogen rate (NR)			
40 kg N ha ⁻¹	5.50 a	5.45 a	5.50 a
60 kg N ha ⁻¹	5.70 a	5.67 a	5.65 a
80 kg N ha ⁻¹	4.20 b	4.30 b	4.25 b
100 kg N ha ⁻¹	2.40 c	2.50 c	2.50 c
SEM	0.35	0.40	0.25
Interactions (WMxNR)	Ns	Ns	Ns

Similarly, significant differences were also recorded in bacterial count due to nitrogen rates. Statistical analysis showed that bacterial count ranged between 2.40×10^6 and 5.70×10^6 cfu/g. The highest number of bacteria was recorded when 60 kg N ha⁻¹ was applied while the lowest number was obtained when 100 kg N ha⁻¹ was applied. The result showed a downward trend when more than 60 kg N ha⁻¹ was applied. There was no interaction between water management and nitrogen rates. Agricultural activities such as, drainage, irrigation, uses of pesticides and fertilizers have significant implications for the microorganisms present in the soil [38]. The soil microorganisms are sensitive to changes in the surrounding soil [39], and have shown that microbial population changes after fertilization [40].

3.2 Effect of water management and nitrogen rates on microbial biomass carbon

Table 3 shows the effect of water management and nitrogen rates on microbial biomass carbon in the lowland soil. Statistical analysis indicated that there were significant differences in microbial biomass carbon due to water management practices. Continuous ponding of irrigation water had the highest microbial biomass carbon with a value of 720 mg/kg while the lowest was recorded by alternate ponding (AF30-7-30-7-30-7) with a value of 300 mg/kg.

Table 3. Main effects and interactions of water management and nitrogen rates on microbial biomass carbon.

Factors/interactions	mg/kg		
	2009	2010	2011
Water management (WM)			
CF	720 a	700 a	710 a
AF30-7-30-7-30-7	300 c	300 c	310 c
AF60-7-30-7	500 b	510 b	520 b
AF90-7	610 b	600 b	600 b
SEM	0.25	0.35	0.40
Nitrogen rates (NR)			
40 kg N ha ⁻¹	620 b	600 b	610 b
60 kg N ha ⁻¹	600 b	610 b	620 b
80 kg N ha ⁻¹	700 a	720 a	700 b
100 kg N ha ⁻¹	300 c	300 c	310 c
SEM	0.03	0.05	0.15
Interactions(WMxNR)	Ns	Ns	Ns

Similarly, significant differences were observed in microbial biomass carbon with the application of different rates of nitrogen fertilizer. Application of 80 kg N ha⁻¹ had the highest microbial biomass carbon with a value of 720 mg/kg while the lowest was obtained by application of 100 kg N ha⁻¹. There was no interaction between water management and nitrogen rates. Recognition of the importance of soil microorganisms has led to increased interest in measuring the nutrients held in their biomass [41]. Besides living plants roots and organisms, soil microbial biomass is a living portion of soil organic matter. Soil microbial biomass is considered to act both as the agent of biochemical changes in soil and as a repository of plant nutrients such as nitrogen (N) and phosphorus (P) in agricultural ecosystems [42].

4. Conclusion

Lowland rice fields often have a lot of organic matter left on the soil surface after harvest. This is incorporated into the soil during land preparation. Application of irrigation water and chemical fertilizer stimulated the activity of microorganisms in the soil as seen in the trial. Continuous ponding had the highest microbial biomass carbon from the water management strategies. Application of 80 kg N ha⁻¹ had the highest microbial biomass carbon after which there was a decline. Chemical fertilizer in the cropping system resulted in poor microbial activity and productive potential of the soil beyond 80 kg N ha⁻¹. Thus, raising the question of sustainable production of lowland rice with chemical fertilizer alone.

References

- [1] D.S.Jenkinson and J.N. Ladd, "Microbial Biomass in Soil: Measurement and Turnover," In: Soil Biochemistry, Paul, E.A. and J.N. Ladd (Eds.). Marcel Dekker, New York, USA, pp:415- 471,1991.
- [2] J.L.Smith, and E.A. Paul, The Significance of Soil Microbial Biomass Estimations. In: Soil Biochemistry, Bollag, J.M. and G. Stotzk, (Eds.) Dekker Ltd., New York, pp 206, 1990
- [3] D.S.Schimel, "Terrestrial ecosystem and carbon cycle," Global Change Biology., 1: 77-91, 1995

- [4] D.D.Poudel, W.R. Horwarth, W.T. Lanini, S.R Temple and A.H.C. Van- Bruggen, "Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low input and conventional farming systems in northern California," *Agriculture Ecosystem and Environment.*, 90: 125-137, 2002.
- [5] R.Hengeveld, "Measuring ecological biodiversity," *Biodiversity Letter.*, 3: 58- 65, 1996.
- [6] F.Schinner, and R. Sonnletner, "Bodenökologie : Mikrobiologie und Bodenenzymatik," Springer Verlag, New York, pp 105, 1996.
- [7] M.R.Hyman, C.Y. Kim and D.J. Arp," Inhibition of ammonia monooxygenase in *Nitrosomonas europaea* by carbon disulfide," *Journal Bacteriology*, 172: 4775-4782,1990 .
- [8] D.J.Khonje, E.C. Varsa and B. Klubek, "The acidulation effects of nitrogenous fertilizers on selected chemical and microbiological properties of soil," *Communication in Soil Science and Plant Analysis*, 20: 1377-1398, 1989.
- [9] P.Mader, A. Flie-Bach, D. Dubois, L. Gunst, P. Fried and U. Niggli, "Soil fertility and biodiversity in organic farming," *Science*, 296: 1694-1697, 2002.
- [10] D.W.Lotter,. "Organic agriculture,," *Journal of Sustainable Agriculture*. 21:59-128, 2003.
- [11] Anonymous, "Organic farming enters the mainstream," *Nature*, 428: 783- 783,2004.
- [12] G.S.Kang, V. Beri, O.P. Rupela and B.S. Sidhu, "A new index to assess soil quality and sustainability of wheat based cropping systems," *Biology, Fertilizer and . Soils*, 41: 389-398, 2005.
- [13] J.P.Reganold, L.F. Elliott and Y.L. Unger, 1987. Long-term effects on organic and conventional farming on soil erosion. *Nature*, 330: 370-372 1987.
- [14] M.Pulleman, A. Jongmans, J. Marinissen and J. Bouma, 2003. Effects of organic versus conventional arable farming on soil structure and organic matter dynamics in marine loam in the Netherlands. *Soil Use and Management.*, 19: 157-165, 2003
- [15] P.Mader, A. Flie-Bach, D. Dubois, L. Gunst, P Fried and U. Niggli, "Soil fertility and biodiversity in organic farming," *Science*, 296:1694-1697, 2002.
- [16] F.Oehl, E. Sieverding, P. Mader, D. Dubois, K. Ineichen, T. Boller and A. Wiemken, "Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi," *Oecologia*, 138: 574-583, 2004.
- [17] L.Horrigan, S.L. Robert and P. Walker, "How sustainable agriculture can address the environmental and human health harms of industrial agriculture," *Environment. Health Perspectives.*, 110: 445-456,2002.
- [18] C.Macilwain, "Is organic farming better for the environment," *Nature*, 428: 797-798, 2004.
- [19] H. Giles, " Is organic food better for us *Nature*," 428: 796-797, 2004.
- [20] G.S.Kang, V. Beri, O.P. Rupela and B. Sidhu, "A new index to assess soil quality and sustainability of wheat based cropping systems," *Biology, Fertilizer and. Soils*, 41 : 389-398, 2005.
- [21] D.S. Jenkinson,. and D.S. Powlson, "The effect of biocidal treatments on metabolism in soil," V. A method for measuring soil biomass. *Soil Biology and Biochemistry*, 8: 209-213,1976.
- [22] D.S.Jenkinson and J.N. Ladd, " Microbial Biomass in Soil: Measurement and Turnover," In: *Soil Biochemistry*, Paul, E.A. and J.N. Ladd (Eds.). Marcel Dekker, New York, USA., pp: 415-471,1991.
- [23] D.S.Powlson, and D.S. Jenkinson, " A comparison of organic matter, biomass, adenosine triphosphate and mineralisable nitrogen contents of plowed and direct-drilled soils," *Journal of Agricultural Science.*, 97: 713- 721,1981.
- [24] J.L.Smith, and E.A. Paul, 1990. The Significance of Soil Microbial Biomass Estimations. In: *Soil Biochemistry*, Bollag, J.M. and G. Stotzky, (Eds.) Dekker Ltd., New York, pp 206, 1990
- [25] A.Fliebach, and P. Mader, "Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems," *Soil Biology and Biochemistry*, 32: 757-768, 2004.
- [26] A.D.Peacock, M.D. Mullen, D.B. Ringelberg , D.D. Tyler, D.B. Hedrick, P.M. Gale and D.C. White, "Soil microbial community responses to dairy manure or ammonium nitrate applications," *Soil Biology and Biochemistry* , 33: 1011-1019, 2001.
- [27] T.G.Shepherd, S. Saggiar, R.H. Newman, C.W. Ross and J.L Dando,"Tillage- induced changes in soil structure and soil organic matter fractions," *Australian Journal of Soil Research.*, 39: 465-489, 2001.
- [28] F.Caravaca, G. Masciandaro and B. Ceccanti, "Land use in relation to soil chemical and biochemical properties in a semiarid Mediterranean environment," *Soil Tillage Research*, 68: 23-30, 2002.
- [29] S.Saggiar, M.D. McIntosh, C.B. Hedley and H. Knicker, "Changes in soil microbial biomass, metabolic quotient and organic matter turnover under *Hieracium* (*H. Pilosella* L.)," *Biology. Fertilizer and. Soil*, 30: 232-238, 1999.
- [30] J.H.Warcup, "The soil plate method for isolation of fungi from soil," *Nature*,166: 117-117, 1950.

- [31] L.F.Johnson, and A.E. Curl, "Method for the research on ecology of soil borne pathogens." Burgess publishing company Minneapolis pp 247, 1972.
- [32] A.Fliebach, and P. Mader, "Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems," *Soil Biology and Biochemistry*, 32: 757-768, 2004.
- [33] J.M.Anderson, and J.S.I. Ingram, "Tropical Soil Biology and Fertility" A Handbook of Methods. 2nd Edn., CAB. International, Oxfordshir Wallingford, UK., 1993
- [34] J.B.Santos, O. Arf; A.R.M. Cassioloto and J.Paz-Ferreiro 2005. Tillage system and water management effects on respiration and microbial biomass carbon under upland rice in Brazil. *Univercicle Estadual Paulista Sao Paulo* pp 1-4, 2005.
- [35] D.J.Khonje, E.C. Varsa and B. Klubek, "Theacidulation effects of nitrogenous fertilizers on selected chemical and microbiological properties of soil," *Communication in Soil Science and Plant Analysis*, 20: 1377-1398, 1989.
- [36] P.Mader, A. Flie-Bach, D. Dubois, L. Gunst, P. Fried and U. Niggli, "Soil fertility and biodiversity in organic farming," *Science*, 296: 1694-1697, 2002.
- [37] J.B.Santos, O. Arf; A.R.M. Cassioloto and J . Paz-Ferreiro 2005. Tillage system and water management effects on respiration and microbial biomass carbon under upland rice in Brazil. *Univercicle Estadual Paulista Sao Paulo* pp 1-4, 2005.
- [38] R.Hengeveld, "Measuring ecological biodiversity," *Biodiversity Letter.*, 3: 58- 65, 1996.
- [39] F.Schinner, and R. Sonnletner,"*Bodenokologie Mikrobiologic und Bodenenzymatik*," Springer Verlag, New York, pp 105, 1996..
- [40] M.R.Hyman, C.Y. Kim and D.J. Arp,"Inhibition of ammonia monooxygenase in *Nitrosomonas europaea* by carbon disulfide," *Journal of Bacteriology*, 172: 4775-4782,1990
- [41] D.S. Jenkinson,. and D.S. Powlson, "The effect of biocidal treatments on metabolism in soil," V. A method for measuring soil biomass. *Soil Biology and Biochemistry*, 8: 209-213,1976.
- [42] D.S.Jenkinson and J.N. Ladd, " Microbial Biomass in Soil: Measurement andTurnover," In: *Soil Biochemistry*, Paul, E.A. and J.N. Ladd (Eds.). Marcel Dekker, New York, USA., pp: 415-471,1991.