IS MICROBIAL ACTIVITY IN ANIMAL MANURE DRIVEN MORE BY STOICHIOMETRY THAN INDIVIDUAL NUTRIENT CONCENTRATIONS?

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ABSTRACT

The stoichiometric characteristics of organic resourcesare known to influence availability of nutrients to decomposers, microbial activity, rates of decomposition and nutrient release.However, information is scanty on the influence of manure stoichiometry on microbial activity. In this paper we report preliminary analysis of the effect oforganic carbon (C), nitrogen (N), phosphorus (P) and potassium (K) concentrations and their stoichiometric ratioson microbial activity inanimal manure.For the first time, this study established a significantly negative association between total microbial counts and the concentrations of C and stoichiometric ratios (C: N, C: P and C: K) in manure.Microbial activity did not significantly vary with the N:P ratio. We concluded that overall microbial activity in animal manure is determined by stoichiometric ratios rather than individual nutrient concentrations. We recommendmore detailed studies to determine whether or not this pattern is consistent across different groups of microbial decomposersin animal manure.

Keywords: Colony forming units, manure, microbial activity, nutrient concentrations, stoichiometry.

INTRODUCTION

Animal manure contains substantial quantities of nutrients needed for plant growth and it is one of the soil amendments used to counter nutrient limitationsin the soil (Fulhage, 2000). Manure is also a source of organic matter, which promotes growth of beneficial soil organisms, soil structure(Edmeades, 2003) and soil carbon (C) sequestration (Maillard and Angers, 2014; Zhang *et al*., 2015). The stoichiometric characteristics (e.g. C:N, N:P and C:P ratios) of organic resourceshave been reported to significantly influence the availability of nutrients to decomposers,microbial activity, rates of decomposition and nutrient release(Enríquez*et al*., 1993;Güsewell and Gessner, 2009; Keiblinger*et al*., 2010; Manzoni *et al*., 2010).Stoichiometric characteristics also influencemicrobial C use efficiency, which is an important determinant of C cycling in ecosystems (Keiblinger*et al*., 2010). The C:N ratio is also one of the important factors affecting the composting process; with initial C:N ratio of 25–30 being optimum for composting (Zhu, 2007).

So far much of the analysis on the relationships between stoichiometric ratios and microbial activities has focussed on plant litter. On the other hand, information is scanty on the influence of manure stoichiometry on microbial activity. Since mineralization of nutrients from manure is driven by microbial processes, it is important to explore the role of manure characteristics in this process.Therefore, the objective of this preliminary analysis was to determinethe relationship between microbial activityand nutrient concentrations andstoichiometric ratios in manure.Ourkeyhypothesis was that stoichiometric ratios are more important than individual nutrient concentrations in determining microbial activity in manure.

MATERIALS AND METHODS

Location and description of experimental site.

The experiment was carried out at the Institute for Agricultural Research (IAR), Ahmadu Bello University, Samaru- Zaria, which is located at Latitude 11° 11" N and Longitude 7° 33" E in the Northern Guinea Savanna zone of Nigeria.Samaru-Zaria has a mean annual rainfall of about 1050 mm, spanning the periods from May to September, while the dry season starts from October to April with a mean daily temperature of 24° C (Kowal andKnabe, 1972). The hottest months are those that precede the rains (March to April) and coldest months occur in November to January, October and February are considered as transition months. The global radiation is evenly distributed throughout the year, ranging from 440 cal. cm^2 day^{-1} in August to 550 cal. cm^2 day^{-1} in April to May (Kowal, 1972).

Experiment

Freshcattle manure was collected from the National Animal Production Research Institute (NAPRI),Ahmadu Bello University, Zaria, Nigeriamixed thoroughly and subjected to threestorage treatments, namely: (i) storage in a pit covered with polythene sheet, (ii) heaping on the ground surface and covering with polythene sheet, and (iii) heaping on the ground surface and leaving uncovered. The dung was then left undisturbed and allowed to decompose for four weeks, hereafter referred to as incubation. Then it was removed and stored in the field for 0, 4, 8 and 12 weeks before application as a soil amendment. Manure samples were taken twice from each of these treatmentsand storage periods for chemical analysis and determination of microbial activity. The first set of samples was taken at the end of one month of incubation, while the second set was taken at the end of the field storage periods just before application in the field.

Samples were then digested using wet oxidation, and the organic carbon (C) concentration was determined by the Walkley-Black method (Nelson and Sommers, 1982).

The N concentration was determined by the micro-kjedahl wet digestion method (Bremner, 1982). Potassium (K) concentration was determined by flame photometer, while calcium (Ca) and magnesium (Mg)concentrationswas determined using atomic absorption spectrophotometer. Phosphorus (P) concentrationwas determined by the vanadomolybdate yellow colour method (Juo, 1979). For ease of calculating stoichiometric ratios and subsequent analysis,concentrations of all elements were converted into percentages on dry matter (% DM) basis.

Microbial populations in the different treatments were determined using soil-dilution plate technique.For this purpose, soil extract agar (SEA) was reconstituted according to the instruction of the manufacturer, while the potato dextrose agar (PDA) was prepared as described inHarrigan and McCance (1990).A 10-fold dilution of samples was made by adding 10 g of manure sample to 90 ml of sterile distilled water in 250 ml bottles. After thoroughly shaking and mixing, 10-fold serial dilutionsof up to 10^{-9} wereprepared by transferring 1 ml to universal bottle containing 9 ml of distilled water. Aliquots (0.5ml) of the 10^{-9} dilutions were then transferred in duplicates into SEA and PDA plates and spread using flamed bent glass spreaders.The inoculated plates were placed in an incubator then incubated at an ambient temperature of 30° C for seven days and the colony forming units (CFU) of bacteria and fungi were counted.The CFU is a unit used to estimate the number of viable bacteria or fungal cells in a sample (Goldman and Green, 2008). The CFU was used as a proxy for total microbial populations in the manure samples.

The CFUs and nutrient contents obtained after one month of incubation and after three months of storage were compared using a *T*-test assuming unequal variance. In addition, the relationship between CFUsand nutrient contents andstoichiometric ratios were exploredusing correlation analysis.Initially, the data collected after one month of incubationand after three months of storagewere analysed separately. The same analysis was also run after combining the two datasets. In addition, we explored regression analysis in order to see whether CFUs vary with stoichiometric ratios. For this purpose we transformedall the data into logarithms.

RESULTS AND DISCUSSION

After one month of incubation CFU was 10.5 and this increased to 27 (or 159%) after field storage(Table 1). This changewas statistically significant (T = -3.02, P = 0.004) could be attributed to changes in the nutrient contents of the manure during storage. There was a reduction in C, N, P, K, Mg and Ca concentration during storage although the changeswere not significant in most cases. Significant reduction was recorded in C concentrations and C:K ratios (-26%), while the smallestchange occurredin N concentrations. Among the stoichiometric ratios, C:N and C:K significantly changed during storage (Table 1).

Table 1. Changes in the mean number of colony forming units (CFU), nutrients and stoichiometric ratios between end of incubation and three months after storage

Variable	After one month of incubation	After storage Periods	P value†	Change in mean (%)‡
CFU	10.5	27.2	0.023*	159.2
C (%)	38.3	28.2	0.004**	-26.3
N (%)	1.4	1.4	0.787	-1.7
P (%)	0.6	0.6	0.252	-8.7
K (%)	2.6	2.3	0.523	-9.3
Mg (%)	0.6	0.6	0.838	-4.0
Ca (%)	0.8	0.6	0.225	-19.0
C:N	27.6	20.6	0.005**	-25.2
C:P	66.2	55.8	0.235	-15.7
N:K	0.7	0.7	0.752	-4.2
N:P	2.5	2.7	0.423	9.0
C:K	19.4	14.3	0.064	-26.4
K:P	4.2	4.1	0.846	-2.3

†P values are for comparing values after one and three months using t-test assuming unequal variance.
‡Percentage change in mean from end of incubation period and storage times.

The correlationsbetween CFUs and all nutrients and elemental ratios were not statistically significant after one month of incubation(results not presented). This is probably because the time was too short for such changes to take place.After storage, CFUs were significantly ($P<0.05$) negatively correlated with concentrations of C,Ca andthe C:N, C:P and C:K ratios in manure(Table 2). Similar results emerged from the combined data, but with the addition of a significant C:Kand K:Prelationship with CFU.On the other hand the correlations with N:Pratios and individual concentrations of N, P, K and Mg were not significant ($P>0.05$).The regression analysis (Figure 1) confirms that total microbial counts are negatively related to C concentrations and C:N, C:P and C:K ratios.

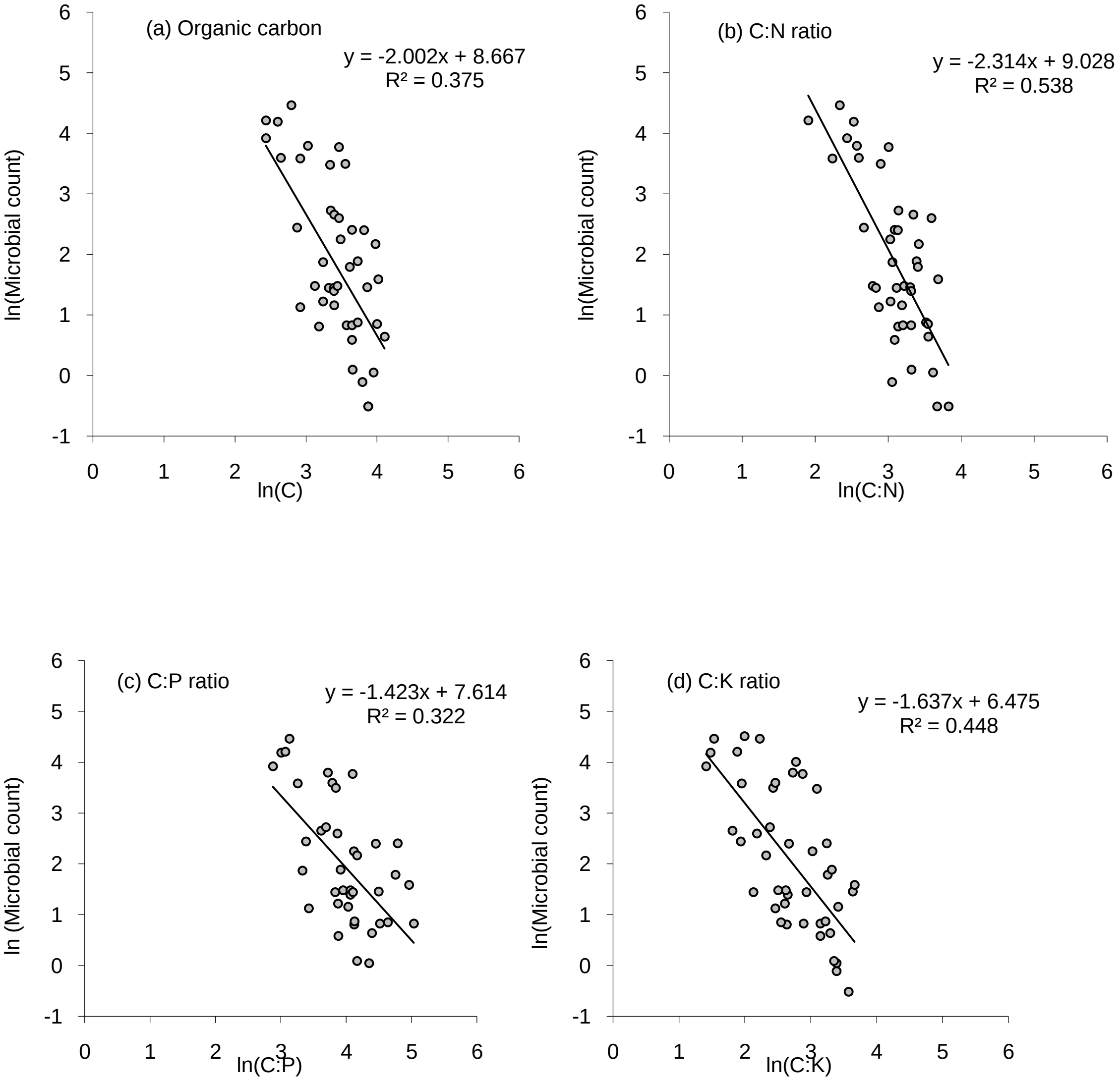

Fig. 1 Variation in total microbial count (colony forming units) with the concentrations of organic carbon (a), C:N ration (b), C:P ratio (c) and C:K ratio (d) of cattle manure on natural logarithmic scale.

For the first time this study also established a negative association between microbial counts and C:K ratios. These results are consistent with earlier reports from studies on plant litter (Enríquez*et al*., 1993; Keiblinger*et al*., 2010). Bacterial growth efficiency is known to decreases with increasing C:N and C:P ratios in the substrate (Enríquez*et al*., 1993).

Table 2.Spearman rank correlation coefficients between colony forming units and initial chemical contents and stoichiometric ratios in manure

	After three months		**Combined data**	
Variables	**Coefficient**	**P value**	**Coefficient**	**P value**
C (%)	-0.623	<0.001***	-0.398	0.005***
N (%)	-0.054	0.802	-0.076	0.605
P	0.170	0.424	-0.021	0.889
K	0.286	0.175	0.276	0.058
Mg	-0.252	0.235	0.078	0.595
Ca	-0.444	0.031*	-0.069	0.641
C:N	-0.605	0.002**	-0.341	0.018**
C:P	-0.673	<0.001***	-0.397	0.006**
N:K	-0.155	0.466	-0.286	0.049
N:P	-0.208	0.328	-0.031	0.836
C:K	-0.676	<0.001***	-0.526	<0.001***
K:P	0.222	0.294	0.301	0.038*

Significant at P = 0.05; ** at P = 0.01; *** at P = 0.001.

Sample sizes were 24 for analysis at time of application and 48 for the overall.

The N:P ratio is known to be a critical determinant of microbial activity and the relative importance of different microbial decomposers in plant litter (Güsewell and Gessner, 2009). However, in this study the correlations between N:P ratios and microbial activity were not significant. Such relationship could probably be uncovered if the analysis were done accordingtodecomposers groups. For example, low N:P ratios have been shown to promote bacterial decomposition while high N:P ratios promote fungal decomposition (Güsewell and Gessner, 2009).

CONCLUSION

The main conclusion from this analysis is that stoichiometric ratios are more important in determining microbial populationsthan the individual nutrient concentrations in manure. However, detailed studies are needed to determine whether or not this pattern is consistent across different microbial decomposers in animal manure.

REFERENCES

Bremner, J.M. (1982). Total nitrogen.In: Black, C. A. (ed) Methods of Soil Analysis Part II. Chemical and Microbiological Properties.American Society of Agronomy, Madison, Wisconsin, 1149-1178 pp.

Edmeades, D.C.,(2003).The long-term effects of manures and fertilisers on soil productivity and quality: a review.Nutrient Cycling in Agroecosystems66, 165–180.

Enríquez, S., Duarte, C.M., Sandjensen, K. (1993). Patterns in decomposition rates among photosynthetic organisms – the importance of detritus C:N:P content. Oecologia 94, 457–471.

Fulhage, C.D. (2000). Reduce environmental problems with proper land application of animal manure. University of Missouri Extension. USA.

Goldman, E.; Green, L.H.,(2008).Practical Handbook of Microbiology, Second Edition, CRC Press, Taylor and Francis Group, 864 pp.

Güsewell, S., Gessner, M.O., (2009). N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology 23, 211–219.

Harrigan, W.F.,McCance, M.E.,(1990).Laboratory methods in Food and Dairy Microbiology.London Academic Press.

Juo, A.R.S. (1979). Selected methods for soil and plant analysis.IITA Manual Series.No.1, Ibadan, Nigeria.

Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle,.I, Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., Boltenstern, S.Z., (2010).The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiology and Ecology 73, 430–440.

Koerselman, W., Meuleman, A.F.M., (1996). The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33, 1441–1450.

Kowal, J.M. (1972). Radiation and Potential crop protection in Samaru, Nigeria. Savanna, 1: 89-101.

Kowal, J.M. and D.T. Knabe (1972). An Agro-Climatological Atlas of Northern Nigeria with explanatory notes. Ahmadu Bello University, Zaria Press.

Maillard,É., Angers, D.A., (2014). Animal manure application and soil organic carbon stocks: a meta-analysis. Global Change Biology 20, 666–679.

Manzoni, S., Trofymow, J.A., Jackson, R.B., Porporato, A., (2010). Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecological Monograph 80, 89–106.

Nelson, D.W.,Sommers, L.E.,(1982). Total carbon, organic carbon and organic matter. In: Page,A.L. Miller, R.H. and Keeney, D.R. (eds). Methods of Soil Analysis No. 9, Part II, Chemical and Microbiological properties.American Society of Agronomy, Madison, Wisconsin.Pp 539-579.

Tanimu, J., (2012).Effect of cow dung management and urea fertilizer on some soil chemical properties and maize (Zea mays l.) yield in the northern guinea savanna of Nigeria.PhD Thesis, Ahmadu Bello University, Nigeria.

Zhang, W., Liu, K., Wang, J., Shao, X., Xu, M., Li, J., Wang, X., Murphy, D.V., (2015).Relative contribution of maize and external manure amendment to soil carbon sequestration in a long term intensive maize cropping system.SciReports5, 10791. DOI: 10.1038/srep10791.

Zhu, N.,(2007).Effect of low initial C/N ratio on aerobic composting of swine manure with rice straw.BioresourcesTechnology 98, 9–13.