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### Mechanism(s) Underlying Interactions Between Cattle Manure and Mineral Fertilizer in a Maize Field Soil in Ghana

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#### Abstract

A 70-day laboratory incubation study was conducted to unravel the mechanism(s) underlying synergistic interactions between organic and inorganic nutrient inputs to the soil. Soil from a maize farmer's field at Kpongu in the Upper West Region of Ghana was amended with a factorial combination of 0, 50% and 100% levels of the recommended rates (RR) of inorganic fertilizer and cattle manure and incubated at room temperature in plastic cups. Deionized water was added to maintain soil moisture at 70% field capacity throughout the incubation period. Cups were covered with gas-permeable parafilm. Soil sampling was done at 7, 28, 42, 56 and 70 days after incubation. The soil was analyzed for available phosphorus, organic carbon, microbial biomass carbon, nitrates, ammonium, soil urease activity, iron, and copper at each sampling. The addition of 100% RR NPK + 50% RR manure often results in higher amounts of the measured parameters, synergistic interactions and supply of nutrients to the soil, with longer residual effects. While all the under-studied mechanisms (improved nutrient synchrony, priming effects, general fertility improvement fertility) contribute to synergistic interactions, the improved nutrient synchrony mechanism is the most prominent. Farmers can therefore manage the timing of the nutrient inputs well to capitalize on this mechanism for improved soil fertility.

**Keywords**: Integrated nutrient management; Nutrient synchrony; Organic-inorganic fertilizer interactions; Priming.

# Mécanisme(s) sous-jacent(s) aux interactions entre le fumier de bovin et les engrais minéraux dans un sol de champ de maïs au Ghana

#### Résumé

Une étude d'incubation en laboratoire de 70 jours a été menée pour démêler les mécanismes sousjacents des interactions synergiques entre les apports de nutriments organiques et inorganiques dans le sol. Le sol d'un champ de maïs à Kpongu, dans la région du Haut-Ouest du Ghana, a été modifié avec une combinaison factorielle de 0, 50 et 100 % des taux recommandés (RR) d'engrais inorganiques et de fumier de bovin et incubé à température ambiante dans des tasses en plastique.

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De l'eau désionisée a été ajoutée pour maintenir l'humidité du sol à 70 % de la capacité du champ tout au long de la période d'incubation. Les gobelets étaient recouverts de parafilm perméable aux gaz. L'échantillonnage du sol a été effectué 7, 28, 42, 56 et 70 jours après l'incubation. Le sol a été analysé pour déterminer s'il contenait du phosphore, du carbone organique, du carbone de biomasse microbienne, des nitrates, de l'ammonium, de l'uréase du sol, du fer et du cuivre à chaque échantillonnage. L'ajout de 100 % de RR NPK + 50 % de fumier RR entraîne souvent des quantités plus élevées des paramètres mesurés, des interactions synergiques et l'apport de nutriments au sol, avec des effets résiduels plus longs. Bien que tous les mécanismes sous-étudiés (meilleure synchronisation des éléments nutritifs, effets d'amorçage, amélioration générale de la fertilité, fertilité) contribuent aux interactions synergiques, le mécanisme amélioré de synchronisation des éléments nutritifs est le plus important. Les agriculteurs peuvent donc bien gérer le moment des apports en nutriments afin de tirer parti de ce mécanisme pour améliorer la fertilité des sols.

Mots Clés: Gestion intégrée des éléments nutritifs; synchronization des éléments nutritifs; geinteraction entre les engrais organiques et inorganiques; amorçage.

#### Introduction

Sole mineral fertilizer application does not often meet crop nutrient requirements in Africa, due to economic constraints and environmental factors (FAO, 2006). Organic nutrient sources alone also do not suffice to meet increasing food demand (FAO, 2006) because they are usually low in major nutrient concentrations (Vanlauwe and Giller, 2006) and require bulky amounts to meet crop nutrient demand. In this light, one of the appropriate options for maintaining soil fertility has been to combine both organic and inorganic nutrient sources to tap a combined effect of their advantages. A judicious combination of mineral fertilizer with organic sources of nutrients may result in synergistic interactions (Vanlauwe et al., 2001a; Mucheru et al., 2002), evident in crop yield. The tern "added benefits" describes a synergistic interaction from combined organic and inorganic nutrient source application where yield is significantly higher than the yield from the sum of individual applications while added disadvantages describe a turn of events from antagonistic interaction where yield from combinations is significantly lower than the sum of yields

from sole applications. Several trials established in the various sub-regions of Africa aimed at quantifying potential added benefits in treatments with combined applications of organic resources and mineral N. Brempong et al. (2017) reported of higher yields and economic returns from combining 100% of the rate of cattle manure with half the rate of mineral fertilizer, compared to a sum of their applications. Vanlauwe et al. (2001b) reported additional increase of 488 and 579 kg grains ha<sup>-1</sup> when the 45 kg ha<sup>-1</sup> urea + 45 kg ha organic manure and 90 kg ha<sup>-1</sup> urea + 90 kg <sup>1</sup> organic manure were applied, respecha tively. Okalebo et al. (2003) applied wheat straw and soya bean haulms with urea and obtained an added maize yield of 684 kg grain ha<sup>-1</sup> as compared to a sum of yields from individual applications. In rare cases where added disadvantages were recorded, there were faults with one or both nutrient sources or environmental factors were not favourable (Mucheru et al., 2002).

Mechanisms underlying synergistic interactions leading to added benefits in crop yield may include 'improved nutrient synchrony' (Vanlauwe *et al.*, 2002b), 'general soil fertility

improvement' (Vanlauwe et al., 2001b) and 'priming effects' (Kuzyakov et al., 2000; Giller, 2002). In the improved nutrient synchrony scenario, there is a temporal immobilization of nitrogen (N) by microbes following combined application (Myers et al., 1994; Palm et al., 1997). This immobilization is powered by the high supply of energy from carbon (C) to microbes to drive decomposition processes. The immobilized N is made available at a later stage of plant growth when the manure has decomposed, and some microbes have lysed to make nutrients available. In effect, the peak of nutrient supply coincides with the highest crop nutrient demand stage, so nutrients are efficiently used and little or none is lost to the environment. In the case of general fertility improvement, the organic nutrient source adds organic matter which improves soil conditions such as water and nutrient holding capacities, pH, cation exchange capacity, micronutrient concentrations (Vanlauwe et al., 2001b; Palm et al., 1997; Mutuo et al., 2000; Wallace 1996; Zingore et al., 2008), which in turn enhances the efficiency of the applied nutrients in the fertilizer. These processes consequently lead to better crop growth and yield.

Priming effect refers to strong short - term extra changes in the turnover of soil nutrients caused by the addition of easily decomposable organic materials (Kuzyakov et al., 2000). Usually, the extra nutrients are not from the sources of the applied nutrients but from sensitization of organic matter and microbial biomass to release more, after the addition of easily decomposable organic materials (Dalenberg and Jager, 1989; Pulleman and Tietema, 1999; Magid et al., 1999). The most important mechanisms for real priming effects are the acceleration or retardation of soil organic matter turnover due to increased activity or amount of microbial biomass.

Though the mechanisms underlying interactive effects have been identified elsewhere, not much has been done to identify them in Ghanaian soils. There is the need to identify the paramount mechanism at play so it can be capitalized and enhanced for added benefits upon application of mineral and organic nutrient sources. This need has led to the objective to unravel the mechanism underlying synergistic interactions leading to added benefits from such applications. The study hypothesis is that the increase in yield after the addition of both inorganic fertilizer and manure to the soil is merely due to the introduction of more nutrients to the soil and nothing else. The study was a follow up on a previous field study where an added benefit in maize grain yield was observed from combining same rates of mineral fertilizer and cattle manure (Brempong et al., 2017). Various soil fertility factors and nutrient indices were evaluated to arrive at the underlying mechanisms.

### Materials and Methods Study site, soil collection and analyses

This 70-day laboratory incubation study was conducted at the soil science laboratory of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi- Ghana. The 70 days was to simulate the number of days the Akposoe maize variety takes to reach physiological maturity on the field and capture various stages of the maize growth. Table 1 shows the sampling days of the incubation and their corresponding stages of maize growth.

Soil was collected from an untreated portion of a farmer's field at Kpongu in the Upper West region of Ghana (latitude 09°57'48.6" N and longitude 002°30'31.4" W and at an elevation of 286 m above sea level) at the end of the growing season around November. The soil was stored at room temperature before it was transported to the Soil Science laboratory

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### of KNUST.

### Soil and manure analyses

The soil was kept at room temperature (25°C) for a month before the start of the study. Initial physico-chemical analysis was done to characterize the soil before incubation, as shown in Table 2.

Soil texture was determined using particle size distribution (Anderson and Ingram, 1993). Gravimetric moisture content was determined at 105°C. The core sampling technique (Stone, 1991) was used for bulk density determination. The electronic method with distilled water was used to determine soil pH. Soil organic carbon was determined by the Walkley and Black method (Nelson and Sommers, 1982). Inorganic nitrogen was determined by the indophenol blue and the salicylic methods. Bray P1 method (Bray and Kurtz, 1945) was used to determine the available phosphorus. Microbial biomass carbon was determined using the fumigation technique (Anderson and Ingram, 1993). Urease activity was determined by the urea solution method. Iron and copper concentrations were determined by the atomic absorption spectrophotometer (AAS) method (FAO, 2008).

The manure samples were air-dried for five days, milled with Perten's laboratory mill 3310 and analyzed for total N (Kjeldahl digestion), phosphorus (Bray P - 1), potassium (Flame photometry method) and carbon (wet oxidation method) and dry matter content as described in Table 3. Total N, total P and K were determined by the above-mentioned methods.

#### Treatments application for the incubation

Nine combined treatments (three levels of mineral fertilizer by three levels of cattle manure) were applied in the study. The treatments are outlined in Table 4. Mineral

Table 1. Sampling days in the incubation
period and corresponding stages of growth
maize. Adapted from weedsoft.unl.edu

Sampling days after incubation	Stage of maize growth
7	Germination and seedling emergence
28 to 35	Vegetative development
35 to 42	Tasselling
42 to 56	Grain filling
56 to 70	Grain filling to physiological maturity

Table 2. Initial physicochemical and
biological properties of the soil used for
incubation

Property	Value
Soil texture	Sand
-Clay (%)	- 3.20
-Silt (%)	- 3.98
-Sand (%)	- 92.82
Soil moisture (%)	3.2
Bulk density (gcm <sup>-3</sup> )	1.5
Soil pH (1:1 $H_2O$ )	6.44
Organic carbon (%)	0.42
Inorganic nitrogen (mg kg <sup>-1</sup> )	8.72
Available phosphorus (mg kg <sup>-1</sup> )	1.99
Microbial biomass C (mg kg <sup>-1</sup> )	0.002
Urease activity (mg NH4 kg <sup>-1</sup> hr	<sup>1</sup> ) 19.4
Iron (mg kg <sup>-1</sup> )	8.64
Cupper (mg kg <sup>-1</sup> )	1.02

fertilizer was applied as urea (contains 46% N) for N application, triple superphosphates (contains 46%  $P_2O_3$ ) for  $P_2O_5$  application and muriate of potash (contains 63%  $K_2O$ ) for  $K_2O$  application. To calculate the treatments to be added based on the field scale provided in Table 4, the bulk density of the field was determined.

The volume of the disposable cup at the height at which soil was to be filled was also determined. The bulk density of the field was used

Table 3. Cattle manure characterization Nutrient

Property	Value
Carbon (%)	23.65
Total Nitrogen (%)	2.76
Total Phosphorus (%)	0.41
Total Potassium (%)	0.59
Dry matter content (%)	51.73
C:N Ratio	8.58

to determine the mass of soil to be put into the cup at the filling volume. The following calculations were made:

weight of soil (g) = Bulk density (gcm<sup>-3</sup>) 1 ha = 2 x  $10^6$  kg soil (based on the assumption that one-hectare furrow slice has  $2 \times 10^6$  kg soil).

The amounts of urea, triple superphosphates and murate of potash (NOP) to be applied were calculated according to their recommendations on per hectar basis with the soil in a disposable cup (based on the assumption that one-hectare furrow slice has  $2 \times 10^6$  kg soil) and their percentage nutrient constitution. Urea contains 46% N, triple superphosphates contain 46% P<sub>2</sub>O<sub>5</sub> and MOP contains 60% K<sub>3</sub>O.

Same steps were followed to apply the manure at all the as listed in Table 4. The amounts of urea, triple super-phosphates and muriate of potash were further calculated based on their constituent percentages.

Treatments $(\%)$	Ν	$P_2O_5$	K <sub>2</sub> O	Manure	
	kg ha -1			t ha <sup>-1</sup>	
Control	0	0	0	0	
0 % RRF, 50 % RRM	0	0	0	2.5	
0 % RRF, 100% RRM	0	0	0	5	
50 % RRF, 0 % RRM	30	20	20	0	
50% RRF, 50% RRM	30	20	20	2.5	
50 % RRF, 100 % RRM	30	20	20	5	
100 % RRF, 0 % RRM	60	40	40	0	
100 % RRM, 50 % RRM	60	40	40	2.5	
100 % RRF, 100 % RRM	60	40	40	5	

Table 4. Treatment description for the incubation study

RRF is recommended rate of fertilizer and RRM is recommended rate of manure (Agricultural Extension Handbook, 1977). N  $P_2O_3$  and  $K_2O$  are hereafter referred to as 'NPK'.

# Experimental design and incubation procedure

A factorised design in CRD arrangement with three treatment replications was used in the study. Thus, there were 27 cups in a set of the three treatment replications. The set of the three replications was repeated five times, thus making a total of 135 cups. (The five time repetition was to allow for the continuous evaluation of the soil even after the destructive soil sampling at every sampling routine; Destructive because soil that was collected could not be returned to the cups to continue incubating). Roots, stones, and debris were removed from the fresh soil. The soil was not air-dried to minimize the disturbance of microbial activity. The calculated mass of the soil (207.43 g) was poured in the cups and amended with the recommended amounts of manure and mineral fertilizer. Deionized water (9.34 ml) was added to make soil moisture up to 70 % field capacity (FC). The soil was mixed thoroughly with the water and the amendment and covered with gaspermeable parafilm to minimize water losses and kept on a lab bench with temperature ranging from 25.6 °C to 33.3 °C. The moisture content was monitored and adjusted to maintain it at field capacity which called for a periodic change of the parafilm. Destructive soil sampling was done at 7, 28, 42, 56 and 70 days after incubation for analysis, on a set of treatment triplicates at each sampling time. The incubated soil was analyzed for pH, soil moisture content (SMC), available P, organic C, microbial biomass carbon (MBC), nitrate-nitrogen (NO<sub>3</sub>-N), ammonium nitrogen (NH<sub>4</sub>-N), soil urease activity and micronutrients (Fe and Cu) for the incubation period.

#### Determination of mechanisms *Priming Effect*

Priming effect =  $N_{Combined} - (N_{Fertilizer} - N_{Control}) - (N_{Manure} - N_{Control}) - N_{Control}$ 

[adopted from Kuzyakov et al. (2000)]

where 'N <sub>Combined</sub>' is nutrient released from combined application plot, 'N <sub>Fertilizer</sub>'is nutrient released from fertilizer amended plot, 'N <sub>manure</sub>' is the nutrient released from manure amended plot, 'N <sub>control</sub>' is the nutrient released from control plots.

*Nitrogen and phosphorus synchrony indices* Nitrogen synchrony Index (NSI) and phosphorus synchrony Index (PSI) was used to determine the synchrony between nutrient release from various treatments and crop nutrient requirement at various sampling times of the incubation. The amounts of mineral N and available P measured in the soil at various sampling times were transformed into unitless values with the aid of a linear scoring function (appendix 1) with scoring values ranging from 1 to 10. The transformed values of mineral N and available P were used to estimate the N synchrony index and the P synchrony index as follows:

 $NSI = (\sum_{t=i}^{n} Ni)/n$  and  $PSI = (\sum_{t=i}^{n} Pi)/n$ 

where Ni is N synchrony score value, Pi is P synchrony score value and n is the number of times nutrients were measured during the incubation.

An index of 1 meant the corresponding nutrient input supplied only 10% of the required nutrient at a growth stage while an index of 10 meant a 100% of the required nutrient was supplied at that growth stage.

#### Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) using GENSTAT statistical package (version 12) and significant means were separated with Fischer's least significant difference at 5 %.

#### Principal component analysis

Principal Component Analysis (PCA) was

carried out to determine the relative contributions of each of the mechanisms to interactive effects using nitrogen and phosphorus synchrony indices, priming effect and improvement in soil N and P stock as data set.

#### Results

### Effect of manure and mineral fertilizer application on soil fertility parameters *Inorganic* $N(NH_4^+ and NO_3^-)$ release

There was a significant (p = 0.03) interactive effect of manure and mineral fertilizer on soil mineral nitrogen as shown in figure 1A. A sharp increase in soil N relative to the initial soil concentration (17.44 kg ha<sup>-1</sup>) was observed at 7 days after incubation (DAI) from all the nutrient inputs except the control. There was a general steady decline from all the treatments till 42 DAI, after which N concentrations started to rise again. The 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure maintained the highest N concentration till 42 DAI; the beginning of the grain-filling phase. All the nutrient inputs except the control,  $30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{t}$  manure, and 0 NPK + 5 t manure met the maize tasseling N demand (49.5 kg ha<sup>-1</sup>) (IFA, 1992) from about 35 to 42 DAI.

At the incubation period that represents the initial stages of grain filling (from 42 to 56 DAI), all the nutrient inputs except the control, 0 NPK + 2.5 t manure and 0 NPK + 5 t manure supplied N above the grain filling N demand ( $32.3 \text{ kg ha}^{-1}$ ) (IFA, 1992). At 70 DAI which coincides with physiological maturity of the maize, only 60:40:40 kg ha<sup>-1</sup> NPK + 0 t M, 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure and 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure contained N above the grain filling demand with 60:40:40 kg ha<sup>-1</sup> NPK + 0 t manure supplying the highest.

#### Available phosphorus release

Figure 1B shows the combined effect of manure and mineral fertilizer application on P

release. Phosphorus release was generally slow during the first 28 DAI except for 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure which peaked at 28 DAI. The use of 30:20:20 kg ha<sup>-1</sup> NPK + 2.5 t manure released the highest P, 7 DAI.

None of the nutrient inputs met the maize tasseling P demand (25 kg ha<sup>-1</sup>) at 35 to 42 DAI except 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure. Only 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure and 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure supplied P above the grain filling P demand (22.5 kg ha<sup>-1</sup>) during the grain filling period at 56 DAI. All the nutrient inputs but the control supplied P above the grain filling P demand at 70 DAI (at physiological maturity).

# Contribution of manure and mineral fertilizer application rates to nutrient synchrony

All the nutrient inputs had their highest nitrogen synchrony indices (NSI) of 10 at 7 DAI (Table 5). The same occurred at 28 DAI when maize crops would still be at the vegetative stage except for 30:20:20 kg ha<sup>-1</sup> NPK and 5 t manure.

At 42 DAI, 30:20:20 kg ha<sup>-1</sup> NPK + 5 t manure, 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure, 30:20:20 kg ha<sup>-1</sup> NPK and 60:40:40 kg ha<sup>-1</sup> NPK had NSI of 10. The 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure and 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure had indices of 4 and 8 respectively at the beginning of the grain filling stage (56 DAI). However, by the end of the grain filling stage, all the nutrient inputs had NSI of 1. Cumulatively, the recommended full rates of inorganic fertilizer (60:40:40 kg ha<sup>-1</sup>) with or without manure recorded higher NSI between 6.4 and 7 (Table 5). The least cumulative NSI was obtained from 5 t manure.

The indices showing the synchrony between P

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Figure 1. The combined effect of cattle manure and mineral fertilizer application on Nitrogen (A) and Phosphorus (B) release. Bars represent the least significant difference between treatment means at every sampling date.

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	Nitrogen Synchrony Index (NSI)							
Treatments applied	7 DAI	28 DAI	42 DAI	56 DAI	70 DAI	Mean		
$30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{ t manure}$	10	10	3	1	1	5		
$30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 5 \text{ t manure}$	10	10	9	1	1	6.2		
$60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{ t manure}$	10	10	10	4	1	7		
$60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 5 \text{ t manure}$	10	10	5	8	1	6.8		
30:20:20 kg ha <sup>-1</sup> NPK	10	4	10	1	1	5.2		
60:40:40 kg ha <sup>-1</sup> NPK	10	10	10	1	1	6.4		
2.5 t manure	10	10	7	1	1	5.6		
5 t manure	10	2	2	1	1	3.2		
	Phosphorus Synchrony Index (PSI)							
$30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{ t manure}$	10	6	1	1	1	3.8		
$30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 5 \text{ t manure}$	10	4	1	1	1	3.4		
$60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{ t manure}$	10	10	1	1	1	4.6		
$60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 5 \text{ t manure}$	10	4	1	1	1	3.4		
30:20:20 kg ha <sup>-1</sup> NPK	10	4	1	1	1	3.4		
$60:40:40 \text{ kg ha}^{-1} \text{ NPK}$	10	4	1	1	1	3.4		
2.5 t manure	10	3	1	1	1	3.2		
5 t manure	10	3	1	1	1	3.2		

Table 5. Nitrogen synchrony index

release and P demand by maize are shown in Table 5. On the average, PSI was low (below 5) for all the nutrient inputs throughout the incubation, with the highest PSI of 4.6 from  $60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 2.5 \text{ t}$  manure and the lowest PSI from 2.5 and 5 t manure. From the table, all the nutrient inputs had the highest phosphorus synchrony index at 7 DAI. The highest P synchrony index was observed from 60:40:40 kg ha <sup>-1</sup> NPK + 2.5 t manure at 28 DAI while the index from the remaining treatments decreased compared to the value recorded at 7 DAI with 2.5 t and 5 t manure recording the least P synchrony index. Phosphorus synchrony indices were lowest (1) for all the nutrient inputs from 42 DAI upwards.

#### **Priming effect**

# Contribution of manure and mineral fertilizer application to N priming

Priming effect on soil N concentration as influenced by combined manure and mineral fertilizer applications is indicated in figure 2A. Since the actual combined effects were the focal points, only those four treatments among the nine were considered for a priming effect. A positive priming effect was observed from 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure throughout the incubation period until after 56 DAI (corresponding to the tail end of grain filling on the field). The use of 30:20:20 kg ha<sup>-1</sup> NPK + 2.5 t manure, on the other hand, had a negative priming effect throughout the

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incubation period until after 60 days. The remaining combined treatments had alternating positive and negative priming effects throughout the incubation period.

### Organic carbon priming effect from combined manure and mineral fertilizer application

Figure 2B shows an organic carbon priming effect as influenced by the combined application of manure and mineral fertilizer. A positive priming effect was observed from 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure throughout the incubation period with the highest effect at 70 DAI. The  $30:20:20 \text{ kg ha}^{-1}\text{NPK} +$ 5 t manure started with a negative priming effect which rose to a positive value at 28 DAI, peaked at 56 DAI, and declined again to a negative effect at 70 DAI. The application of 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure had a positive priming effect from 7 to 56 DAI. The application of 30:20:20 kg ha<sup>-1</sup> NPK+ 2.5 t manure had a negative priming effect on organic C at 7, 56, and 70 DAI.

#### **General fertility improvement**

# *Effect of manure and mineral fertilizer application on Soil organic carbon*

There was a significant interactive effect (p=0.04) from combined manure and mineral fertilizer on soil organic carbon (Figure 3). Combined application of 30:20:20 kg ha<sup>-1</sup> NPK + 5 t manure produced the highest SOC at 7 and 56 DAI, aside from which none of the combined nutrient management options affected SOC.

#### *Effect of manure and mineral fertilizer application on micronutrients* Iron

Combined manure and mineral fertilizer had a significant interactive effect (p=0.035) on soil iron (Fe) concentration (Figure 4A) with 30:20:20 kg ha<sup>-1</sup> NPK + 5t manure supplying the highest Fe at 7 DAI. The iron release peaked for all treatments 42 DAI with the

highest observed under  $30:20:20 \text{ kg ha}^{-1} \text{ NPK} + 0 \text{ t manure.}$ 

#### Copper

The combined application of manure and mineral fertilizer significantly influenced (p= 0.04) Cu with the highest Cu content observed under 30:20:20 kg ha<sup>-1</sup> NPK + 5 t manure at 7 and 42 DAI whilst the lowest was recorded under 60:40:40 kg ha<sup>-1</sup> NPK + 0 t manure at 7 DAI (Figure 4B). The highest copper content was recorded under the application of 30:20:20 kg ha<sup>-1</sup> NPK + 2.5 t manure at 28 and 56 DAI. Copper content from all the combined nutrient inputs peaked at 42 DAI with the highest value observed under 30:20:20 kg ha<sup>-1</sup> NPK + 5 t manure.

### Effect of manure and mineral fertilizer application on microbial biomass carbon (MBC)

There were small increases in MBC under the combined manure and mineral fertilizer application (Figure 5) from 0 to 28 DAI. The 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure gave the highest MBC at 28 DAI. Microbial biomass carbon peaked at 56 DAI and 42 DAI under the application of 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure and 30:20:20 kg ha<sup>-1</sup> + 0 t manure, respectively. The amount of MBC from all the nutrient management options declined at 70 DAI with the control performing best.

# Principal component analysis of the mechanisms

Five principal components cumulatively explained the variation in interaction between manure and mineral fertilizer as shown in table 6.

Principal component one (PC1) explained 41.27 % of the variation which was dominated by the N synchrony index (from the rotated component matrix table, Table 8). Principal component two (Table 6) explained about 28.13 % of the variation in the interac-



Figure 2. Effect of combined manure and mineral fertilizer application on soil nitrogen priming (A) and organic carbon (B) priming. Bars represent the least significant difference between treatment means at every sampling date.

tion and priming effect dominated this component.

The third component also explained 15.02 % of the variation and the priming effect again is the main contributing variable for this component. Mineral N, representing the general fertility improvement mechanism is

highly loaded on component four which explains 11.13 % of the variation in the data. Component five explains 4.45 % of the total variation in the data with the N synchrony index heavily loaded on it.



Figure 3. The combined effect of manure and mineral fertilizer application on SOC. Bars represent the least significant difference between treatment means at every sampling date.

### Discussion

## Improved N and P release - crop demand synchrony

Should the improved nutrient synchrony mechanism be at play, combined applications must have met N and P demand at the critical stages of the crop in question (Vanlauwe et al., 2001 a). The IFA (1992) reported that the critical nutrient demand stages of maize growth are the tasseling and grain filling stages requiring 49.5 and 32.3 kg ha<sup>-1</sup> N respectively. All the combined nutrient inputs (Figure 1A) except 30:20:20 kg ha  $^{-1}$  NPK + 2.5 t manure synchronized the required crop N demand with supply at the tasseling stage (35 to 42 DAI). The use of  $30:20:20 \text{ kg ha}^{-1} \text{ NPK}$ +2.5 t manure could not supply enough N for this critical stage due to the low amount of N supplied by this combination compared to the higher doses. At the initial stages of grain filling (from 42 DAI), all the combined nutrient inputs released N above the grain filling N demand but according to the order of supplied nutrients rates, hence the higher the supplied rate, the higher the amount of N released as reported by Fashina et al. (2002). At the period of incubation representing the end of the grain filling stage to physiological maturity (56 to 70 DAI), only 60:40:40 kg ha<sup>-1</sup> NPK + 5 t manure and 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure met the grain filling N demand, because the manure had probably improved the nutrient holding capacity (Adugna, 2016) of the soil by improving cation and anion exchange capacities of the soil, so that the effect of the full dose of fertilizer was prolonged. The calculated NSI (Table 5) showed that 60:40:40 kg ha  $^{-1}$  NPK + 2.5 t manure and 60:40:40 kg ha  $^{-1}$  NPK + 5 t manure best synchronized (supplied 100%) crop N release with demand within the first 28 DAI; thus the vegetative phase. This might have occurred because of the relatively large amount of readily available nutrients (FAO,



Figure 4. The combined effect of manure and mineral fertilizer application on soil Fe (A) and Cu (B) content. Bars represent the least significant difference between treatment means at every sampling date.



Figure 5. The combined effect of manure and mineral fertilizer application on microbial biomass carbon. Bars represent the least significant difference between treatment means at every sampling date.

2006) from the full dose of fertilizer coupled with the better soil conditioning effect from the 2.5 t or 5 t manure. However, the fact that 60:40:40 kg ha <sup>-1</sup> NPK + 2.5 t (mean NSI=70%) manure and 2.5 t manure alone (mean NSI = 56%) met N demand better than 60:40:40 kg ha <sup>-1</sup> NPK + 5 t manure (mean NSI = 68%) and 5 t manure alone (mean NSI=32%), considering the entire incubation period, is indication that there is a limit to manure application beyond which a luxury consumption of N by microbes is introduced or higher microbial activity from more manure may cause higher immobilization rates at critical stages of crop growth.

The PSI values (Table 5) indicated that all the nutrient inputs met the full P requirement at 7 DAI, but this was only maintained by 60:40:40 kg ha<sup>-1</sup> NPK + 2.5 t manure at 28 DAI. Only 10 % or less of the required P was supplied by all the nutrient management options at 42, 56, and 70 DAI. Thus, to a large extent, most of the nutrient inputs did not meet maize nutrient demand at various critical stages of growth (Vanlauwe *et al.* (2001a) for Prelease.

#### Priming of soil N and OC

The application of  $60:40:40 \text{ kg ha}^{-1} \text{ NPK} + 5 \text{ t}$  manure (Figure 2A) might have provided a

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Measurements		Principal Component Table						
	PC1	PC2	PC3	PC4	PC5			
Eigen values	2.06	1.41	0.75	0.56	0.22			
% Contribution	41.27	28.13	15.02	11.13	4.45			
Cumulative percent (%)	41.27	69.40	84.42	95.55	100			
		Rotated Component Matrix						
Vectors	1	2	3	4	5			
Mineral N	0.318	0.583	-0.219	0.700	-0.144			
N synchrony index	0.641	-0.130	-0.017	-0.033	0.755			
P released	-0.375	0.499	-0.587	-0.353	0.376			
P synchrony index	0.579	0.028	-0.371	-0.509	-0.517			
Priming effect	0.110	0.627	0.685	-0.354	0.014			

Table 6. Principal Component Analysis

large response in the activation of microbial metabolism initiated by the microbial succession from the addition of organic matter to the soil (Blagodatskaya and Kuzyakov, 2011). This must have led to the high N release and the consequent positive priming effect of 41.77, 20.56, 26.16, and 22.49 mg N / kg at 7, 28, 42, and 56 DAI respectively; similar to the findings of Hejnak et al. (1996) who reported that the size of the priming effects increases with the amount of the added organic substances. Moreover, the full dose of urea (an ammoniacal fertilizer) applied as N fertilizer and the bacteria decomposition of the manure (O'Leary et al., 2002) must have released lots of ammonium which have been reported to cause more priming effect in the soil than nitrates (Stout, 1995).

With the relatively low amount of N supplied through 30: 20: 20 kg ha<sup>-1</sup>, the increased activated microbial energy from the 2.5 t manure caused the immobilization of more fertilizer N leaving very little behind throughout the incubation period leading to the

negative priming effect of -7.78, -10.68, -44.46 and -5.93 mg/kg observed at 7, 28, 42, and 56 DAI respectively from the application of 30: 20: 20 kg ha  $^{-1}$  + 2.5 t manure. The positive priming effecton OC at 7, 28, 42, and 56 DAI from 60: 40: 40 kg ha  $^{-1}$  NPK + 2.5 t manure (Figure 2B) and the positive priming effect from 60: 40: 40 kg ha  $^{-1}$  NPK + 5 t manure at 7, 28 and 56 DAI) is evidence to the above claim that nutrient amounts in 30:20:20 kg ha  $^{-1}$  only met microbial immobilization and energy needs (Kuzyakov *et al.*, 2000).

# Effect of manure and mineral fertilizer on soil nitrogen and phosphorus

Considering the low C: N ratio of the manure, N release should have been high at the initial stages of the incubation (7 to 28 DAI) but a decline in N release was rather observed from most of the nutrient inputs (Figure 1A) probably due to low initial microbial biomass. The cropping history of the study site indicated that manure had never been applied; hence a low microbial population (MBC > 20 mg/kg, Table 2) acted on the applied nutrients and released low rates of N at the initial stages

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of incubation. In a related study, Jangid *et al.* (2008) observed that microbial diversity in terms of species richness and evenness was higher in soils amended with poultry litter than inorganic fertilizer. The introduced substrate (manure) caused microbial numbers to increase leading to the rise in N release after some time of the incubation period. Das *et al.* (2017) also found increases in microbial numbers and activity following composted cattle manure application.

Phosphorus release was generally slow at the initial stages of incubation for most of the nutrient inputs (Figure 1B) probably due to immobilization of P following application which was later released after 56 DAI when lysed microbes had also made nutrients available to the soil. Lazcano *et al.* (2013) found similar decline in soil P concentration right after organic amendment due to immediate P immobilization.

Soil microbes had to break down less of the full mineral fertilizer dose since it was reduced by 50 %, implying less energy use, similar to reports of Demoling *et al.* (2007), hence the more organic C retained by the application of 30: 20: 20 kg ha<sup>-1</sup> NPK + 5t manure (Figure 3).

The highest micronutrient supply from the manure could have been the reason for high Fe content from 30: 20: 20 kg ha<sup>-1</sup> NPK + 5 t manure (Figure 4A) at 7 DAI and 30: 20: 20 kg ha<sup>-1</sup> NPK + 2.5 t manure at 70 DAI.

The highest Cu content observed from  $30:20:20 \text{ kg ha}^{-1} \text{NPK} + 5 \text{ t}$  manure at 42 DAI and  $30:20:20 \text{ kg ha}^{-1} \text{NPK} + 2.5 \text{ t}$  manure at 28 and 56 DAI (Figure 4B) suggested the supply of micronutrients by manure. A similar effect was observed for Fe from both individual nutrient inputs.

# Effect of manure and mineral fertilizer application on soil MBC

The enhancing effect of manure on microbial biomass (Xu *et al.* 2008) was also observed from the combined application of 60: 40: 40 kg ha<sup>-1</sup> NPK + 2.5 t manure at 28 DAI. At 56 DAI, enough nutrients were still being supplied by 60: 40: 40 kg ha<sup>-1</sup> NPK + 5 t manure to support microbial numbers and the consequent highest MBC.

# The relative contribution of the key mechanisms to added benefits

The findings from the PCA (Tables 6) showed that the mechanism contributing most to the synergistic interaction between manure and mineral fertilizer is improved nitrogen synchrony which dominated PC1 (41.27 %) and PC5 (4.45 %). The next most contributing mechanism was the priming effect which dominated PC2 (28.13 %) and PC3 (15.02 %). The least contributing mechanism to synergy was the general fertility improvement mechanism which was represented with N and Prelease and dominated PC4 (11.13 %).

#### Conclusion

The laboratory incubation study demonstrated that synergistic interaction occurs when the full rate (60:40:40 kg ha<sup>-1</sup> NPK) of fertilizer with half-rate (2.5 t ha<sup>-1</sup>) of manure are combined. Also, all the studied mechanisms (improved nutrient synchrony, priming, and general fertility improvement) contribute to synergistic interactions between organic and inorganic nutrient sources but the improved nutrient synchrony mechanism contributes most, followed by priming effects and general fertility improvement in that order.

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### Summary statement

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### Appendices

Appendix 1. Linear functions used to calculate NSI and PSI at various stages of incubation *1.1 Nitrogen synchrony index functions* 





1.2 Phosphorus synchrony index functions

