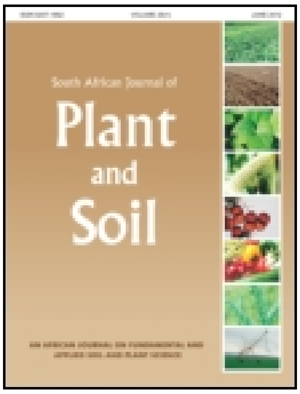

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A laboratory incubation study was conducted to determine the effects of liming on microbial activity and N mineralization in two Bizana soils amended with broiler manure. The experimental layout was a 4 x 3 complete factorial experiment with three replicates, arranged in a randomized design. Soil pH, CO_2 evolution, and mineral N concentration were measured. After 56 days the soil pH ranged from 4.50 to 5.74 and 4.99 to 5.94, in the Magusheni and Nikwe soils, respectively. The effect of liming on microbial activity and N mineralization differed between the soils. In the Nikwe soil (acid saturation 4.0%), microbial activity and N mineralization increased as the rate of broiler manure application was raised, but liming had no effect. In the Magusheni soil (acid saturation 25%), microbial activity increased as both lime and chicken manure application rates increased, but liming reduced N mineralization, suggesting N immobilization was being driven by an active microbial population in the limed soils. The rates of lime and/or chicken manure application, percentage Ca^{2+} and soil acid saturation were important factors influencing microbial activity and N mineralization, but the effect of soil pH on N mineralization was not evident in either of the soils.

Keywords: Biological activity, chicken manure, lime, nitrogen mineralization, soil acidity.

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Introduction

Many studies have evaluated the effects of acidity or liming of low pH soils on the mineralization of soil organic N in acid soils (Thompson *et al.,* 1954; Dancer *et al.,* 1973; Nyborg & Hoyt, 1978; Tabatabai & Al-Khafaji, 1980; Simard & N'dayegamiye, 1993; Weier & Gilliam, 1986; Lyngstad, 1992; Clay *et al.,* 1993; Curtin *et al.*, 1998), but relatively few have investigated these effects in soils amended with organic wastes. Factors shown to influence these effects are soil pH, type of soil and organic material used and the concentration of nutrients other than N in the soil (Fu *et al.*, 1987; Rees *et al.*, 1993), emphasizing the need to study different systems individually. Animal manures and other organic amendments usually contain a relatively small proportion of plant available N whilst the remainder must be mineralized through the action of soil microbes before it is available for plant uptake (Nyamangara *et al.*, 2005). As with plants, soil microbial activity is adversely affected by high soil acidity (Alexander, 1980), which, in turn, can reduce the rate of bio-degradation of added organic amendments. Hence, soil acidity is expected to affect cycling and overall availability of N and other major nutrients in the soil–plant system (Condron *et al.*, 1993).

In the high rainfall areas (> 600 mm p.a.) of the Eastern Cape Province, soils are acidic (pH < 5.5) in nature and require regular lime applications to maintain maximum crop production (Mandiringana *et al.*, 2005; Fyfield *et al.*, 2000; Fyfield, 2002). The major proportion of these soils are cultivated by smallholders, who rely on crop production for part of their food security and use animal manures as fertilizers, including cattle, goat and sheep manure sourced from their livestock pens and broiler manure which they purchase commercially (Silwana, 2000; Mkile, 2001). The amelioration of soil acidity by liming and application of animal manure is expected to stimulate soil microbial activity, which may either reduce or increase the release of N from the acid-soluble pool (Isirimah & Keeney 1973; Edmeades *et al.*, 1981). No research has been conducted on the effects of liming on microbial activity and N mineralization in acid soils of the Eastern Cape that have been amended with animal manures in general and broiler manure in particular. Broiler manure was selected because Judge (2001) reported that its addition to acid soils raised the soil pH. The objective of the present study was to determine the effects of liming of acid soils on microbial activity and N mineralization in soils amended with broiler chicken manure.

Material and methods

Experimental material

Topsoil (0-15 cm) of Magwa Connemara family (Soil Classification Working Group, 1991) from Magusheni (30° 53' S; 29° 53' E), and Sweetwater Newton family from Nikwe (30° 51' S; 29° 37' E) in Bizana (Eastern Cape Province, South Africa) were sampled from natural veld. For convenience, the Magwa and Sweetwater soil families will be referred to as Magusheni and Nikwe soils, respectively. The soils and their general properties have been described previously (Booyens, 2001). The soils were air-dried and screened through a 2 mm sieve for analysis. Soil pH was determined in deionized water with a 1:1 (w/v), soil:water ratio. Mineral N (NH_4-N and NO_3-N) was extracted with 2 M KCl solution (1:5 soil dry weight basis) and determined by steam distillation (Keeney & Nelson, 1982). Total C and N were determined by a dry combustion procedure using a LECO CHN 1000 Auto-Analyzer. The exchangeable Ca, Mg, K, and Na were extracted with 1

M ammonium acetate at pH 7.0 and determined by atomic absorption spectrophotometry. The 1 M KCl extraction was used for exchangeable acidity (Al + H) and exchangeable Al (Non-Affiliated Soil Analysis Working Group, 1990). Exchangeable acidity in the extract was determined by titration to a phenolphthalein endpoint with NaOH and exchangeable Al by atomic absorption. Acid saturation was calculated as:

$$\text{Acid saturation} = \frac{(\text{exchangeable Al + H}) \times 100}{\text{exchangeable Ca + Mg + K + Na + Al + H}(\text{cmol}_c\ \text{kg}^{-1})}$$

$$\text{Ca saturation} = \frac{(\text{exchangeable Ca}) \times 100}{\text{exchangeable Ca + Mg + K + Na + Al + H}(\text{cmol}_c\ \text{kg}^{-1})}$$

(FSSA, 1989)

The saturation percentage of other cations was calculated in a similar fashion. Effective cation exchange capacity (ECEC) was estimated through the summation of exchangeable Ca, Mg, K, Na and exchangeable acidity. Particle size distribution was determined by the hydrometer method (Gee & Bauder, 1986). Soil water content at field capacity was determined by the moisture retention plate method (Topp *et al.*, 1993). Soil analyses are given in Table 1.

The calcitic lime ($CaCO_3$) used in the present study had a fineness of 2500 µm and a CCE of 95%. The broiler manure available under the trade name GROMOR is registered as an organic fertilizer (National Plant Food CC, 2000). Pre-treatment of the manure before analysis involved air drying at room temperature for 1 week. The pH was determined using a manure:water ratio of 1: 2.5 (w/v). Total C, total N and inorganic N (NH_4-N and NO_3-N) were determined using the same procedures as for the soils (Table 2). Moisture content was determined from loss in weight after drying at 65°C for 48 h. The soils, liming material, and chicken manure were shipped to Colorado State University in the US where this study was conducted.

Incubation experiment

A laboratory incubation experiment evaluated the effects of liming on microbial activity and N mineralization in two soils amended with broiler manure. Soils were not part of the factorial design because we determined that the two soils were so chemically different that they represented different populations and should be considered separately. For each soil, rates of lime and manure application were arranged in a 4 x 3 complete factorial experiment with 3 replicates using a randomized design. Samples (100 g) of air dried sieved soil (< 2 mm) were placed into plastic sample cups (10 cm tall x 10 cm diameter). Lime treatments for the Magusheni soil involved the application of calcitic lime at rates of 0, 4.1, 8.2 and 12.2 g $CaCO_3$ kg^{-1}. These rates are equivalent to field application rates of 0, 8.1, 16.3 and 24.4 Mg lime ha^{-1} (assuming 1 ha weighs 2×10^6 kg of soil in the 15 cm surface layer). The less acid Nikwe soil received lime rates of 0, 3.1, 6.2 and 9.4 g $CaCO_3$ kg^{-1} or 0, 6.2, 12.5 and 18.7 Mg lime ha^{-1}. For both soils the highest rate was expected to raise the soil pH to 6.0 using the SMP buffer method (Shoemaker, McLean & Pratt, 1961).

Table 1 Characteristics of the soils used in this study

Soil property	Magusheni soil	Nikwe soil
Total C (%)	4.6	3.4
Total N (%)	0.3	0.2
C/N ratio	15:1	17:1
Soil pH (H_2O)	4.78	5.06
NH_4-N (mg kg^{-1})	21.5	5.9
NO_3-N (mg kg^{-1})	4.7	5.9
ECEC ($cmol_c$ kg^{-1})	5.86	8.85
Ca saturation (%)	29.3	46.1
Mg saturation (%)	40.1	33.6
K saturation (%)	3.7	14.9
Na saturation (%)	2.7	1.1
Acid saturation (%)	24.0	4.3
Exch. Al ($cmol_c$ kg^{-1})	1.27	0.20
Exch. Al+H ($cmol_c$ kg^{-1})	1.40	0.38
Sand (%)	27	30
Silt (%)	28	26
Clay (%)	45	44
Water content at FC (%)	35.3	26.9

Table 2 Characteristics of the broiler manure used in this study

pH (KCl)	Total N (g kg^{-1})	Total C (g kg^{-1})	C:N ratio	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	Water (%)
6.31	34	340	10:1	2.7	<0.01	12.2

When lime ($CaCO_3$) reacts with an acid soil CO_2 gas is released as a by-product. In order to differentiate between CO_2 evolution due to soil biological activity and abiotic CO_2 evolution (CO_2 from $CaCO_3$), additional lime treatments were prepared and treated with amounts of $CaCO_3$ similar to the lime increments used in the present study. The magnitude of this abiotic CO_2 evolution in limed treatments was estimated by subtracting CO_3-C before and after the experimental period as described in Persson *et al.* (1989).

In both soils, broiler manure treatments involved the application of the manure at rates of 0, 2.5 and 5 g (oven dry basis) manure kg^{-1} soil. These rates correspond to 0, 5 and 10 Mg ha^{-1} containing 0.12 kg H_2O kg^{-1}. All treatments were homogenized and the soil water content was raised to 75% of field capacity. The plastic cups were immediately placed in 1l glass jars, sealed airtight and incubated in the dark at 25 ± 1°C for a period of 56 d.

Carbon dioxide (CO_2) evolution, used as the indicator of microbial activity (Castellanos & Pratt, 1981), was determined at 0, 2, 4, 7, 14, 21, 28, 35, 42, 49 and 56 d after commencing the incubation. The CO_2 in the atmosphere of the jars was sampled with a gas syringe and the concentration of CO_2 was determined with an infrared gas analyzer. After each

CO_2 sampling, each jar was aerated for 10 min. The weight of the incubated soils was monitored weekly to ensure constant water content during the incubation period. The mass of CO_2 evolved per container was calculated as follows (Silberberg, 1996):

$$n = PV/RT$$

where n = µg C g^{-1} soil, P = Pressure (atm), V = volume (ml), R = universal gas constant (82.05 ml atm K^{-1} mol^{-1}), T = temperature (°K)

At the end of the incubation period, soils were again mixed thoroughly and air-dried before chemical analysis. Soil pH and mineral N (NH_4-N and NO_3-N) were measured as described previously. Net N mineralization in the incubated soils was estimated as follows (Kaboneka *et al*., 1997):

$$\text{Net } N_m = N_{m \text{ amended soil}} - N_{m \text{ unamended soil}}$$

where N_m = N mineralization (mg N kg^{-1} soil); unamended soil = (no lime and manure added).

Statistical procedures

All statistical analyses were performed using Statistical Analysis Software General Linear Model (SAS GLM) procedure (SAS Institute, 2005). Treatment means were compared using the least significant difference test, at the 5% level of significance (Steel *et al.*, 1997). Correlation was determined by Pearson's correlation coefficient.

Results and discussion

Soil pH

In both soils increasing the rate of lime application resulted in a linear increase in soil pH (Table 3). In Table 3, soil pH changes less than 0.5 units (even though statistically significant) were not considered to be practical changes with respect to soil management, sampling and analytical uncertainty (Edwards & Beegle, 1988). Soil pH varied from 4.50 (0 Mg ha^{-1}) to 5.74 (24.4 + 10 Mg ha^{-1}) in the Magusheni soil and from 4.99 (10 Mg ha^{-1}) to 5.94 (18.7 + 5 Mg ha^{-1}) in the Nikwe soil. In both soils, the highest pH values were observed in samples that received the highest lime rates, but these rates failed to raise the soil pH to 6.0, although the pH of 5.94 in the Nikwe soil was close (Table 3). The most likely reason why the desired pH levels were not attained could be the higher levels of nitrification, which are discussed under microbial activity and nitrogen mineralization below.

Broiler manure applications had little effect on soil pH in the present study. However, other researchers (Rodella *et al.*, 1995; Judge, 2001) noted increases in soil pH following its addition to acid soils.

Table 3 Effects of factorial combinations of calcitic lime and broiler chicken manure rates on the pH of Magusheni and Nikwe soils after 56 days of laboratory incubation

Soil	Lime rate ($Mg\ ha^{-1}$)	Manure rate ($Mg\ ha^{-1}$) 0	5	10
		pH (H_2O)		
Magusheni	0	4.50	4.67	4.72
	8.1	4.94	4.94	5.06
	16.3	5.31	5.34	5.36
	24.4	5.66	5.72	5.74
	$LSD_{0.05}$		0.034	
Nikwe				
	0	5.09	5.06	4.99
	6.2	5.40	5.32	5.31
	12.5	5.68	5.64	5.65
	18.7	5.91	5.94	5.91
	$LSD_{0.05}$		0.028	

Soil microbial activity and nitrogen mineralization

The results suggest that, in all treatment levels, the combination lime and broiler manure stimulated soil microbial activity more than the addition of lime and broiler manure alone (Table 4). The CO_2 evolved in the Magusheni soil ranged from 98.0 (8.1 Mg ha^{-1}) to 136.2 µg C g^{-1} (24.4 Mg ha^{-1}) for lime alone, from 135.2 (5 Mg ha^{-1}) to 201.0 µg C g^{-1} (10 Mg ha^{-1}) for broiler manure alone, and from 154.2 (8.1 + 5 Mg ha^{-1}) to 268.7 µg C g^{-1} (24.4 + 10 Mg ha^{-1}) for lime plus broiler manure treatments. In contrast, in the Nikwe soil results suggest that, in all treatment levels, CO_2 evolution increased with increasing broiler manure application, however, liming and the associated increase in soil pH had no apparent effect on CO_2 evolution (Table 4). The CO_2 evolved ranged from 51.7 (6.2 Mg ha^{-1}) to 54.1 µg C g^{-1} (12.5 Mg ha^{-1}) for lime alone, from 135.3 (5 Mg ha^{-1}) to 191.3 µg C g^{-1} (10 Mg ha^{-1}) for broiler manure alone, and from 127.3 (12.5 + 5 Mg ha^{-1}) to 194.2 µg C g^{-1} (18.7 + 10 Mg ha^{-1}) for lime plus broiler manure treatments.

The trend in amount of CO_2 evolved corresponded with that of N mineralization (Table 5). These results suggest that, in the Magusheni soil the addition of lime alone or lime combined with broiler manure led to varying levels of temporal N

Table 4 Effects of factorial combinations of calcitic lime and broiler chicken manure rates on CO_2 evolution from Magusheni and Nikwe soils after 56 days of laboratory incubation. Values reflect soil respiration after correction of CO_2 from $CaCO_3$

Soil	Lime rate ($Mg\ ha^{-1}$)	0	5	10
		CO_2 evolution[#] (µg C g^{-1} soil)		
Magusheni	0	76.3	135.2	201.0
	8.1	98.0	154.2	226.8
	16.3	120.4	175.2	247.9
	24.4	136.2	196.1	268.7
	$LSD_{0.05}$		10.8	
Nikwe	0	55.3	135.3	191.3
	6.2	51.7	130.2	190.1
	12.5	54.1	127.3	192.1
	18.7	54.0	127.6	194.2
	$LSD_{0.05}$		7.5	

[#]Values were obtained by the summation of CO_2 respired from day 0 through day 56.

Table 5 Effects of factorial combinations of calcitic lime and broiler chicken manure rates on inorganic N (NH_4-N + NO_3-N) and net mineralization N contents of the Magusheni and Nikwe soils after 56 days of laboratory incubation

Soil	Lime rate ($Mg\ ha^{-1}$)	NH_4-N			NO_3-N			Inorganic N†			Net N mineralization††		
		Manure rate ($Mg\ ha^{-1}$)											
		0	5	10	0	5	10	0	5	10	0	5	10
		($mg\ N\ kg^{-1}$)											
Magusheni	0	6.96	24.2	40.9	67.7	62.9	62.9	74.7	87.2	103.9	----	12.4	29.2
	8.1	1.87	7.70	13.8	71.7	71.8	79.8	73.6	79.5	93.6	-1.05	4.78	18.9
	16.3	1.85	2.71	9.19	72.0	74.8	79.9	73.9	77.5	89.1	-0.82	2.84	14.4
	24.4	1.52	2.18	4.57	68.4	78.10	82.9	69.9	80.3	87.5	-4.81	5.57	12.8
	$LSD_{0.05}$		3.46			2.64			2.76			2.90	
Nikwe													
	0	1.41	1.46	1.66	27.4	42.5	56.9	28.8	44.0	58.6	----	15.2	29.9
	6.2	1.29	1.59	1.59	26.7	40.4	58.2	28.0	42.0	59.8	-0.80	13.2	31.0
	12.5	1.27	1.45	1.64	28.6	41.9	57.6	29.9	43.4	59.2	1.08	14.6	30.5
	18.7	1.29	1.93	1.89	29.1	43.4	58.9	30.4	45.3	60.8	1.63	16.5	32.0
	$LSD_{0.05}$		NS			2.21			2.35			2.59	

†Inorganic N = NH_4-N + NO_3-N

††Net N mineralization at a defined level is the difference between Net N mineralization in the treatment less that in the control. For example, Net N mineralization at 8.1 $Mg\ ha^{-1}$ lime (-1.05 $mg\ kg^{-1}$) is derived from inorganic N (73.6 $mg\ kg^{-1}$) minus inorganic N in the control (74.7 $mg\ kg^{-1}$).

immobilization. The rate of N mineralization ranged from –0.82 (16.3 $Mg\ ha^{-1}$) to –4.81 mg k g^{-1} (24.4 $Mg\ ha^{-1}$) for lime alone, from 12.4 (5 $Mg\ ha^{-1}$) to 29.2 $mg\ kg^{-1}$ (10 $Mg\ ha^{-1}$) for broiler manure alone, and from 2.84 (16.3 + 5 $Mg\ ha^{-1}$) to 18.9 $mg\ kg^{-1}$ (8.1 + 10 $Mg\ ha^{-1}$) for lime plus broiler manure treatments. Conversely, in the Nikwe soil N mineralization also increased as the rate of broiler manure application was increased, but the application of lime and the corresponding increase in soil pH had no apparent effect. The rate of N mineralization ranged from –0.80 (6.2 $Mg\ ha^{-1}$) to 1.63 $mg\ kg^{-1}$ (18.7 $Mg\ ha^{-1}$) for lime alone, from 15.2 (5 $Mg\ ha^{-1}$) to 29.9 $mg\ kg^{-1}$ (10 $Mg\ ha^{-1}$) for broiler manure alone, and from 13.2 (6.2 + 5 $Mg\ ha^{-1}$) to 32.0 $mg\ kg^{-1}$ (18.7 + 10 $Mg\ ha^{-1}$) for lime plus broiler manure treatments (Table 5). In general, the rate of total CO_2 evolution and N mineralization in the Nikwe soil followed the order of 10 Mg > 5 Mg > 0 $Mg\ ha^{-1}$ broiler manure, regardless of lime rate or final soil pH values.

The distinct difference in microbial activity and N mineralization patterns between the two soils were apparently associated with differences in specific soil properties, such as Ca^{2+} percentage and soil acid saturation. Havlin *et al.* (1999) reported that high Ca^{2+} saturation indicates a favourable pH for plant growth and microbial activity and will usually mean low concentrations of exchangeable Al in acidic soils. In the Nikwe soil microbial activity did not respond to liming because Ca^{2+} saturation was relatively high (46.1%) while acid saturation value was low (4.3%). In contrast, Ca saturation and soil acid saturation were 29.3% and 24.0, in the Magusheni soil. Thus, in comparison to the Nikwe soil, Ca saturation is lower by 36% whereas the acid saturation percentage is more than five fold in the Magusheni soil. Soil microbial activity was adversely affected by the levels of acidity in this soil, as indicated by the low activity in unlimed treatments. Hence, the higher carbon dioxide evolution in limed relative to unlimed treatments suggest that liming the Magusheni soil created a more favourable environment for soil microorganisms. Furthermore, liming caused N immobilization due to a higher microbial demand for nitrogen. Overall these results indicate that microbial activity and N mineralization were limited by C and N availability and soil acidity in the Magusheni soil. In contrast, for the Nikwe soil only C and N availability was limiting.

Table 5 also shows that in both the Magusheni and Nikwe soils, NO_3-N was the dominant inorganic N form. In the Magusheni soil, except for 24.4 $Mg\ ha^{-1}$ lime alone, the rate of nitrification was significantly lower in unlimed relative to the limed treatments. However, increasing the liming rate did not always result in a significant increase in nitrification rate. Others have also shown that, in general, liming of acid soils tends to increase nitrification levels (Dancer *et al.*, 1973; Nyborg & Hoyt 1978). In the Nikwe soil, in all broiler manure treatments, the rate of nitrification was similar in unlimed relative to the limed treatments. This suggests that in the Nikwe soil liming had no apparent effect on nitrification whereas in the Magusheni soil liming boosted nitrification levels. However, it is interesting to note that in the Magusheni soil, 90 % or more of inorganic N in limed treatments was in the NO_3-N form irrespective of the soil pH level whilst in the Nikwe soil appreciable nitrification was observed in all treatments. A decrease in soil pH is normally observed following nitrification of NH_4-N to NO_3-N, because the nitrification of 1 mole of NH_4-N produces 2 moles of hydrogen (H^+) ions. The H^+ ions released during nitrification lowers pH of the soil (Helyar, 1976; Haynes & Swift, 1988; Tyson & Cabrera,

Table 6 Correlations coefficients (r^2) between N mineralization and selected soil characteristics for the Magusheni and Nikwe soils

	Magusheni	Nikwe
Soil characteristics	N mineralization	
Soil pH	-0.28ns	-0.017ns
Total Soil C	0.32ns	0.33ns
Total Soil N	-0.29ns	-0.08ns
C:N ratio	-0.08ns	0.30ns
CO_2 evolved	0.63***	0.95***

*** = significant at P<0.001;

ns = not significant.

1993; Wong & Swift, 2003). Therefore, it is most likely that the slightly lower than expected pH values that were observed for the highest lime rates (18.7 and 24.4 Mg ha^{-1}) could be partly due to the effect of nitrification on soil pH.

Correlation analysis

In both soils mineralization of N was significantly correlated with microbial activity (evolution of CO_2) only, and not with soil pH, soil Total C, soil Total N, and C:N ratio (Table 6).

These results confirmed that microbial activity is a reliable indicator of the amount of N mineralized during incubation as pointed out by Iratini & Arnold (1960), Agbim *et al.* (1977) and Castellanos & Pratt (1981). Conversely, the lack of correlation between soil pH and N mineralization contradicts the findings of Fu *et al*. (1987) who reported N mineralization in soils amended with organic wastes to be highly dependent on and positively correlated with soil pH.

Conclusions

Liming resulted in a linear increase in soil pH on both soils, however, broiler manure applications had no apparent effect on pH. The effects of liming on microbial activity and N mineralization varied greatly between the two soils. In the Nikwe soil, microbial activity and N mineralization increased as broiler manure application rates increased, however, no lime effects were observed. In the Magusheni soil microbial activity was enhanced by liming at all rates of broiler manure application, but the lime-induced acceleration in microbial activity led to N immobilization over the duration of the experiment. These results demonstrate the importance of reducing the availability of soil Al to improve microbial activity, but also that liming can lead to a lag in the rate at which manure N becomes available for plant uptake. In this study, the amounts of lime and broiler manure applications, Ca^{2+} and the soil acid saturation were factors that were associated with the level of microbial activity and N mineralization, whereas the effect of soil pH on N mineralization was not evident.

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