

Effects of temperature and amendments on nitrogen mineralization in selected Australian soils

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Abstract The effects of temperature (18, 24, and 37 °C) and form of nitrogen (N) input from various sources (organic—green waste compost, biosolids, and chicken manure; inorganic—urea) on N transformation in three different Australian soils with varying pH (4.30, 7.09, and 9.15) were examined. Ammonification rate (ammonium concentration) increased with increase in temperature in all soil types. The effect of temperature on nitrification rate (nitrate concentration) followed 24>37>18 °C. Nitrification rate was higher in neutral and alkaline soils than acidic soil. Mineral N (bioavailable N) concentration was high in urea treatments than in organic N source treatments in all soil types. Acidic soil lacked nitrification activity resulting in low nitrate (NO₃) buildup in urea treatment, whereas a significant NO₃ buildup was noticed in green waste compost treatment. In neutral and alkaline soils, the nitrification activity was low at 37 °C in urea treatment but with a significant NO₃ buildup in organic amendment added soils. Addition of organic N sources supplied ammonia oxidizing bacteria thereby triggering nitrification in the soils (even at 37 °C). This study posits the following implications: (1) inorganic fertilizer accumulate high NO₃ content in soils in a short period of incubation, thereby becoming a potential source of NO₃ leaching; (2) organic N sources can serve as

possible source of nitrifying bacteria, thereby increasing bioavailable N (NO₃) in soils regardless of the soil properties and temperature.

Keywords Organic amendment · Nitrate leaching · Fertilizer · Nitrogen · Bioavailability · Soil

Introduction

Agricultural productivity is currently straining to meet the food demands of a growing population. Reflecting the global demands and increasing costs of mineral fertilizers, using organic amendments (OAs) is rapidly increasing and their share of agricultural land continues to rise in many countries (Park et al. 2011). Globally, significant quantities of OAs such as plant residues (3.8×10^9 Mg year⁻¹), biosolids (10×10^7 Mg yr⁻¹), and animal manures (7×10^9 Mg year⁻¹) are produced (Thangarajan et al. 2013). Recycling these OAs in agriculture has several advantages such as improving soil health, crop yield, soil carbon (C) content, and microbial biomass and activity. Nevertheless, improper application of OAs can cause environmental problems like nutrient eutrophication (NO₃ leaching) and greenhouse gas emission (Quilty and Cattle 2011).

High concentration of NO₃ in drinking water is considered to be harmful to human health (Hmelak Gorenjak and Cencič 2013). Nitrate can also degrade surface water quality, resulting in eutrophication, algal blooms, and fish poisoning (Di and Cameron 2002). Transfer of NO₃ from farming systems to groundwater and the ongoing degradation of water resources by agricultural practices is a challenging problem worldwide (Danielopol et al. 2003; Spiertz 2010). Furthermore, Ruidisch et al. (2013) noted that the risk of nutrient leaching via surface runoff into rivers and lakes or percolation through the unsaturated zone into groundwater is a major environmental

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concern especially in regions affected by very frequent and intense rainfalls.

Though the application of OAs generally result in an increase in N mineralization potential of soils (Benitez et al. 2003), the quantity of residual N that is available for plants (i.e., bioavailable N) is influenced by the receiving soil and amendment characteristics, climatic conditions (temperature and rainfall), soil microbial communities, application rate, application timing, and method of application (Douglas and Magdoff 1991). Out of all these factors influencing bioavailability of N from OAs, soil temperature and microbial communities play a major role in determining the fate of N (Joergensen et al. 1990). Urease activity increases with increase in temperature (Suter et al. 2011), and the presence of sizeable nitrifier communities (i.e., ammonia oxidizing bacteria; AOB) helps the rapid conversion of one bioavailable N form to the other (NH_4 to NO_3). When compared with synthetic fertilizers, it is difficult to predict the pattern and amount of bioavailable N released from organic N sources to soil (Pereira et al. 2005). While N mineralization in OAs has been extensively studied (Azeez and Van Averbek 2010; Fanguero et al. 2010; Khalili and Nourbakhsh 2012), only a few analyses (Flavel and Murphy 2006; Khalil et al. 2005) have compared N mineralization in different soils treated with various OA sources at a range of temperatures and related the mineralization potential to AOB and NO_3 leaching. The present study aims to compare N transformation between inorganic and organic N sources as influenced by temperature and soil type. It also examines the interaction between N transformation of OAs as measured by N mineralization potential and AOB contributed by these OAs.

In our study, we attempted to (1) compare the influence of temperature on N mineralization in three soils amended with 4 N sources, (2) compare the influence of N source (OA type and inorganic fertilizer) on N mineralization, (3) examine the nitrifier communities (AOB) as affected by OA addition, and (4) estimate the potentially bioavailable (mineralizable) organic N. We used the results to predict the possibilities of NO_3 production and leaching from OAs and inorganic fertilizer added soils.

Materials and methods

Soil and organic amendments

Seventeen soils from various regions of Australia with a wide range of pH ($[\text{OH}^-]$ ranging from 1.99×10^{-10} to 1.41×10^{-5}) were collected from a depth of 0–15 cm. Three different organic N sources and one inorganic N source with a wide range of C:N ratios were used in this study. Green waste compost (GWC) was collected from Jeffries Compost, Soil and Mulch Experts in Adelaide; biosolids (BS) was collected

from Boneo Water Recycling Plant, South East Water, in Victoria, and chicken manure (CM) was collected from SA Manure Supplies, South Australia. Commercially available urea (U) was utilized as inorganic N source. Soils and OAs were air-dried, sieved through 2-mm mesh, and stored at room temperature (24 °C) prior to analysis. Total C and N were determined by combustion using LECO CNS-2000 analyzer. Other physicochemical properties of the soils and amendments (Table 1) were determined using the analytical methods outlined in Rayment and Lyons (2010).

Short-term nitrification assay

The short-term nitrification assay (SNA) measurements were made on the collected 17 soils following the method of Bramley and White (1990). Briefly, 200-g soil was leached overnight with 0.005 M KCl in a Büchner funnel fitted with a Whatman no. 1 filter paper. Four replicates of 5 g (dry-weight equivalent) of the leached soil samples were taken in 50 mL centrifuge tubes. To each of the 4 tubes, 20 mL of 0.005 M KCl, 0.3 % (W V⁻¹) agar, and 10 mL of 0.01 M $(\text{NH}_4)_2\text{SO}_4$ were added. The tubes were shaken at room temperature for 8 h. After 1 and 8 h shaking, two tubes (per shaking time) were removed from the shaker, centrifuged, filtered, and the filtrate was frozen and stored at −20 °C for NO_3 quantification.

Laboratory incubation

Three out of 17 soils with varying pH and SNA value were randomly selected for the incubation study. The experiment comprised of five N treatments (three organic N sources, one inorganic N source, and one control soil without N source) for each soil and at each temperature. Briefly, 200 g (dry-weight equivalent) of soil was mixed thoroughly with the N source at a rate of 900 mg N kg⁻¹ in a plastic zip lock bag. The treatments were replicated thrice. The moisture content of the mixture was maintained at maximum water holding capacity (75 %) and the bags were kept open for gas exchange. The amount of water loss due to evaporation as measured by weight loss was corrected at an interval of 2–3 days. The experimental design was completely randomized, and amended soils were randomly arranged and incubated in three different temperatures (18, 24, and 37 °C). In total, there were 45 treatment bags (3 soils × 5 treatments × 3 replicates) for each incubation temperature.

Soils were sampled after each incubation period of 1 h, 2, 3, 4, 5, 6, 7, 14, 21, and 28 days, and were analyzed for mineral N (NH_4 and NO_3). Five grams (dry-weight equivalent) of moist incubated soil were weighed in a Falcon tube and extracted with 50 mL of 2 M KCl solution. The mixture was shaken end-over-end at 70 rpm for 1 h and then filtered using a syringe filter (0.45 µm). The extracted solution was stored at −20 °C prior to mineral N analysis.

Table 1 Selected physiochemical properties of soil and amendments used in this study (mean \pm standard deviation)

Sample	pH	Carbon (%)	Nitrogen (%)	C:N ratio	Total NH ₄ (mg kg ⁻¹)	Total NO ₃ (mg kg ⁻¹)	Total mineral N (mg kg ⁻¹)
Soil 1**	4.30 \pm 0.06	2.76 \pm 0.09	0.11 \pm 0.01	25	6.66 \pm 0.47	*	6.66
Soil 2	4.40 \pm 0.05	7.75 \pm 0.01	0.55 \pm 0.00	14	9.23 \pm 0.25	8.44 \pm 0.54	17.6
Soil 3	4.46 \pm 0.05	0.47 \pm 0.01	0.02 \pm 0.00	23	2.65 \pm 0.32	*	2.65
Soil 4	4.74 \pm 0.02	1.25 \pm 0.01	0.03 \pm 0.00	42	3.49 \pm 0.48	1.05 \pm 0.07	4.52
Soil 5	4.89 \pm 0.04	0.64 \pm 0.06	0.02 \pm 0.00	32	5.28 \pm 0.30	9.14 \pm 0.27	14.5
Soil 6	4.91 \pm 0.02	3.89 \pm 0.07	0.24 \pm 0.01	16	14.2 \pm 0.70	28.9 \pm 0.54	43.1
Soil 7	5.62 \pm 0.09	0.40 \pm 0.02	0.01 \pm 0.00	40	1.39 \pm 0.16	*	1.39
Soil 8	5.65 \pm 0.07	0.85 \pm 0.06	0.02 \pm 0.00	42	3.67 \pm 0.28	2.47 \pm 0.34	6.14
Soil 9	5.73 \pm 0.08	0.85 \pm 0.03	0.04 \pm 0.00	21	7.23 \pm 0.12	9.44 \pm 0.33	16.6
Soil 10	5.91 \pm 0.03	0.36 \pm 0.04	0.01 \pm 0.00	36	3.53 \pm 0.27	1.54 \pm 0.24	5.07
Soil 11	5.95 \pm 0.05	2.84 \pm 0.19	0.16 \pm 0.01	18	12.2 \pm 0.28	5.33 \pm 0.37	17.5
Soil 12	6.12 \pm 0.06	3.01 \pm 0.01	0.21 \pm 0.00	14	15.4 \pm 1.29	4.69 \pm 0.49	20.1
Soil 13**	7.09 \pm 0.06	0.92 \pm 0.02	0.04 \pm 0.00	23	6.81 \pm 0.19	26.3 \pm 0.77	33.1
Soil 14	7.62 \pm 0.02	0.68 \pm 0.04	0.04 \pm 0.00	17	2.34 \pm 0.31	4.63 \pm 0.20	6.89
Soil 15	7.71 \pm 0.03	0.72 \pm 0.02	0.03 \pm 0.00	24	8.19 \pm 0.31	3.70 \pm 0.34	11.8
Soil 16	8.02 \pm 0.09	1.10 \pm 0.05	0.04 \pm 0.00	27	5.44 \pm 0.49	3.44 \pm 0.33	8.88
Soil 17**	9.15 \pm 0.05	1.91 \pm 0.02	0.12 \pm 0.00	16	12.0 \pm 0.53	6.64 \pm 0.37	18.6
GWC		17.1 \pm 0.42	1.43 \pm 0.03	12	195 \pm 17.1	37.7 \pm 2.36	233
CM		17.2 \pm 1.60	3.11 \pm 0.10	6	198 \pm 28.3	7732 \pm 77.7	7930
BS		26.0 \pm 0.19	4.17 \pm 0.02	6	389 \pm 19.1	60.6 \pm 5.11	450
U		20.4 \pm 0.04	46.5 \pm 0.04	0.44	0	0.00	0

*Below detectable level (<0.013 mg kg⁻¹)

** Soils used in the incubation experiments

GWC green waste compost, CM chicken manure, BS biosolids, and U urea

Potentially bioavailable N, PAN_{anaerobic} (anaerobically mineralizable N)

The three soils selected for laboratory incubation study were analyzed for potentially bioavailable N by using short-term anaerobic soil incubation method described by Rayment and Lyons (2010). Potentially bioavailable N (NH₄) in soil and soil with amendments were examined. This method involves two separate extractions which are detailed below.

For immediate extraction of NH₄, 50 mL of 2 M KCl was added to 5-g samples (soil; soil and amendments), and the mixture was shaken in an end-over-end shaker for 1 h. The mixture was then centrifuged, filtered (0.45 μ m), and the filtrate was stored in -20 °C for NH₄ quantification. For anaerobic incubation and post-incubation extraction of NH₄, 5-g samples (soil; soil and amendments) was placed in bottle, and 10 mL deionized water was then added to it, tightly capped, and incubated at 40 °C. After 7 days of incubation, 40 mL of 2.5 M KCl was added and shaken in an end-over-end shaker for 1 h, centrifuged, filtered (0.45 μ m), and the filtrate was stored at -20 °C for NH₄ quantification. Potentially bioavailable N was calculated using the following equation:

$$\text{Potentially bioavailable N}_{\text{anaerobic}} = [\text{NH}_4\text{D}_7 - \text{NH}_4\text{D}_{\text{initial}}] \quad (1)$$

Where NH₄D₇ is the concentration of NH₄ after 7 days incubation (mg kg⁻¹), and NH₄D_{initial} is the concentration of NH₄ in the immediate extraction (mg kg⁻¹).

Ammonium concentration in Eq. 1 was calculated using the following equation:

$$\text{NH}_4\text{D}_n = A \times V/W \quad (2)$$

Where NH₄D_n is the concentration of NH₄ after n days of incubation (mg kg⁻¹), A is the concentration of NH₄ concentration in sample solution (mg kg⁻¹), V is the total volume of solution added (2 M KCl or 2.5 M KCl+deionized water) (L), and W is the weight of soil used (kg).

Most probable number count

The AOB numbers in the three selected soils and the soils mixed with N sources were estimated. Briefly, the most probable number count (MPN) media described by Schmidt and Belser (1994) was prepared, sterilized, and 4-mL aliquots was placed in sterile tubes. Serial dilutions of soil/soil and amendment mixture (10^{-1} to 10^{-9} dilution) were made with 1 mM

sterilized phosphate buffer. One milliliter of each dilution was inoculated into MPN media tubes containing NH_4 solution, and each inoculation was replicated five times. Inoculated MPN tubes were incubated at 28 °C in the dark. At intervals of 1 week for a maximum of 12 weeks, each tube was tested for NO_3 production by observing color change from blue green to yellow due to pH drop, which indicated acid production during the nitrification process. Griess–Ilosvay colorimetric spot test and Merckoquant (1.10020) strip test were also carried out to confirm the nitrification process in the positive MPN tubes. AOB population each week was estimated using Information Collection Rule MPN calculator (version 2.0) (US EPA 2009).

Chemical analysis

Mineral N (NH_4 and NO_3) was determined by using modified methods employed by Miranda et al. (2001) and Mulvaney (1996) using BioTek SynergyTM HT multi-mode microplate reader.

Nitrogen mineralization rate

Net N mineralization (N_{minR}), ammonification (N_{ammR}), and nitrification (N_{nitR}) rates were calculated using Eqs. 3, 4, and 5, respectively, as follow:

$$N_{\text{minR}} = \frac{[(N_f - C_f) - (N_i - C_i)]}{(f - i)} \quad (3)$$

Where N_{minR} is the net N mineralization rate in the amendment ($\text{mg N kg soil}^{-1} \text{ day}^{-1}$), N_f and N_i are the total N mineralized in the amendment and soil mixture ($\text{mg NO}_3 + \text{NH}_4 \text{ kg soil}^{-1}$) on the final and initial sampling dates, respectively, C_f and C_i are the total N mineralized in the control soil

($\text{mg NO}_3 + \text{NH}_4 \text{ kg soil}^{-1}$) on the final and initial sampling dates, respectively, and f and i are the final and initial sampling dates (day), respectively.

$$N_{\text{ammR}} = \frac{[(N_{\text{af}} - C_{\text{af}}) - (N_{\text{ai}} - C_{\text{ai}})]}{(af - ai)} \quad (4)$$

Where N_{ammR} is the net ammonification rate in the amendment ($\text{mg N kg soil}^{-1} \text{ day}^{-1}$), N_{af} and N_{ai} are the total NH_4 in the amendment and soil mixture ($\text{mg NH}_4 \text{ kg soil}^{-1}$) on the peak NH_4 concentration date and initial sampling date, respectively, C_{af} and C_{ai} are the total NH_4 in the control soil ($\text{mg NH}_4 \text{ kg soil}^{-1}$) on the peak NH_4 concentration date and initial sampling date, respectively, and af and ai are the peak NH_4 concentration date and initial sampling date (day), respectively.

$$N_{\text{nitR}} = \frac{[(N_{\text{nf}} - C_{\text{nf}}) - (N_{\text{ni}} - C_{\text{ni}})]}{(nf - ni)} \quad (5)$$

Where N_{nitR} is the net nitrification rate in the amendment ($\text{mg N kg soil}^{-1} \text{ day}^{-1}$), N_{nf} and N_{ni} are the total NO_3 mineralized ($\text{mg NO}_3 \text{ kg soil}^{-1}$) in the amendment and soil mixture on the final and initial sampling dates, respectively, C_{nf} and C_{ni} are the total NO_3 mineralized ($\text{mg NO}_3 \text{ kg soil}^{-1}$) in the control soil on the final and initial sampling dates, respectively, and nf and ni are the final and initial sampling dates (day), respectively.

Statistical analysis of N mineralization results

To evaluate the potential availability of the organic N in amendments, a nonlinear exponential regression mathematical model proposed by Stanford and Smith (1972) was used as follows (Eq. 6):

$$N_{\text{min}} = N_0 (1 - \text{Exp}^{-K_0 t}) \quad (6)$$

Where N_{min} is the amount of N mineralized in aerobic incubation ($\text{mg N kg soil}^{-1} \text{ day}^{-1}$) associated with OAs at a specific time (t), N_0 is the potentially bioavailable N (mg N kg soil^{-1}), k_0 is the mineralization rate constant (per day), and t is the time of incubation (day).

Analysis of variance was used to test the effects of amendment type, soil, and temperature on mineralization rate (N_{min}) using IBM[®] SPSS[®] Statistics (version 21.0).

Results and discussion

Soil and amendment properties

Selected physiochemical characteristics of the 17 soils and 4 N sources used in the study are listed in Table 1. Soil pH ranged from 4.30 to 9.15 with C:N ratio ranging from 14 to

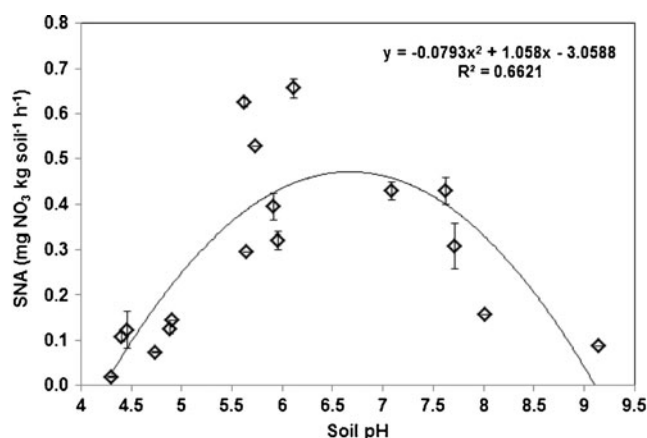


Fig. 1 Short-term nitrification assay (SNA) value plotted against soil pH in the 17 soils collected from various regions in Australia. The vertical bars indicate standard errors

Table 2 Peak ammonium and nitrate concentration in the three soils (acidic, neutral, and alkaline) amended with various N sources (green waste compost, biosolids, chicken manure, and urea) incubated for 28 days at 3 different temperatures (18, 24, and 37 °C) (mean±standard deviation)

Soil	N source	18 °C			24 °C			37 °C		
		Peak NH ₄ (mg kg ⁻¹)	Peak NO ₃ (mg kg ⁻¹)	Peak NH ₄ (mg kg ⁻¹)	Peak NH ₄ (mg kg ⁻¹)	Peak NO ₃ (mg kg ⁻¹)	Peak NH ₄ (mg kg ⁻¹)	Peak NH ₄ (mg kg ⁻¹)	Peak NO ₃ (mg kg ⁻¹)	Peak NO ₃ (mg kg ⁻¹)
Acidic	Control	19.23±2.01 (3)*	9.481±1.23 (28)*	14.31±0.72 (7)*	3.650±0.24 (21)*	52.39±3.7 (21)*	14.46±0.06 (28)*			
	GWC	31.43±2.47 (5)*	80.22±9.54 (28)*	41.53±7.32 (6)*	91.71±7.52 (28)*	77.57±4.47 (7)*	103.6±6.51 (21)*			
	BS	282.4±13.9 (4)*	39.90±6.40 (21)*	264.2±54.5 (7)*	49.01±5.14 (28)*	319.8±11.2 (3)*	0.170±0.02 (5)*			
	CM	378.7±29.6 (5)*	101.6±10.2 (28)*	527.5±8.59 (7)*	50.46±6.54 (21)*	606.7±2.38 (3)*	0.230±0.04 (5)*			
Neutral	U	1,343±32.0 (6)*	1.461±0.02 (2)*	1,342±51.5 (4)*	2.169±0.15 (28)*	1,342±54.5 (2)*	0.370±0.01 (7)*			
	Control	12.84±1.71 (3)*	36.96±8.11 (21)*	5.54±0.371 (3)*	42.92±3.59 (7)*	9.340±2.48 (1)*	53.89±13.2 (21)*			
	GWC	17.46±1.63 (3)*	52.79±9.25 (28)*	11.44±0.33 (3)*	50.80±10.0 (28)*	18.65±0.86 (3)*	84.24±9.24 (21)*			
	BS	220.9±67.1 (5)*	279.0±12.8 (28)*	235.6±17.7 (4)*	256.7±26.1 (28)*	282.6±6.15 (6)*	97.50±15.2 (28)*			
Alkaline	CM	294.2±4.49 (6)*	335.5±6.33 (28)*	263.1±5.07 (4)*	406.3±16.5 (28)*	427.8±12.2 (5)*	126.5±5.63 (21)*			
	U	1490±66.7 (6)*	481.3±2.14 (28)*	1416±18.2 (4)*	731.0±15.2 (28)*	1422±74.2 (3)*	36.49±8.25 (28)*			
	Control	17.00±4.16 (14)*	17.81±2.01 (4)*	5.330±0.05 (4)*	20.18±1.23 (21)*	9.590±0.31 (1)*	159.9±21.2 (21)*			
	GWC	9.851±0.67 (3)*	32.15±2.14 (21)*	6.32±0.230 (3)*	40.48±3.16 (28)*	15.66±1.67 (1)*	189.8±21.4 (28)*			
	BS	283.7±81.3 (2)*	244.6±24.1 (28)*	194.2±0.82 (4)*	205.7±17.1 (28)*	243.3±8.37 (1)*	368.9±31.3 (28)*			
	CM	260.9±12.1 (1)*	444.2±19.5 (28)*	273.8±9.42 (7)*	419.7±16.2 (21)*	429.6±17.0 (5)*	579.9±34.7 (28)*			
	U	1,534±46.8 (2)*	603.8±24.1 (28)*	1,499±13.1 (3)*	768.3±25.1 (28)*	1,358±30.8 (2)*	38.34±5.10 (28)*			

GWC green waste compost, CM chicken manure, BS biosolids, and U urea

* Values within parenthesis represent the day when the peak value was obtained

Table 3 Net N ammonification rate and nitrification rate in the three soils (acidic, neutral, and alkaline) amended with various N sources (green waste compost, biosolids, chicken manure, and urea) and incubated for 28 days at 3 different temperatures (18, 24, and 37 °C)

Treatment	Acidic soil			Neutral soil			Alkaline soil		
	18 °C	24 °C	37 °C	18 °C	24 °C	37 °C	18 °C	24 °C	37 °C
Ammonification rate (N_{ammR}) (mg NH_4 kg $^{-1}$ day $^{-1}$)									
Control	0.71	1.15	1.95	0.93	1.09	1.65	3.62	4.34	5.21
GWC	1.85	2.53	3.02	0.41	3.47	4.57	0.54	0.54	1.23
BS	3.97	4.06	10.44	2.40	7.57	14.23	6.23	8.23	10.92
CM	57.87	82.0	114.0	7.80	33.90	35.66	123.9	258.3	21.19
U	103.0	144.0	169.2	181.6	154.8	166.5	638.3	769.6	664.0
Nitrification rate (N_{nitR}) (mg NO_3 kg $^{-1}$ day $^{-1}$)									
Control	0.24	0.11	0.52	1.18	0.28	0.95	0.21	0.26	0.21
GWC	2.86	3.63	5.41	1.00	0.80	1.97	0.78	0.97	6.31
BS	1.46	1.98	0.00	10.5	8.07	2.48	8.44	6.86	12.7
CM	2.93	2.29	0.00	9.85	13.9	−0.43	12.2	10.9	18.6
U	0.00	0.04	0.00	15.4	25.1	0.43	21.3	27.1	1.04

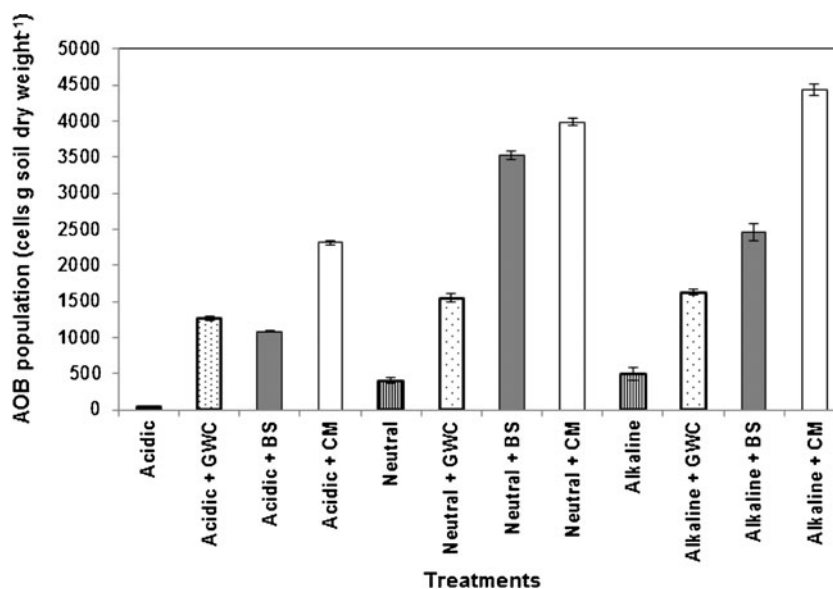
GWC green waste compost, CM chicken manure, BS biosolids, and U urea

42. Of the organic N sources, GWC exhibited higher C:N ratio (12) than CM (6) and BS (6). All the OAs had high extractable mineral N ($\text{NH}_4 + \text{NO}_3$) content when compared to the mineral N content in soils. Chicken manure (7,930 mg kg $^{-1}$) had the highest of the three followed by BS (450 mg kg $^{-1}$), and then GWC (233 mg kg $^{-1}$). Of the amendments, CM had the highest NO_3 concentration (7,732 mg kg $^{-1}$), while BS had the highest NH_4 concentration (389 mg kg $^{-1}$). pH of the three soils selected for N mineralization incubation study are as follows: Soil 1 (acidic soil; pH 4.3), Soil 13 (neutral soil; pH 7.09), and Soil 17 (alkaline soil; pH 9.15). The total N content of the alkaline soil (0.12 %) was higher than the acidic (0.11 %) and neutral soils (0.04 %). The extractable mineral N was higher in neutral soil (33 mg kg $^{-1}$) than in alkaline (18 mg kg $^{-1}$) and acidic soils (7 mg kg $^{-1}$). The extractable NO_3 content was

negligible (<0.013 mg kg $^{-1}$) in acidic soil. Total C was higher in acidic soil (2.76 %) than in alkaline (1.91 %) and neutral soils (0.92 %).

Short-term nitrification assay

The SNA value of the 17 soil samples was calculated as the difference between NO_3 produced after 1 and 8 h incubation. Short-term nitrification assay values were plotted against their respective soil pH and the data was fitted by quadratic equation (Fig. 1). Though optimum pH for nitrifier activity was generally close to original soil pH (Bramley and White 1990), in our study, however, the SNA was very low in low pH soils (pH<4), increased with increase in soil pH (4–8), but declined at high soil pH (pH>8). De Boer and Kowalchuk (2001) also reported

Fig. 2 Estimate of AOB population in soils and soils treated with OAs (GWC, BS, and CM). The vertical bars indicate standard errors

that the absence of nitrifier activity in highly acidic soils (pH<4.5) resulted in low nitrification. However, the addition of organic N substrate increased the soil pH and nitrifying activity in these highly acidic soils. The soils selected for the mineralization study in the incubation experiment are acidic, neutral, and alkaline with SNA values of 0.02, 0.43, and 0.09 mg NO₃ kg⁻¹ h⁻¹, respectively.

Temperature effect

Table 2 shows the peak concentrations of mineral N (bioavailable N) during the course of 28-day incubation period in soils of varying pH (acidic, neutral, and alkaline) treated with different N sources (GWC, BS, CM, and U) and incubated at different temperatures (18, 24, and 37 °C). Ammonium concentration increased in the treatments and reached a peak value on or before day 7 in incubation. Results show that net ammonification rate (Eq. 4, Table 3) increased with an increase in temperature in all the soils regardless of the soil pH which is consistent with the conclusions reached by Myers (1975). Ammonium concentration was greater in inorganic urea N amended soils (~1,500 mg NH₄ kg soil⁻¹) than in soils amended with organic N sources (Table 2). Similar results of

higher NH₄ concentration in urea treatments (~400 mg kg⁻¹) than in organic N (chicken manure, swine manure, and cattle manure) treatments (~15–200 mg kg⁻¹) were obtained by Eneji et al. (2002). Though there was a slight increase in NH₄ concentration in control soils, this increase was not significant compared to amended soils throughout the incubation period regardless of the incubation temperatures. In acidic soil, increase in temperature did not largely affect the NH₄ buildup in urea treatment, whereas NH₄ concentration increased with increase in temperature in all OA treatments (Table 2). These results are consistent with Agehara and Warncke (2005) findings in which urea released more N (91–96 %) than the organic materials (blood meal, alfalfa pellets, and partially composted chicken manure). In that same study, the increase in temperature (15–25 °C) did not affect the N release from urea but did increase the N release for organic materials by 15 % (mean value). Increase in temperature did not affect NH₄ concentration in neutral and alkaline soils. However, the net ammonification rate (Eq. 4) was higher at 37 °C than 18 and 24 °C (Table 3). There was a significant NH₄ concentration observed even on day 0 at 37 °C (data not shown).

In contrast to large NH₄ build up in acidic soils across all temperatures, NO₃ build up was negligible regardless of the

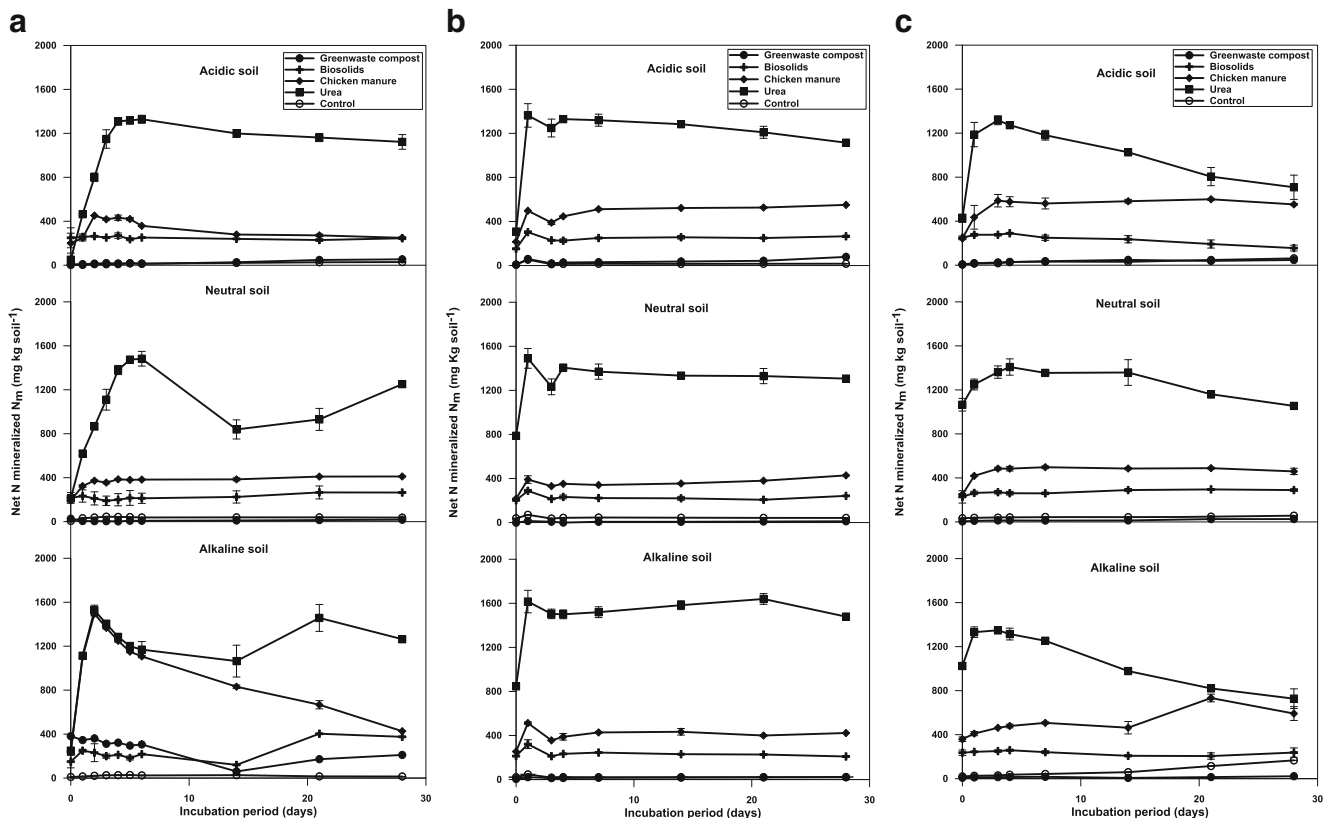


Fig. 3 a Net N mineralized (N_m) during the 28-day incubation period in three different soils treated with various N sources at 18 °C. The vertical bars indicate standard errors. b Net N mineralized (N_m) during the 28-day incubation period in three different soils treated with various N

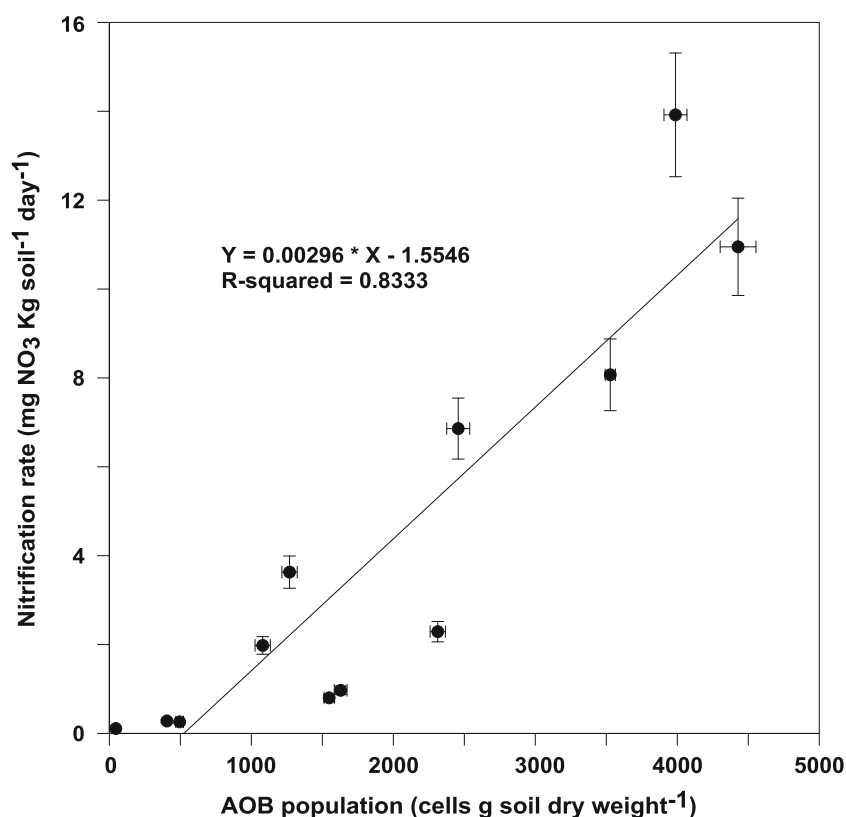
sources at 24 °C. The vertical bars indicate standard errors. c Net N mineralized (N_m) during the 28-day incubation period in three different soils treated with various N sources at 37 °C. The vertical bars indicate standard errors

incubation temperature. There was only a slight NO_3 build up in neutral soils, but a significant NO_3 build up in alkaline soils (Table 2). Low SNA in acidic soil when compared to the other two soils explains the insignificant NO_3 build up in acidic soil (Fig. 1). Similar results of higher nitrification in high pH soils than in acidic soils observed by Khalil et al. (2005) was attributed to the high microbial activity in high pH soil, and it has been suggested that because of the sensitivity of AOB to low pH, some highly acidic soils lack nitrification activity (De Boer and Kowalchuk 2001). However, at 37 °C, NO_3 build up was observed in GWC treatment in acidic soil. Optimum temperature for nitrification in neutral soil was 24 °C in all treatments except for GWC treatment. Malhi and McGill (1982) studied the effect of temperature on nitrification in three Alberta soils and reported that the optimum temperature for nitrification in soils was 20 °C, and at a higher temperature (>30 °C) nitrification activity had virtually ceased. Rising temperature increased NO_3 concentration in all OAs-treated alkaline soils, but the optimum temperature for nitrification in urea treatment in alkaline soil was 24 °C. Optimum temperature for substantial nitrification was associated with the environmental conditions (Norton and Stark 2011) where the soil was formed (Stark 1996), climatic zones (Malhi and McGill 1982), soil type and texture (Griffin et al. 2002), soil drying rate after wetting (Griffin et al. 2002), and moisture content (Agehara and Warncke 2005).

Amendment effect

Generally, a reduction in NH_4 due to the formation of NO_3 (nitrification) was observed in N sources amended soils. Highest and lowest NH_4 concentrations were observed in U and GWC amended soils, respectively. After day 7, a decrease in NH_4 concentration was observed in all treatments (except for urea treatment in acidic soil), and the concentration reached below detectable level on day 28 in OA treatments of neutral and alkaline soils at 18 and 24 °C. Acidic soils lacked nitrification activity as indicated by low SNA value ($0.02 \text{ mg NO}_3 \text{ kg}^{-1} \text{ h}^{-1}$), and hence there was no NO_3 build up in control and urea treatment. However, there was a significant build up of NO_3 in GWC-treated acidic soils ($103.6 \text{ mg NO}_3 \text{ kg soil}^{-1}$) compared to other OAs. Results of the MPN study indicate acidic soils had a very low AOB count (45 cells per gram soil dry weight) when compared to neutral (405 cells per gram soil dry weight) and alkaline soils (495 cells per gram soil dry weight) (Fig. 2). The estimates of AOB population in the OA amended soils (mean value of 2,471 cells per gram soil dry weight), based on maximal counts of AOB reached after 12 weeks of incubation, were 8-fold higher than in the soils with no amendments (mean value of 318 cells per gram soil dry weight). In particular, AOB population increased by 34-fold in acidic soils when treated with OAs (mean value of 1,554 cells per gram soil dry weight) (Fig. 2). Adding OAs had contributed

Fig. 4 Mean nitrification rate and the estimated numbers of AOB in soils and soils treated with OAs (GWC, BS, and CM). The vertical and horizontal bars indicate standard errors



to the AOB population in all the soils and triggered nitrification even in acidic soil lacking nitrification. Organic amendments are rich sources of nitrifiers (AOB and ammonia oxidizing archaea; AOA) (Yamamoto and Nozomi 2012), which can influence the growth and activities of soil microbial communities (Chu et al. 2008).

Chicken manure amended soils had high NO_3 concentration to start with ($\sim 100 \text{ mg NO}_3 \text{ kg soil}^{-1}$) (data not shown), and a decrease in NO_3 concentration during the initial incubation days was observed in CM-treated acidic soils. In general, NO_3 concentration was higher in urea-treated soils

($768 \text{ mg NO}_3 \text{ kg soil}^{-1}$) than in soils treated with OAs ($579 \text{ mg NO}_3 \text{ kg soil}^{-1}$) (Table 2). This is comparable to the findings of Xie and MacKenzie (1986) who stated that about 1 to 5 kg manure-N was found to be equivalent to 1 kg of urea-N in terms of increasing soil NO_3 concentrations.

Nitrogen mineralization, nitrification, and NO_3 leaching

Figure 3a–c shows the net N mineralized (N_m) throughout the aerobic incubation period for amended and unamended soils at all temperatures. Comparing N_m in treatments with urea and

Table 4 Parameters of simple exponential model used to describe mineralized N data in acidic, neutral, and alkaline soils at 18, 24, and 37 °C incubation studies

Temperature	Soil	N Source	Mineralization parameters*		Regression coefficient (R^2)
			N_0	K_0	
18 °C	Acidic	GWC	71.57	0.058	0.9695
		BS	436.73	0.040	0.9784
		CM	— ^a		
		U	1,343.60	0.187	0.9990
	Neutral	GWC	31.07	0.082	0.8833
		BS	343.47	0.062	0.9721
		CM	1001.91	0.022	0.9879
		U	1,488.71	0.229	0.9971
	Alkaline	GWC			0.8954
		BS	565.28	0.052	0.9775
		CM	— ^a		
		U	1575.72	0.144	0.9981
24 °C	Acidic	GWC	93.76	0.042	0.9379
		BS	— ^a		
		CM	692.61	0.066	0.9895
		U	1,368.60	0.228	0.9901
	Neutral	GWC	19.72	0.051	0.8724
		BS	350.00	0.072	0.9561
		CM			
		U	1,491.97	0.289	0.9924
	Alkaline	GWC	75.51	0.014	0.9174
		BS	395.23	0.070	0.9447
		CM	749.49	0.048	0.9535
		U	1617.56	0.289	0.9996
37 °C	Acidic	GWC	55.57	0.084	0.9147
		BS	— ^a		
		CM	637.75	0.117	0.9904
		U	1321.74	0.239	0.9921
	Neutral	GWC	29.59	0.088	0.7478
		BS	316.08	0.114	0.9681
		CM	534.59	0.111	0.9873
		U	1,411.32	0.253	0.9957
	Alkaline	GWC	193.40	0.005	0.9393
		BS	352.92	0.055	0.9842
		CM	828.43	0.090	0.9858
		U	1,369.62	0.174	0.9998

*Simple exponential model, $N_{\min} = N_0 (1 - \text{EXP}^{-K_0 t})$

^a Model not fitted

GWC green waste compost, CM chicken manure, BS biosolids, and U urea

OAs, N_m was low in OA treatments, but there was a steady increase in N_m in OA-treated soils, indicating the slow release of mineral N. A significant difference in net N mineralization rate (N_{minR}) (calculated using Eq. 3 with data produced in Fig. 3) was observed between treatments incubated at the same temperatures ($P \leq 0.05$). Across three incubation temperatures, the net N mineralization rate differed significantly ($P \leq 0.05$) amongst CM-treated acidic soil, BS- and CM-treated neutral soil, and GWC, CM, and U-treated alkaline soils. Across the three soils incubated at the same temperatures, treatments with GWC and CM in 18 °C, GWC in 24 °C, and GWC in 37 °C were significantly different in net N mineralization rate ($P \leq 0.05$). Net N nitrification rates as calculated from Eq. 5 in unamended soils after 28 days were ranged from 0.11 to 1.18 mg NO_3 kg soil⁻¹ day⁻¹ (Table 3). The progress of nitrification differed according to the type of N amendment, incubation temperature, and soil type. Nitrification rate was observed to be high in urea treatment when compared to OA treatments. This property of slow and steady build up of NO_3 concentration in OA treatments compared to urea treatments will reduce N loss as NO_3 leaching from organic N sources. A significant decrease in NO_3 leaching from OAs application when compared with mineral fertilizers application was reported by Leclerc et al. (1995) and Ulen (1999). However, Basso and Ritchie (2005), Chambers et al. (2000), and Yan et al. (2002) demonstrated that OAs are potential sources of NO_3 leaching when compared to inorganic fertilizers. This is due to the slow N release from OAs compared to inorganic fertilizers, at times when no crop uptake had occurred, thus leading to a greater leaching potential (Scheller and Vogtmann, 1995).

Addition of OAs enhanced the nitrification rate in alkaline and neutral soils even at high temperature (37 °C) (Table 3). Among the nitrifiers, AOB are highly sensitive to high temperatures when compared with AOA. AOA are thermophilic and can survive and be active even at 97 °C (Reigstad et al. 2008). Some *Nitrosospira* sp. (AOB) are abundant at low temperatures (4–10 °C) (Siripong and Rittmann 2007) but absent at higher temperatures (30 °C), whereas other species of *Nitrosospira* which are absent at low temperatures are abundant at high temperatures (30 °C) (Avrahami and Bohannan 2007). MPN results (Fig. 2) indicate that alkaline soil has a high AOB population that can survive at low temperatures, and hence incubating them at 37 °C would have reduced the nitrification rate (Table 3). Furthermore, the addition of OAs rich in nitrifiers surviving at high temperatures enhanced the nitrification activity in all soils. More AOB found in the CM treated alkaline soil (4,428 cells per gram soil) correspond to the high nitrification rate (10.95 mg NO_3 kg soil⁻¹ day⁻¹) obtained in the soil mixture (Fig. 4). Ammonium is tightly bound by the negatively charged surfaces of clay complexes in soil and is not easily available for plants. In contrast, NO_3 with a negative charge is generally present in soil solution and is easily available for plants yet can

be lost by leaching (Gilmour et al. 2003). This property of OAs enhancing nitrification by supplying AOB to in soils lacking nitrification activity will enhance the conversion of tightly bound NH_4 into NO_3 . Consequently, this will improve soil N bioavailability for plants but also the potential risk of NO_3 loss by leaching.

Potentially bioavailable N and rate constants

Mathematical description of N mineralization is important for predicting the ability of soils and OAs to supply N over time and predicting the potential risk of N loss as NO_3 leaching (Pereira et al. 2005; Zarabi and Jalil 2013). The results of the exponential model (Eq. 6) used for describing mineralized N_{min} and to predict potential bioavailable N (N_0) and the mineralization rate constant (k_0) in amendments added to different soils at varying temperatures are presented in Table 4. The model proposed by Stanford and Smith (1972) is for mineralization of soil N, and this explains the lack of fit of this model for some of the treatments with substrate N (Hernández et al., 2002). N_0 ranged from 13.92 to 381.0 mg kg⁻¹ in GWC-amended soils, from 258.64 to 403.0 mg kg⁻¹ in BS-amended soils, from 411.0 to 1001.91 mg kg⁻¹ in CM-amended soils, and from 1,317.47 to 1,681.99 mg kg⁻¹ in U-amended soils. The most likely explanation for low N mineralization potential in GWC-amended soils is the presence of a recalcitrant fraction of N in the GWC. The composting process stabilizes OAs and reduces the fraction of N held in soluble form (Gigliotti et al. 2002). Application of composted amendment to soil will reduce the risk of NO_3 leaching in soil (Flavel and Murphy

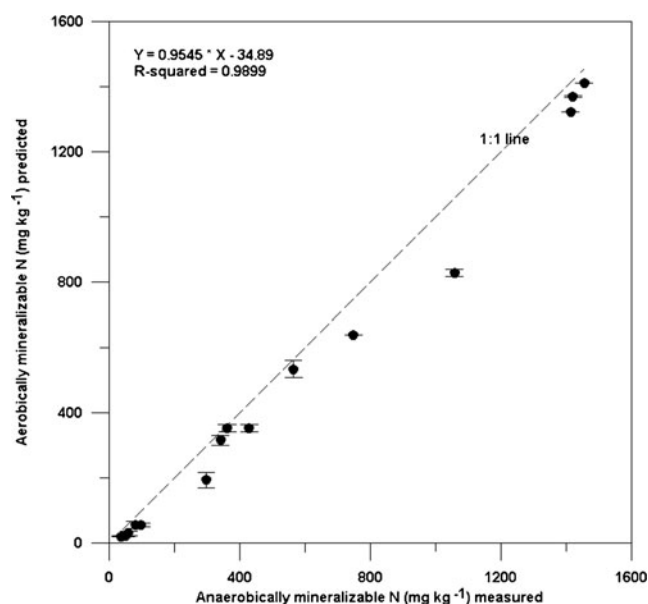


Fig. 5 Relationship between anaerobically mineralizable N ($PAN_{anaerobic}$ —measured) and aerobically mineralizable N (N_{min} —predicted). The vertical bars indicate standard errors

2006). The average k_0 values were 0.053, 0.07, 0.08, and 0.26 day⁻¹ in GWC, BS, CM, and U-treated soils, respectively. Zarabi and Jalil (2013) also observed high k_0 for CM-treated soils compared to other amendments. Low k_0 values obtained for OAs treatments compared to urea treatments indicate that the organic N in OAs is resistant to mineralization (Gil et al. 2011).

Potentially bioavailable N calculated in anaerobic incubation (7 days) ranged from 35 to 98, 340 to 428, 748 to 1,057, and 1,412 to 1,454 mg kg⁻¹ in GWC, BS, CM, and urea-treated soils, respectively. A comparison of potentially bioavailable N measured from anaerobic incubation ($PAN_{\text{anaerobic}}$) (Eqs. 1 and 2) to that predicted from aerobic incubation (N_0) (Eq. 6) was made (Fig. 5), which indicates a strong correlation was evident between anaerobic and aerobic incubations. However, NH_4 released by anaerobic incubation was slightly higher than that predicted from aerobic incubation. The most likely explanations for this result are as follows: firstly, dissimilatory reduction of NO_3 present in the soil to NH_4 (Silver et al. 2001), and secondly, anaerobic conditions may have inhibited nitrification and eventually minimized N loss at nitrous oxide (N_2O) or nitrogen gas (Pett-Ridge et al. 2013). Similar results of slightly higher NH_4 from anaerobic incubation than from aerobic incubation were observed by Bordoloi et al. (2013), Ros et al. (2011), and Soon et al. (2007). Factors such as soil texture, groundwater level, and land use play a major role in determining bioavailable (mineralizable) N under field conditions. Hence, the bioavailable N predicted using aerobic and anaerobic incubation is likely to vary between laboratory incubation and field studies (Ros et al. 2011).

Conclusions

Optimum temperature for N mineralization varies with both soil and N amendment type. Addition of inorganic fertilizers increased mineral N concentration in soils in a rapid phase which may cause loss of N into the environment as ammonia volatilization, NO_3 leaching, or N_2O emission. In contrast, adding OAs delayed the N mineralization rate, and, consequently, there will be a steady supply of N to plants which will in turn reduce the N loss. The major result of this study was that the addition of OAs contributed nitrifiers to soils, triggered nitrification, and increased N bioavailability (NO_3) even in soil lacking nitrification activity. This increase in bioavailable N due to OA being added to soil will support plant growth and may also lead to N loss in the form of NO_3 leaching and N_2O emission. Generally, difference in loss of bioavailable N under field condition was attributed to poor synchronicity between crop demand and N supply. Hence, future studies on OA addition to acidic soil in pot and field experiments with plants are necessary to ensure efficient N use in soils lacking nitrification activity.

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