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Nitrous oxide emission from upland soil amended with different animal manures

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Abstract

The nitrous oxide (N₂O) emission of from arable soil following the application of manure is expected to vary by different animal manure types used. This study was conducted to determine the relationship between the type of animal manure used to amend soil and the amount of N₂O emitted during the cultivation of sweet potato (*Ipomoea batatas*). An additional objective was to study the characteristics of nitrogen (N) and carbon (C) in different animal manures. Composted manures from chickens, cows, and pigs were applied to the soil at rates of 0, 10, and 20 Mg ha⁻¹, respectively. The availability and concentration of N and C varied by manure type. The concentration of NH₄⁺ was greater in pig manure (4638 mg kg⁻¹) than in chicken (551 mg kg⁻¹) and cow manure (147 mg kg⁻¹). The mean cumulative N₂O emission rate across soil application rates was also the highest with pig manure (11.9 kg ha⁻¹ year⁻¹), followed by chicken and cow manure, with emission rates of 10.8 and 10.1 kg ha⁻¹ year⁻¹, respectively. The majority of N₂O measured during the sweet-potato-growing season was produced from aerobic nitrification. Dissolved organic carbon (DOC) concentrations in animal manures did not affect cumulative N₂O emission rates, and no significant relationship was observed throughout the growing season between the concentration of DOC in soil and daily N₂O emission. Cumulative N₂O emission rates depended on the type of animal manure and might be governed by NH₄⁺ concentration, rather than by total N concentration in animal manure type.

Keywords: Denitrification, Greenhouse gas, Nitrification, Nitrogen use, Sweet potato

Introduction

Nitrous oxide (N₂O) is one of greenhouse gases that destroys ozone layer in the stratosphere. Predictive models suggest that atmospheric N₂O may increase from the present concentration of 328 µg/L up to 354–460 µg/L by 2100 (IPCC 2013). Agricultural soil that incorporates nitrogen (N) fertilizer is a major source of N₂O, accounting for approximately 4.3–5.8 Tg N₂O–N year⁻¹, which is 23–31% of the global annual emission [1].

Atmospheric N₂O is emitted from soils by the microorganisms associated with nitrification and denitrification after the application of N fertilizer and organic matter such as manure, compost, and peat moss. The application

of organic matter increases the N and carbon (C) sources necessary for nitrification and denitrification, respectively [2, 3], while microbial activity is enhanced, O₂ is consumed, and anaerobic microsites can develop [4].

Many researchers have reported that the amendment with organic matter could significantly increase N₂O emissions from arable soil [5–13]. Several factors have been identified that affect the N₂O emission from arable soils, including the N fertilization [12–16], pH [17, 18], soil moisture, and temperature [19, 20]. In particular, the content and availability of N and C in organic matter could be one important factor affecting N₂O emission from arable soil. Huang et al. [8] reported the cumulative emission of N₂O was negatively correlated with the C:N ratio in plant residues and positively correlated with dissolved organic carbon (DOC) concentration. As a specific fraction of soil organic carbon, DOC represents the easily degradable portion that is available to microorganisms [21, 22]. Both N₂O-producing processes (nitrification

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and denitrification) are directly controlled by the supply of inorganic N substrates (NH_4^+ and NO_3^-) [23].

Emissions of N_2O from manure-amended fields will vary with animal type due to differences in diet, feed conversions, and management of the manure that result in differences in composition of components such as N and C. Several laboratory-based studies demonstrated the influence of manure type on N_2O emission from soil [24, 25]. However, these experiments were conducted in the absence of plants. Additionally, few studies have been conducted that compare N_2O emission rates from field soil amended with manure from different types of livestock. Therefore, this study was conducted to determine the relationship between the amount of N_2O emitted from soil amended with animal manure (especially, chicken, cow, and pig) under field conditions, and to characterize N and C in the different animal manures.

Materials and methods

Site description and characteristics of manure

The study was conducted on an experimental farm operated by Pusan National University, Miryang, Korea (35°30N, 128°43E). The farm's upland soil belongs to the Bongsan series (fine loamy, mixed, mesic family of Typic Hapludults), and is moderately well-drained with a 7–15% slope. Soil pH was 6.4 and total N concentration was 1.05 g kg^{-1} . Specific physical and chemical properties of the studied soil are shown in Table 1. Precipitation and temperature data were obtained from a weather station in Miryang (Korea Meteorological Administration), located 1 km from the study site.

Composted chicken, cow, and pig manure were used in this study:

Table 1 Selected characteristics of the studied soil

Parameters	Value
Soil separate	
Sand (%)	43.4
Silt (%)	44.5
Clay (%)	12.1
Soil texture	Loam
Bulk density (g cm^{-3})	1.34
pH (1:5 with H_2O)	6.4
Organic matter (g kg^{-1})	15.6
Total nitrogen (g kg^{-1})	1.05
Available phosphate (mg kg^{-1})	142
Exchangeable cation	
K ($\text{cmol}_c \text{ kg}^{-1}$)	0.42
Ca ($\text{cmol}_c \text{ kg}^{-1}$)	5.76
Mg ($\text{cmol}_c \text{ kg}^{-1}$)	1.25

Table 2 Chemical properties of composted animal manures used in this study

Manure	T-N (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	T-C (g kg^{-1})	DOC (mg kg^{-1})
Chicken	18.0	551	4.83	425	1271
Cow	7.90	147	120	309	460
Pig	13.9	4638	3.97	540	204

T-N total nitrogen, T-C total organic carbon, DOC dissolved organic carbon

1. Chicken manure was obtained from a commercial broiler farm (*Synnong Fertilizer*). It contained bark, husk, sawdust, rice bran, dolomitic limestone, and a leavening agent.
2. Cow manure was obtained from a farming association article of incorporation. It contained sawdust, wood chips, and a leavening agent.
3. Pig manure was obtained from a commercial pig farm (*JG Bio*). It contained sawdust.

Specific characteristics of each manure are shown in Table 2.

Field experiment

A field experiment was conducted to determine if animal manure type and application rate impacted N_2O emission from arable soil. Three composted animal manures (chicken, cow, and pig) were applied to small plots ($2.5 \text{ m} \times 4 \text{ m}$) at rates of 0, 10, and 20 Mg ha^{-1} , respectively. All manures were incorporated into the soil using moldboard plows 2 weeks before transplanting sweet potato (*Ipomoea batatas*) to the field on May 20, 2016. Plant spacing within rows was 20 cm. Inorganic fertilizers in the form of urea, fused phosphate, and potassium sulfate were applied to all plots 2 days after transplanting on June 6, 2016. Experimental plots were arranged in a randomized complete block design with four replications. Sweet potatoes were harvested on October 1, 2016. Weeds were removed from plots by hand throughout the growing season.

Measurement of N_2O emission

Soil surface N_2O flux was measured from 44 plots (control, three animal manures, and two application rates replicated four times) once every 2 weeks during the sweet-potato-growing season (May 2016 through October 2016), and once every month during the fallow season (November 2016 through May 2017) using the static chamber technique [26]. Static chambers made of PVC pipe (24.8 cm diameter \times 15 cm height) were installed at the center of each plot before application of

animal manures and inorganic fertilizers. The chambers remained in place during completely year round both the growing season and the fallow season. Before taking gas samples, a PVC cap with a vent tube and sampling port were placed on the anchors. Gas that accumulated inside the chamber was collected with a 20 ml syringe at 0, 20, and 40 min. The gas samples were transferred from the syringe into 12 ml evacuated glass vials (Exetainer® 12 ml vial-evacuated 838 W, Labco, Wales, UK.) sealed with butyl rubber septa. Concentrations of N₂O were measured using a Gas Chromatograph (Shimadzu GC-2010 plus) with a Porapak Q (80/100 mesh) column and an electron capture detector. The highest grade of prefiltered N₂ was the carrier gas. Calibration was routinely performed using dilutions of a certified gas standard mix (Scott Specialty Gases, Plumsteadville, PA, USA). For quality assurance, four replicated samples were injected and measured.

Daily gas flux (F , g ha⁻¹ day⁻¹) was calculated as the follows:

$$F = (\Delta g/\Delta t) \times d \times (273/T) \times (V/A) \times k \times a$$

where $\Delta g/\Delta t$ is the linear change in gas concentrations inside the chamber (g m⁻³ min⁻¹), d is gas density (g m⁻³) at 273 K and 0.101 MPa pressure, T is the air temperature (K) within the chamber, V is the chamber volume (m³), A is the surface area circumscribed by the chamber (m²), k (min day⁻¹) is the time conversion coefficient, and a (10,000 m² ha⁻¹) is the area conversion coefficient. Soil moisture and temperature were measured on a volumetric basis using a 5TE moisture sensor (Decagon Devices, Pullman, WA, USA) every 2 h during whole experiment period. Air temperature was measured in the chamber at the time of gas sampling and used to calculate N₂O fluxes.

Physical and chemical analysis

The water-filled pore space (WFPS, %) was calculated every day for a year using the equation below:

$$\text{WFPS} = (\theta/\text{soil porosity}) \times 100$$

where θ is the volumetric moisture content (m³ m⁻³). Soil porosity (m³ m⁻³) was calculated using a particle density value of 2.65 Mg m⁻³, and soil bulk density values measured once every month of the study period. Averaged daily volumetric moisture was measured at 5 cm soil depth using a soil moisture sensor (WT1000B, RF sensor, Seoul, Korea) installed in each plot.

Soil samples were also collected from each plot once in every month during the sweet-potato-growing season for analysis of inorganic N (NH₄⁺ and NO₃⁻). Air-dried soil samples (5 g) were extracted using 50 mL of 2 M KCl.

After shaking for 1 h, the samples were filtered through Whatman no. 5 filters and the extracts were analyzed using automated colorimetry [27, 28]. In addition, 5 g of wet soil samples were centrifuged at 6000 rpm for 15 min to remove the pore water. The supernatant liquid was separated using a 0.45- μ m membrane filter and was then measured for DOC concentration [29]. DOC was quantified with a total organic carbon (TOC) analyzer (Model TOC-VCPN; Shimadzu, Japan).

Nitrogen-use of sweet potato

N use of sweet potato was determined using manure N removal (MNR) and apparent N recovery (ANR).

The total N removal was calculated by multiplying the total dry matter yield of sweet potato by the N concentration on dry matter base. The MNR was calculated by subtracting the total N removal from the control (0 Mg ha⁻¹) from total N removal from the manure-applied treatments (10 or 20 Mg ha⁻¹) [30].

ANR (%) for each harvest was calculated as [30]:

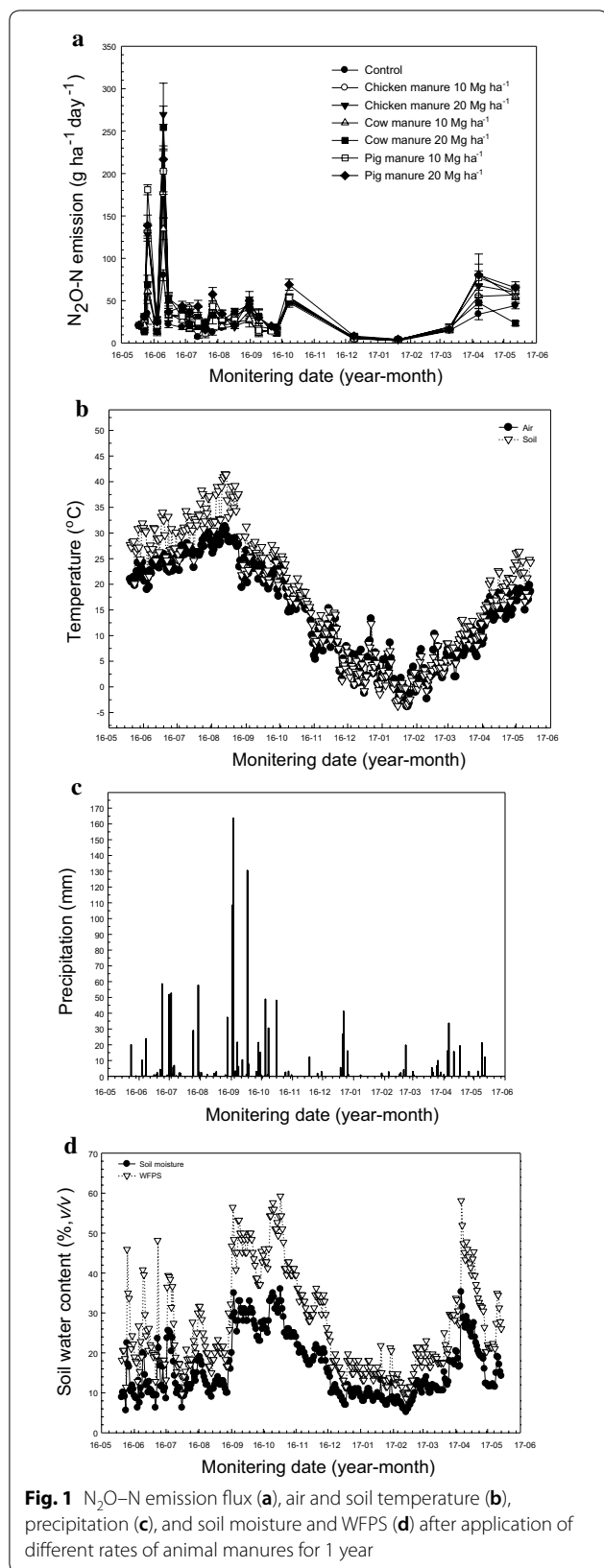
$$\text{ANR} = [(\text{mass of N removed at application rate} \\ - \text{mass of N removed at control}) \\ / \text{mass of N applied at application rate}] \times 100$$

Statistical analysis

All statistical analyses were performed using Statistix version 9.0 (Statistix 2008). Mean values of cumulative N₂O emission, inorganic N and DOC concentrations in soil, MNR, and ANR were analyzed by conducting pairwise comparisons. A least significant difference was used to separate mean effects when the appropriate F test was significant ($p < 0.05$).

Results and discussion

Daily N₂O flux was measured for 1 year (Fig. 1a) to examine the association of N₂O emission with application rate and type of animal manure. N₂O fluxes peaked in May and June and then decreased dramatically from July onward. Similar findings were observed in dairy manure and inorganic fertilizer application in corn and soybean rotation [31]. The first and second N₂O peaks appeared soon after application of animal manures and inorganic N fertilizer, respectively. Animal manures were applied on 20th May and the first N₂O peak took place 6 days later on 26th May. Although the mineralization rate of organic N in manure depends on aeration, moisture condition, and microbial activity in the soil, the release of inorganic N (NH₄⁺ and NO₃⁻) from manure usually takes a few weeks [32, 33]. The previous studies have demonstrated the combination of organic manure and urea showed a significant positive effect on N₂O flux [34, 35]. Besides, N₂O emission tended to be larger in receiving nitrogen



source treatments than in the unfertilized treatment [36, 37]. Hence, higher soil NH_4^+ and NO_3^- availability proceeded as substrate of microbial formation of N_2O by the nitrification and denitrification processes [38]. Therefore, the source of N_2O emission in the first observed peak might be attributed to inherent inorganic N in animal manure [33, 39]. As shown in Table 2, concentrations of NH_4^+ and NO_3^- in animal manures ranged from 147 to 4638 $mg\ kg^{-1}$ and 3.97 to 120 $mg\ kg^{-1}$, respectively. Pig manure contained the highest concentration of NH_4^+ among the studied animal manures, and this contributed to the greatest N_2O flux measured following pig manure treatment on 26th May (Fig. 1a). Urea was applied to the soil on 6th June and a second N_2O peak occurred 4 days later on 10th June. This peak was greater than the first observed peak associated with manure application and might be due to the more rapid release of inorganic N from urea than from manure.

Daily climate data, including temperature, precipitation, and soil water content, were measured for 1 year to examine the relationship between climate data and N_2O flux (Fig. 1b–d). No significant increase was seen in N_2O flux when air and soil temperatures were highest in August, but fluxes were dramatically decreased when air and soil temperatures were lowest (in December and January). Nitrification and denitrification processes are slowed when the soil temperature is below 5 and 2 $^{\circ}C$, respectively [32]. In this study, soil temperature was below 5 $^{\circ}C$ from December to March and below 2 $^{\circ}C$ from January to February (Fig. 1b).

Precipitation and soil water content affected daily N_2O fluxes during the non-growing season. N_2O emissions peaked twice, once in November and again in April, even without the addition of N to the soil. Interestingly, WFPS slightly exceeded 60% only in November and April (Fig. 1d). Similar results were observed by Dobbie and Smith [40] who reported that peak of N_2O fluxes only took place at >60% of WFPS and significant relationship between WFPS and N_2O flux was found. The soil's WFPS could be a useful indicator of the relative potential for aerobic and anaerobic microbial activity in soil [41]. Microbially driven nitrification and denitrification are particularly influenced by WFPS [42]. High O_2 concentrations are known to suppress the activity and synthesis of the denitrification reductases. The N_2O reductase is thought to be the most sensitive to O_2 [43].

Mean cumulative N_2O emissions were highest from soil amended with pig manure across application rates (Table 3). This might be attributed to the higher NH_4^+ concentrations measured in pig manure compared with chicken and cow manure (Table 2). As shown in Fig. 2a–c, relatively higher concentrations of NH_4^+ in soil amended with pig manure were maintained during

the growing season when compared with soil amended with chicken and cow manure. Daily N₂O fluxes were higher in soil amended with pig manure from 6th July to 7th October than in soil treated with chicken and cow manure (Fig. 1a). In contrast, NO₃⁻ concentrations were not higher in soil amended with pig manure during the growing season (Fig. 2d–f). The relationship between daily N₂O emission and inorganic N concentration in soil during the growing season (Fig. 3) implies the majority of N₂O measured in this study could be from aerobic nitrification. The relationship between daily N₂O emissions and the NH₄⁺ concentration in soil was significantly positive in this study, but no significant relationship was seen between daily N₂O emission and NO₃⁻ concentration in soil. Nitrification is the primary pathway of N₂O emission

when soil's WFPS ranges from 30 to 60%, while denitrification is the major process when soil's WFPS is >60% [44, 45]. The WFPS of the soil we studied was mostly less than 60% during the growing season; therefore, we assumed the majority of N₂O was produced from aerobic nitrification. In addition, N₂O emissions resulting from nitrification during the growing season might be governed by the NH₄⁺ concentration in animal manure rather than by the total N concentration in this study.

DOC released from composted animal manure could be a source of available C for use by microorganisms in the denitrification process in soil. Haung et al. [8] reported that cumulative emission of N₂O was positively correlated with DOC concentration in soil. As shown in Fig. 4, mean values of DOC in soil increased as the manure application rate increased. DOC concentration was relatively higher in soil amended with chicken manure than in soils amended with cow and pig manure. This might be attributed to higher DOC concentrations in chicken manure than in cow and pig manure (Table 2). DOC concentration in manures, from the highest to the lowest, was chicken > cow > pig. However, cumulative N₂O–N emission rates ranked from the highest to the lowest were pig > chicken > cow (Table 3). This implied that DOC concentration in animal manures and soil might not affect N₂O soil emissions, and this lack of a relationship was confirmed by correlation coefficient (*r*=0.0004) (data not shown). High O₂ content in the soil we studied might alleviate the effect of DOC on the denitrification process.

Table 3 Cumulative N₂O–N emission from soils amended with different rates of animal manures

Application rate (Mg ha ⁻¹)	Animal manure		
	Chicken	Cow	Pig
Cumulative N ₂ O–N emission (kg ha ⁻¹ year ⁻¹)			
0	7.96 ^c	7.96 ^b	7.96 ^c
10	11.4 ^b	10.8 ^a	13.1 ^b
20	13.1 ^a	11.5 ^a	14.7 ^a
Mean ¹	10.8 ^B	10.1 ^C	11.9 ^A

¹ Mean: mean value across application rates. Upper and lower case letters are for column and row comparison. Values with the same letter within a column or row are not significantly

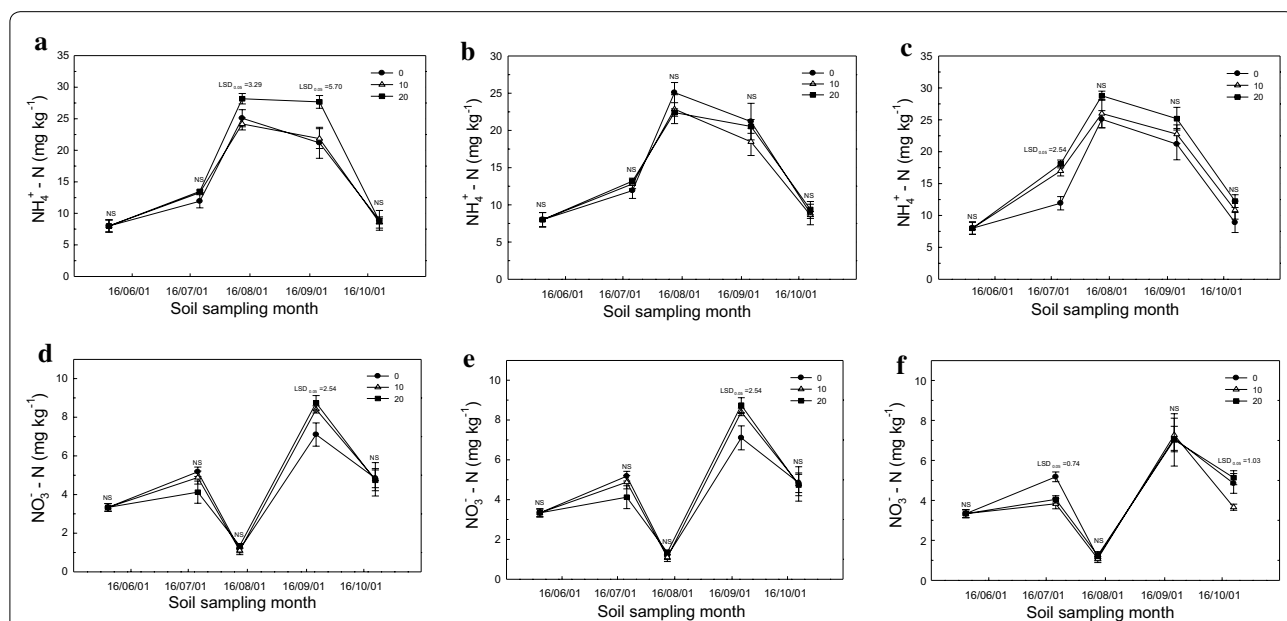
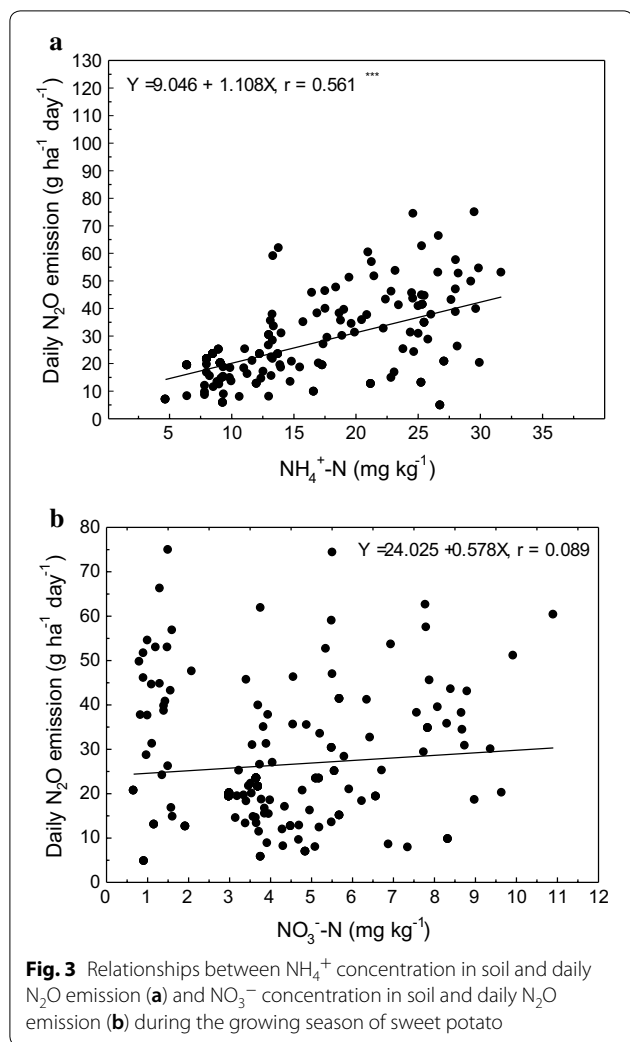
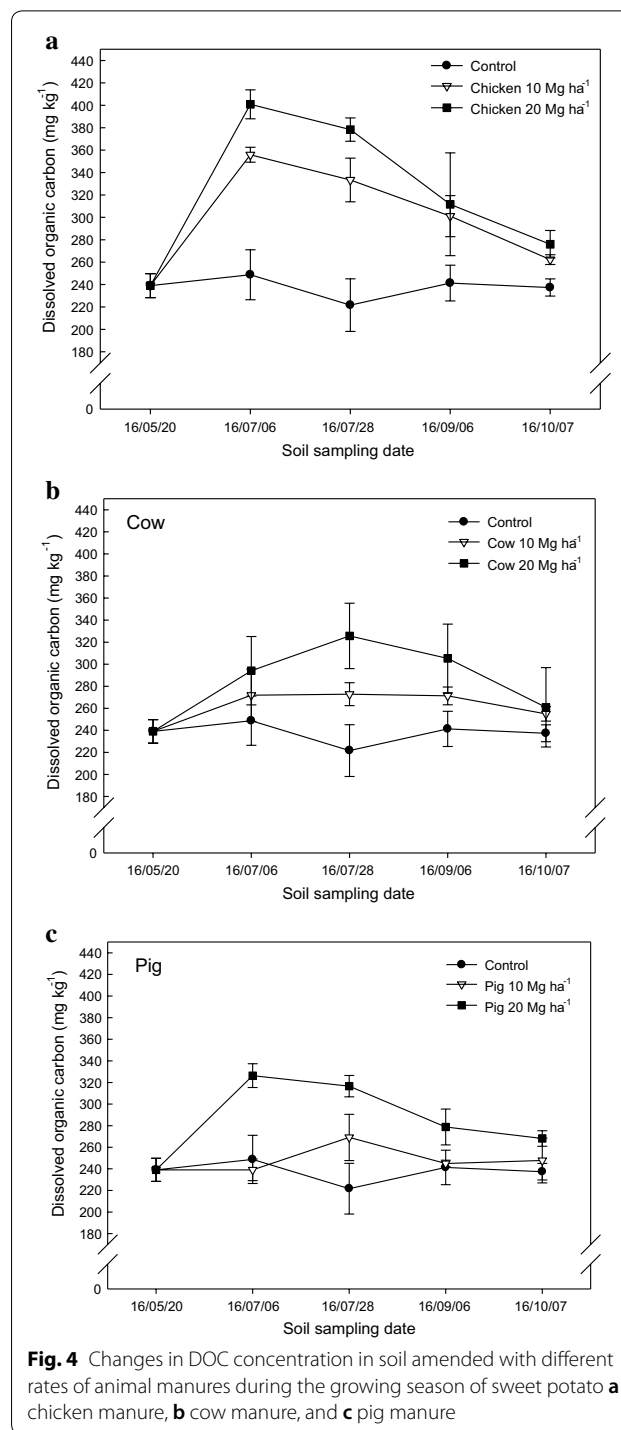


Fig. 2 Changes in inorganic N concentrations in soil amended with different rates of animal manures during the growing season of sweet potato **a**, **d** chicken manure, **b**, **e** cow manure, and **c**, **f** pig manure



As noted earlier, our soil had a WFPS typically <60% throughout the growing season (Fig. 1d). Therefore, we assumed the majority of N_2O was produced from aerobic nitrification rather than by denitrification. Even though the application of manure provided a C source for microbial denitrification, high O_2 concentrations in soil pores might have suppressed the activity and synthesis of the denitrification reductases [43].

Cumulative N_2O emissions during 1 year significantly increased with increasing application rate of all animal manures (Table 3). This increase might be related to change in MNR and ANR by sweet potato with increasing manure application rates. Surplus N in the soil beyond plant requirements might have more opportunity to be converted into N_2O . There was no significant increase in MNR with increasing application rates from 10 to 20 Mg ha^{-1} in all animal manures (Fig. 5a–c). In addition, ANR in chicken manure and pig manure treatments significantly decreased with increasing application



rates (Fig. 5d, f), but there was no significant difference observed between all application rates of cow manure (Fig. 5e). These results mimicked the cumulative N_2O emissions shown in Table 3. Cumulative N_2O emission significantly increased with increasing application rates of chicken and pig manure from 10 to 20 Mg ha^{-1} , but significant increases were not observed

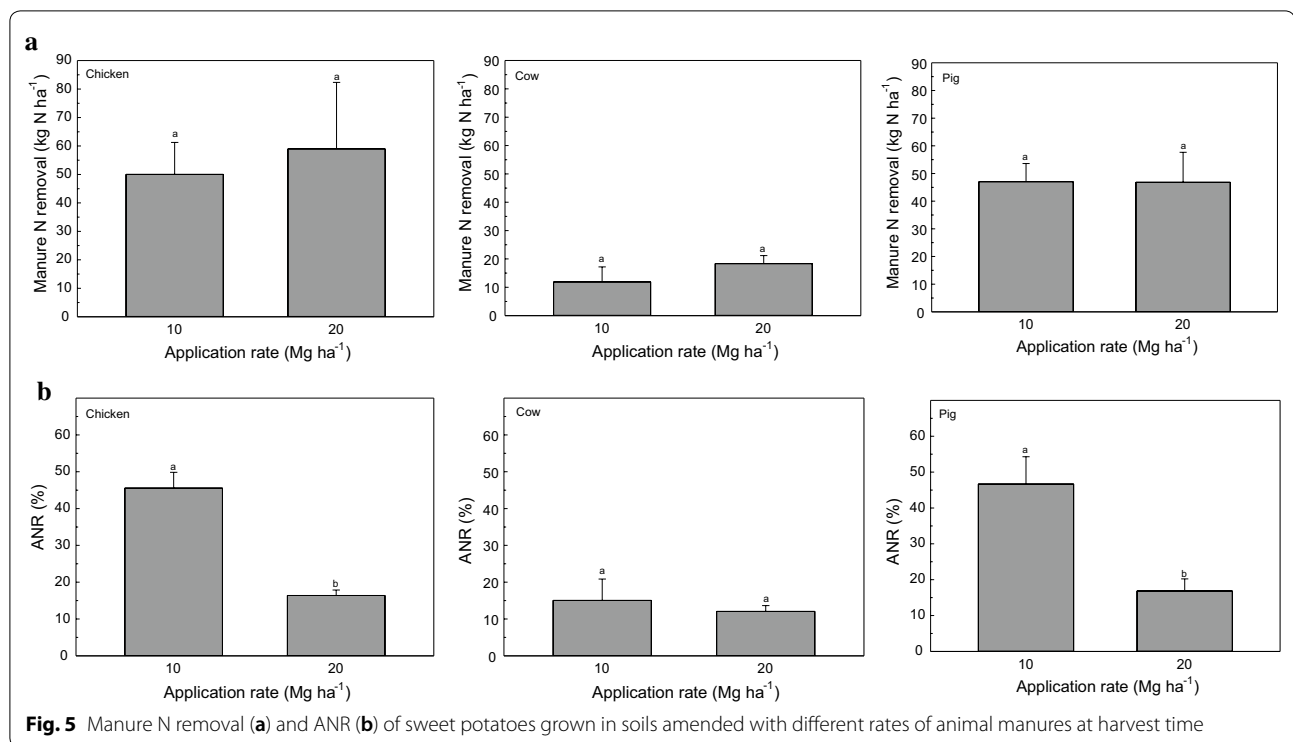


Fig. 5 Manure N removal (a) and ANR (b) of sweet potatoes grown in soils amended with different rates of animal manures at harvest time

when the application rate of cow manure was increased. This implies that excess N in soil amended with animal manures may be lost through N₂O emission.

Authors' contribution

SUK, CR, HHL, and HJP carried out gas and soil sampling, gas and soil analyses, and data organization. SUK, SK, ESJ, and COH participated in interpreting the obtained results and organizing the manuscript. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no competing interests.

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