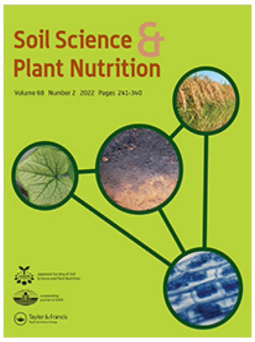

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ORIGINAL ARTICLE

Nitrous oxide and carbon dioxide emissions from two types of soil amended with manure compost at different ammonium nitrogen rates

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ABSTRACT

Ammonium nitrogen (NH_4^+-N) content in soil is a key factor affecting nitrous oxide (N_2O) emissions due to its role as a primary substrate of nitrification. This study aimed at investigating the effects of different application rates of NH_4^+-N on N_2O and CO_2 emissions from two different types of manure compost-amended soil, along with analysis of relative abundances of *narG* and *nosZ* genes under aerobic conditions. Laboratory experiments were conducted using Kochi and Ushimado soils amended with mixed compost (MC: mixture of cattle, poultry, and swine manure) or cattle manure compost (CC) at 3% (dry weight basis). In no compost- and compost-amended soils, $(NH_4)_2SO_4$ was added as a solution equivalent to 160, 200, and 400 mg-N kg^{-1} of soil. Soil samples were aerobically incubated at 70% water-holding capacity (WHC) and 25°C. Emissions of N_2O and CO_2 were measured on days 0, 3, 7, 15, 21, 28, and 42. The abundances of *narG* and *nosZ* genes in Kochi (day 7) and Ushimado (day 21) soils were estimated using qPCR tests. Emissions of N_2O and CO_2 were higher in MC-amended soil because of higher mineral N content and lower C/N ratio of MC than those of CC, regardless of NH_4^+-N rates. Emissions of N_2O and CO_2 were higher in compost-amended Kochi soil due to higher mineral N, total N and C, and clay contents, and possibly because of higher water-filled pore spaces than those in Ushimado soil at the same WHC. In both soils with CC and no compost, raising NH_4^+-N rate from 160 to 200 increased N_2O emissions due to stimulation of nitrification. In contrast, increasing NH_4^+-N rate from 200 to 400 decreased N_2O and CO_2 emissions except for N_2O emissions in MC- and CO_2 emissions in CC- and no compost-amended Ushimado soil possibly due to osmotic stress on microorganisms and limited C availability. Emissions of N_2O were positively related to *narG* gene copy numbers in Kochi soil ($R^2 = 0.78$) due to high N and C contents. Our study revealed that NH_4^+-N rate 400 suppresses N_2O and CO_2 emissions from manure compost-amended soil under aerobic conditions.

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KEYWORDS
Carbon dioxide; compost; NH_4^+-N rate; nitrous oxide; osmotic stress

1. Introduction

Nitrous oxide (N_2O) is a major greenhouse gas with significant global warming potential and direct impacts on stratospheric ozone depletion (Deng et al. 2016). The main anthropogenic processes producing N_2O emissions include the application of organic and inorganic fertilizers containing nitrogen (N) to agricultural soils and combustion of fossil fuels (Signor and Cerri 2013). In agricultural soils, biological N-fixation, crop residue, and sewage sludge are other sources of N, which can have profound impacts on N_2O emissions (Mosier et al. 1998). Emissions of N_2O have increased by 30% over the past four decades to 7.3 Tg-N per year, and two-thirds of total anthropogenic N_2O emissions have come from agricultural soils (Tian et al. 2020; Wang et al. 2021).

N_2O in soil is produced by microbial transformation of mineral N (ammonium N: NH_4^+-N and nitrate N: NO_3^--N) through a series of processes of nitrification and denitrification (Deng et al. 2016). Nitrification is an aerobic process that is primarily regulated by autotrophic and aerobic bacteria (ammonia- and nitrite-oxidizing bacteria) to oxidize ammonium to NO_3^- (Parton et al. 2001). Denitrification is a chained reductive reaction of NO_3^- to N_2, generating intermediate products of NO_2^-, NO, and N_2O under anaerobic conditions by a wide range of heterotrophic bacteria, fungi, and archaea (Otte et al. 2019) and considered to be a main N_2O formation pathway in soils (Sanchez-Garcia et al. 2014). In addition, there is increasing evidence that aerobic denitrification may play an important role when certain bacteria perform NO_3^- respiration in the presence of O_2 (Bateman and Baggs 2005). In general, an abundance of *nosZ*-bearing bacteria promotes the reduction of N_2O to N_2, thereby decreasing the overall emissions of N_2O, while *narG*, which encodes membrane-bound NO_3^- reductase is responsible for increased N_2O emissions (Nie et al. 2016; Lazcano, Zhu-Barker, and Decock 2021).

Factors such as moisture, temperature, mineral N, decomposable organic matter, microorganism populations, soil texture, pH, and drainage capacity affect the production and emissions of N_2O (Boeckx, Beheydt, and Cleemput 2005; Signor and Cerri 2013). The effects of moisture, temperature, and organic matter on N_2O emissions have been examined as follows. Soil temperature and water content directly affect the production and consumption of N_2O through their effects on

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the metabolic activity of microorganisms (Luo et al. 2013). A combined effect of moisture and temperature was explored by Kurganova and de Gerenyu (2010), who stated that N_2O emission rates from wet soils increased significantly in temperatures ranging from 10 to 15°C and from 20 to 25°C. Nitrification and denitrification processes are directly affected by organic matter, and therefore influence N_2O emissions (Mosier 1994). Soil texture and drainage capacity affect gas diffusivity controlling the availability of O_2 in soil for microbial processes, consequently influencing N_2O emissions (Zhu et al. 2020).

The application of mineral N affects nitrification and denitrification, and thereby N_2O emissions (Dobbie and Smith 2003) and NH_4^+-N is a preferred N form for most bacteria and fungi (Muller et al. 2006). Relatively higher N_2O emissions were observed from ammonium sulphate [$(NH_4)_2SO_4$]-amended arable soil due to the stimulation of nitrification (Velthof, Kuikman, and Oenema 2003). Hoang and Maeda (2018) studied the interaction of NH_4^+-N and temperatures and found that NH_4^+-N application at high temperatures suppressed N_2O emissions. Darrah, White, and Nye (1986) investigated physiological and microbiological changes in a fine sandy loam soil following the application of ammonium salts and observed inhibited nitrification due to enhanced osmotic pressure. Effects of osmotic potential on N_2O emissions were explored by Low, Stark, and Lynn (1997) under elevated ammonium contents in a sandy loam soil. Microbial immobilization of N was observed by Sun et al. (2016) under the application of ammonium fertilizer at an annual rate of 50 kg-N ha^{-1} $year^{-1}$. Other studies discussed the effects of NH_4^+-N derived from organic amendment on N_2O emissions. Kim et al. (2019) observed a positive relationship between daily N_2O emissions and soil contents of NH_4^+-N derived from animal manure. Similarly, Li et al. (2017) found a positive correlation between N_2O emissions and NH_4^+-N contents of cattle manure-amended soil. All these studies reported that a single application of mineral N fertilizer or organic amendment significantly affected N_2O emissions.

However, N_2O emissions can be caused by the combined application of ammonium fertilizer and organic amendment because NH_4^+-N is derived from both inorganic fertilizer and the organic amendment. Katoh, Hayashi, and Morikuni (2008) and Jin et al. (2010) observed increased NH_4^+-N contents following the combined application of ammonium fertilizer and cattle manure compost. The addition of mineral N fertilizer to composted swine manure-amended soil induced N_2O emissions 2.9 times higher compared with mineral N fertilizer alone due to higher availability of N and labile organic carbon (C) for nitrifiers and denitrifiers (Qiao et al. 2014). In contrast, Liu et al. (2020) found that the combined application of 25% raw manure-N plus 75% of NH_4^+-N mitigated N_2O emissions with compared to a single application of ammonium fertilizer, but no yield reductions occurred in a maize and wheat rotation system. Cai, Ding, and Luo (2013) also observed decreased N_2O emissions after the application of compost (a mixture of wheat straw, oil cake, and cotton cake) and inorganic N fertilizer together because of the lower amount of mineral N released during the decomposition of compost. Apart from the contradictions in available studies, many of them were field experiments conducted on a single type of soil and did not compare the impacts of NH_4^+-N applications on different types of soil. Therefore, the effects of the addition of NH_4^+-N to different types of soil amended with manure compost remain uncertain.

Organic soil amendment can impact C and N dynamics to promote soil CO_2 emissions (Grave et al. 2015). Ray et al. (2020) found higher cumulative CO_2 emissions from soils amended with chicken manure than those amended with cow manure. In contrast, Termorshuizen et al. (2004) found that C in compost can be sequestered for a long time because labile organic compounds can be protected by hydrophobic humic substances, resulting in reduced CO_2 emissions. Sainju et al. (2012) found that urea-N application increased CO_2 emissions from soil because of increases in the crop yield and the residue in soil. Sosulski et al. (2020) suggested that NH_4^+-N increased CO_2 emissions from soil as a result of availability of N for decomposers of soil organic matter. Ding et al. (2007) revealed that N fertilization resulted in the reduction of CO_2 emissions from soil. Gagnon et al. (2016) found that the application of nitrate fertilizer at a rate of 150 kg-N ha^{-1} reduced CO_2 emissions by 22% than those of ammonium fertilizer. A single application of urea had no significant effect on soil CO_2 emissions in an experiment done by Lin et al. (2021), indicating that oilseed rape cake was the main source of CO_2 production in the combined application with urea. However, more detailed information about the effects of NH_4^+-N on CO_2 emissions from manure compost-amended soil is rarely available.

Studies discussing the effects of NH_4^+-N additions on N_2O and CO_2 emissions from manure compost-amended soil are mandatory to establish strategies to mitigate N_2O and CO_2 emissions from fertilized agricultural soil. Furthermore, the relation of key denitrifying microbial genes to N_2O emissions from soil amended with manure compost and supplemented with NH_4^+-N has not been analyzed in detail. Therefore, the objective of this study was to investigate the effects of different application rates of NH_4^+-N on N_2O and CO_2 emissions from two contrasting types of manure compost-amended soil under aerobic conditions. Further, we analyzed the relative abundances of *narG* and *nosZ* denitrifying genes responsible for N_2O emissions from manure compost-amended soil. These two genes were selected for the analyses because they are the key genes controlling N_2O or N_2 emissions via NO_3^- reduction to N_2O or N_2O reduction to N_2.

2. Materials and methods

2.1. Soil sampling and preparation

Kochi and Ushimado soils with contrasting initial properties were used for laboratory incubation experiments under aerobic conditions. Kochi soil was collected from a vegetable greenhouse at the Faculty of Agriculture and Marine Science, Kochi University, Japan, in April, 2019. Before soil sampling, 18 kg m^{-2} of bark compost was applied to the field for the last five years. Ushimado soil was collected from a paddy field in Ushimado, Okayama Prefecture, Japan, in April, 2016, in which no manure or compost had been amended at least in the past five years. Kochi and Ushimado soils belong to eutric fluvisol and dystric gleysol soil groups (FAO), respectively (Cultivated soil classification committee 1995). Soil samples were air-dried, passed through a 2-mm sieve, and stored until the experiments. Soil texture and cation exchange capacity (CEC) were determined by the sieve and pipette method (Kettler, Doran, and Gilbert 2001) and the Schollenberger method at pH 7 (Sparks et al. 1996), respectively. Total N and C contents in soil were measured by the dry combustion method with a CN coder (MT-700, Yanaco, Japan). Kochi soil showed higher electrical conductivity (EC), CEC, and total N, C, and mineral N contents compared to Ushimado soil. Kochi soil was a sandy clay loam and Ushimado soil was a sandy loam in texture (Table 1). These two soil types were selected to reflect the effects of different properties of soil (total C, N, and mineral N contents) on N_2O and CO_2 emissions after being amended with manure compost at different NH_4^+-N rates.

2.2. Manure compost collection and preparation

Two types of livestock manure compost, cattle manure compost (CC) and mixed compost (MC) were used as soil amendments. The CC containing sawdust was produced at the Research Institute for Livestock Science, Okayama Prefectural Agriculture, Forestry and Fisheries Research Center. The MC (a mixture of cattle, poultry, and swine manure at a rate of 6: 3: 1 on weight basis supplemented with sawdust, rice husk, and bark during the composting process) was produced at the Tetta Town Composting Center, Okayama. Both CC and MC were air-dried and ground into fine powders to assure uniform distribution in soils. Total N and mineral N contents were higher, and the total C content was slightly lower in MC than those in CC (Table 1). The higher C/N ratio and lower N contents of CC than those of MC indicate that CC was more resistant to decomposition and N mineralization.

2.3. Laboratory incubation experiments

Kochi and Ushimado soils were uniformly mixed with two compost types separately at 3% (by weight), considering a common organic matter application rate (approximately 30 Mg ha^{-1}) to agricultural soils. No compost-amended control soil was used as a reference for the comparisons with compost-amended samples in each soil. The application rates of NH_4^+-N to CC-, MC-, and no compost-amended soil were adjusted to 160, 200, and 400 mg-N kg^{-1} of soil (corresponding to 160, 200, and 400 kg-N ha^{-1}, respectively) using $(NH_4)_2SO_4$ as a solution, except Kochi soil, which was amended with MC at 160, the original NH_4^+-N content. These application rates are common for fertilized greenhouse vegetables in Japan (Nishio 2001). Moisture contents of all treatments were adjusted to 70% water-holding capacity (WHC) (equivalent to water-filled pore space (WFPS) 69–72% for Kochi soil and 44–46% for Ushimado soil, which differed due to bulk densities and water absorption by compost particles). The initial WHC levels were maintained during the incubation period by adding deionized water to compensate the loss by evaporation. The treatments were named to K, KCC, and KMC for no compost-, CC-, and MC-amended Kochi soil and U, UCC, and UMC for no compost-, CC-, and MC-amended Ushimado soil followed by NH_4^+-N rates 160, 200, and 400 (−160, −200, and −400).

The experiment consisted of 18 treatments as described above in triplicate (n=3). Soil samples (10 g) were contained in 100-mL glass bottles covered with polyethylene film (0.02 mm thickness) to minimize evaporation, while maintaining gas exchange. The bottles were incubated at 25°C for 42 days and N_2O and CO_2 fluxes were determined on days 0 (incubation start date), 3, 7, 15, 21, 28, and 42.

Table 1. Initial properties of two types of soil and compost.

	Soil type		Compost type	
	Kochi soil	Ushimado soil	Cattle manure compost	Mixed compost
Total N (g-N kg^{-1})	3.9	1.4	16.3	26.0
Total C (g-C kg^{-1})	62.6	14.7	301.6	273.3
C/N ratio	15.9	10.2	18.5	10.5
NH_4^+-N (mg-N kg^{-1})	109.5	9.5	30.5	893.2
NO_3^--N (mg-N kg^{-1})	463.7	19.2	18.1	58.6
pH (H_2O)	5.5	8.5	9.4	9.2
EC (dS m^{-1})	0.8	0.1	7.6	7.5
CEC ($cmol_c$ kg^{-1})	28.3	7.5	-	-
Texture	Sandy clay loam	Sandy loam	-	-

NH_4^+-N: Ammonium N content, NO_3^--N: Nitrate N content, EC: Electrical conductivity, CEC: Cation exchange capacity

2.4. Gas sampling and flux measurement

The incubation bottles were flushed for two minutes with atmospheric air at 50 mL s^{-1} with a mini-pump (MP-2 N, Shibata, Japan) to exchange gas inside the bottles with the ambient air prior to collecting gas samples. The bottles were sealed with butyl rubber septa tightened with aluminum caps for 2 h before collecting gas samples for N_2O and CO_2 measurements. Head space gas samples were injected to gas chromatographs (GC-8A, Shimadzu, Japan) equipped with an electron capture or thermal conductivity detector for N_2O and CO_2, respectively. The gas concentration was determined with respect to a calibration done using two standard gas samples separately for N_2O (1.512×10^{-6} and 0.298×10^{-6} or 20.67×10^{-6} m^3 m^{-3}) and CO_2 (0.35×10^{-3} and 2.19×10^{-3} m^3 m^{-3}). Gas emissions were calculated using the following equation:

N_2O or CO_2 emissions (μg-N or -C kg^{-1} h^{-1}) $= \rho\times C\times10^{-6}\times(V_g+V_L\times\alpha)\times273/(W\times(273+T))/t$

where ρ is the density of N_2O-N (1.25 kg m^{-3}) or CO_2-C (0.5357 kg m^{-3}), C (m^3 m^{-3}) is the concentration of N_2O or CO_2, V_g (m^3) is the volume of the headspace, V_L (m^3) is the volume of the liquid phase, α is the Bunsen absorption coefficients for N_2O (0.539) and CO_2 (0.614) at 25°C, W (kg) is the oven-dried weight of soil, T is the incubation temperature (25°C), and t is an incubation period (2 h). Concentrations of N_2O and CO_2 in the ambient air were deducted from each measured concentration.

Table 2. Cumulative N_2O and CO_2 emissions in the incubation experiment.

		Cumulative N_2O emissions (mg-N kg^{-1})			Cumulative CO_2 emissions (g-C kg^{-1})		
		NH_4^+-N rates (mg-N kg^{-1})					
Soil type	Treatment	160	200	400	160	200	400
Kochi	K-	$13.6 \pm 2.6^{g\dagger}$	121.6 ± 18.1^{d}	62.8 ± 8.4^{e}	4.9 ± 0.2^{de}	5.5 ± 0.5^{cd}	4.5 ± 0.2^{e}
	KCC-	24.9 ± 7.5^{fg}	140.3 ± 10.5^{d}	46.5 ± 1.2^{ef}	5.8 ± 0.3^{c}	6.0 ± 0.1^{c}	4.3 ± 0.3^{e}
	KMC-	412.6 ± 3.3^{a}	260.6 ± 21.1^{b}	196.0 ± 4.4^{c}	10.4 ± 0.3^{a}	10.6 ± 0.9^{a}	7.8 ± 0.5^{b}
Ushimado	U-	3.9 ± 0.3^{e}	30.1 ± 2.1^{c}	18.9 ± 1.9^{d}	2.6 ± 0.5^{f}	2.8 ± 0.2^{ef}	2.6 ± 0.3^{f}
	UCC-	11.4 ± 0.3^{d}	46.7 ± 4.8^{b}	12.2 ± 2.2^{d}	3.3 ± 0.4^{d}	3.3 ± 0.1^{d}	3.2 ± 0.3^{ed}
	UMC-	58.7 ± 6.5^{a}	54.0 ± 2.7^{ab}	49.6 ± 9.2^{b}	6.6 ± 0.3^{a}	5.7 ± 0.1^{b}	4.9 ± 0.1^{c}

†Means with the same letters are not significantly different at $p > 0.05$ within the same soil type × gas.

K: No compost-amended Kochi soil, KCC: Cattle manure compost-amended Kochi soil, KMC: Mixed compost-amended Kochi soil, U: No compost-amended Ushimado soil, UCC: Cattle manure compost-amended Ushimado soil, and UMC: Mixed compost-amended Ushimado soil. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}).

2.5. DNA extraction and quantification of relative abundances of *narG* and *nosZ* genes

The functional genes, *narG* and *nosZ*, were analyzed to identify the relation between relative abundances of those genes and N_2O emissions from compost-amended soil under aerobic conditions. The samples on day 7 of Kochi soil and day 21 of Ushimado soil treatments were used for DNA and functional gene analysis. These sampling days were selected because maximum N_2O emissions appeared within one and three weeks of incubation in Kochi and Ushimado soils, respectively. In addition, the original Kochi and Ushimado soils were also analyzed for reference.

Soil DNA was extracted from 0.4 g of moist soil (triplicates) using a DNA extraction kit (FastDNA Spin Kit for Soil, MP Biomedicals, USA). DNA Clean and Concentrator^{TM}-25 (Zymo Research, USA) was used to purify the eluted DNA (80 µL). Purified DNA was quantified using a spectrophotometer (NanoDrop One, Thermo Scientific, Japan).

For quantification of *narG* and *nosZ* functional genes, a qPCR system (Step One Plus Real-Time PCR, Applied Biosystems, USA) was used. Purified soil DNA samples were subjected to duplicate independent amplifications and genes were quantified according to the method described by Henry et al. (2006). The primer pair *narG* 1960 m-2F, *narG* 2050 m-2R and *nosZ*-2F, *nosZ*-2R were used for quantification of *narG* and *nosZ* genes, respectively. The reaction mixture (20 µL) for *narG* or *nosZ* genes contained 10 µL of qPCR master mix (KAPA SYBR FAST), 1 or 2 µL of each primer, 2 or 1 µL of hundredfold-diluted soil DNA, and 6 or 5 µL of autoclaved ultra-pure water. Thermo-cycling quantitative PCR reaction conditions for *narG* genes were as follows: enzyme activation of 15 minutes at 95°C; 40 cycles of 15s at 95°C for denaturing, 30s at 58°C for annealing, 30s at 72°C for extension, and 15s at 81°C for final extension. Thermo-cycling conditions for *nosZ* genes were similar except the enzyme activation of 10 minutes, annealing of 15s at 60°C, and no final extension. The standard curves for quantification of *narG* and *nosZ* genes were generated using *Pseudomonas stutzeri* NBRC 14165.

2.6. Mineral N, pH, and EC of incubated soil

Soil samples in bottles after gas collections (1:10), the original soil (1:10), and compost samples (1:100) were extracted with 2 mol L^{-1} potassium chloride by shaking at 175 rpm for 1 h, then used to measure NH_4^+-N (the modified indophenol method) and NO_3^- N (the cadmium reduction method) contents using a continuous flow analyzer (QuAAtro 2-HR, Bltec, Japan, Eaton et al. 2005). The pH (H_2O) and EC of water extractions (1:10) of samples were measured using a pH/Ion meter (F-23, Horiba, Japan) and conductivity meter (DS-14, Horiba, Japan), respectively after shaking at 175 rpm for 1 min (Hoang and Maeda 2018).

2.7. Data analysis

Cumulative N_2O and CO_2 emissions were calculated using trapezoidal integration of N_2O and CO_2 fluxes over time. Statistically significant differences in cumulative N_2O and CO_2 emissions among no compost- and compost-amended treatments at three NH_4^+-N rates were examined separately for two types of soil using analysis of variance (two-way ANOVA: compost type×NH_4^+-N rates, $p < 0.05$, Table 2) and Tukey test. Three-way ANOVA (compost type×soil type×NH_4^+-N rates, Table 3) was performed on cumulative N_2O and CO_2 emissions over 42 days. All statistical calculations were performed using the R software (R Core Team 2019).

3. Results

3.1. Emissions of N_2O from compost-amended soil at different NH_4^+-N rates

In no compost-amended Kochi soil (K-160, K-200, and K-400 treatments), peak N_2O emissions appeared on day 3, then decreased gradually to day 42 (Figure 1a). The lowest and highest peak N_2O emissions were seen in K-160 and K-200

Table 3. Responses of cumulative N_2O and CO_2 emissions to compost type, soil type, and NH_4^+-N rates by three-way ANOVA.

Factor	Cumulative N_2O emissions p	Cumulative CO_2 emissions p
Compost type	4.63×10^{-13}	$<2\times10^{-16}$
Soil type	9.23×10^{-13}	$<2\times10^{-16}$
NH_4^+-N rates	0.0119	2.02×10^{-11}
Compost type×Soil type	2.79×10^{-9}	7.09×10^{-8}
Compost type×NH_4^+-N rates	0.0076	4.25×10^{-6}
Soil type×NH_4^+-N rates	0.0309	2.24×10^{-5}
Compost type×Soil type×NH_4^+-N rates	0.014	0.31

treatments, respectively. In CC-amended Kochi soil (KCC-160, KCC-200, and KCC-400 treatments), the same pattern of N_2O emissions as in no-compost treatments was observed (Figure 1b). The highest peak emission of N_2O was in the KCC-200 treatment, while other treatments showed similar peak N_2O emissions. In KMC-200 and KMC-400 treatments, peak N_2O emissions appeared on day 3, whereas in the KMC-160 treatment, the peak appeared on day 7 (Figure 1c). The peak N_2O emission in the KMC-400 treatment was significantly lower than that in the KMC-200 treatment ($p < 0.05$). All N_2O emissions gradually decreased with time except in the KMC-200 treatment, which had an intermediate peak emission on day 14. Among all Kochi soil treatments, MC-amended treatments had higher N_2O emissions than those amended with CC and no compost irrespective of NH_4^+-N rates.

In no compost- and CC-amended treatments of Ushimado soil, peak N_2O emissions appeared on day 14 (Figure 1d, e), where the highest peak N_2O emissions were in U-200 and UCC-200 treatments. In MC-amended Ushimado soil, all peak N_2O emissions appeared on day 21 and the smaller peaks on day 3 (Figure 1f). The peak heights in MC-amended Ushimado soil were similar irrespective of NH_4^+-N rates. When comparing two soil types, Kochi soil treatments had the highest temporal N_2O emissions compared with Ushimado soil treatments (Figure 1).

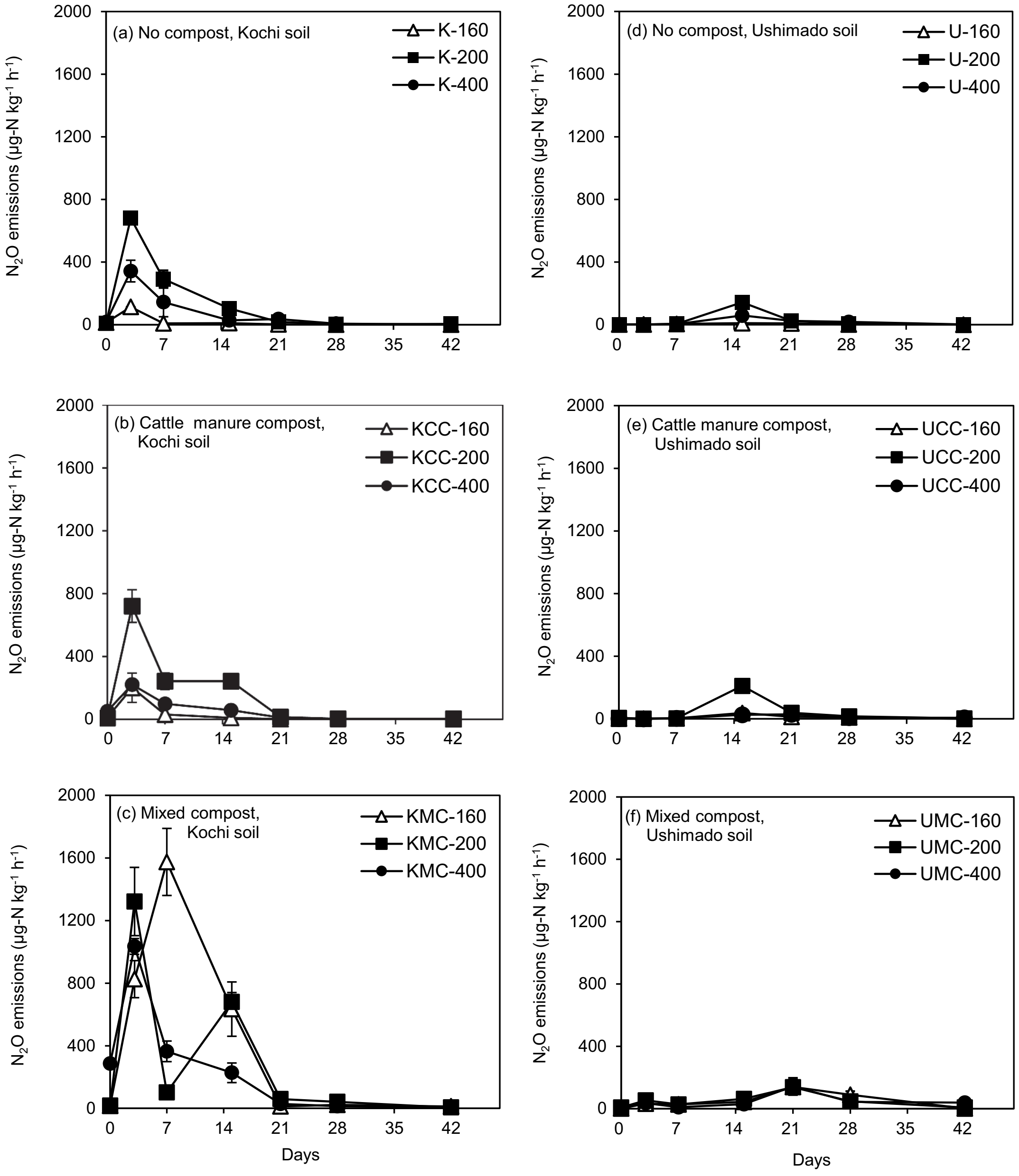

Figure 1. N_2O emissions in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

In no compost- and CC-amended Kochi soil, cumulative N_2O emissions were greater in the order of K-200, KCC-200 > K-400, KCC-400 > K-160, KCC-160 (Table 2). Cumulative N_2O emissions in MC-amended Kochi soil decreased gradually from NH_4^+-N rate 160 (KMC-160) to 400 (KMC-400) (p <0.05). In summary, cumulative N_2O emissions in Kochi soil decreased when the NH_4^+-N rate increased from 200 to 400. Interestingly, in Ushimado soil, the same trends as in Kochi soil were observed at different NH_4^+-N rates, whereas in the UMC-200 treatment, cumulative N_2O emission was not significantly different from those in UMC-160 and UMC-400 treatments (p >0.05, Table 2). According to three-way ANOVA results, the interaction effects among compost type, soil type, and NH_4^+-N rates on cumulative N_2O emissions were significant (p <0.05) in addition to their individual effects (Table 3).

3.2. Emissions of CO_2 from compost-amended soil at different NH_4^+-N rates

In Kochi soil, peak CO_2 emissions occurred within the first week of incubation, then decreased with time (Figure 2a-c). The MC-amended Kochi soil (Figure 2c) showed the highest CO_2 emissions compared with no compost- and CC-

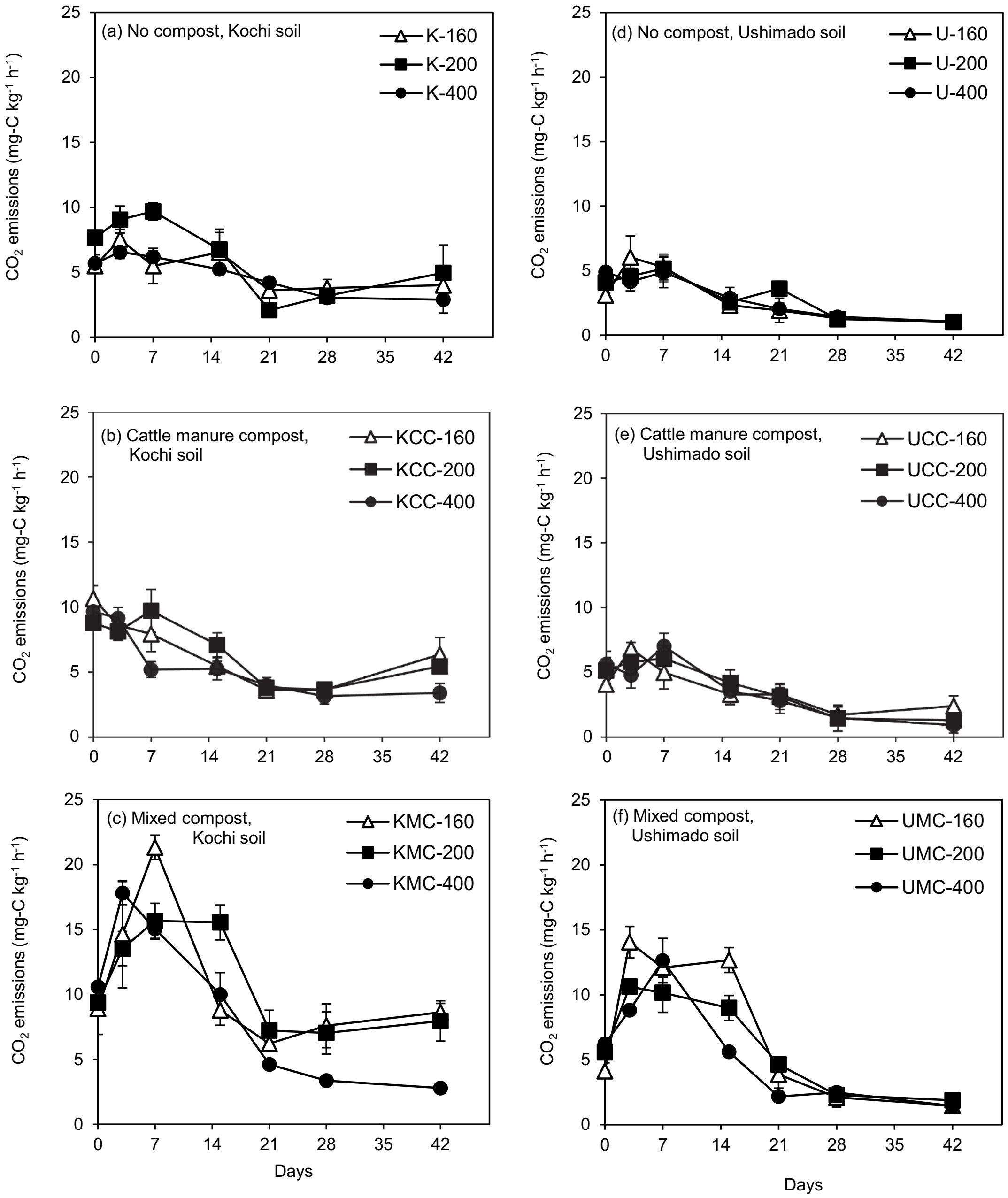

Figure 2. CO_2 emissions in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

amended treatments irrespective of NH_4^+-N rates. In Ushimado soil, CO_2 emissions showed similar patterns, which were slightly lower than those in corresponding Kochi soil treatments (Figure 2d-f).

In Kochi soil, cumulative CO_2 emissions at NH_4^+-N rate 160 were the same level as those at NH_4^+-N rate 200, while those at NH_4^+-N rate 400 were significantly lower than those at NH_4^+-N rate 200 ($p < 0.05$, Table 2). In Ushimado soil, significant differences in cumulative CO_2 emissions across NH_4^+-N rates were observed only when amended with MC (Table 2). In those treatments, cumulative CO_2 emissions were significantly higher in the order of UMC-160 > UMC-200 > UMC-400 ($p < 0.05$). The individual effects of compost type, soil type, and NH_4^+-N rates on cumulative CO_2 emissions were significant ($p < 0.001$, Table 3), whereas the interactions between three factors were not significant ($p > 0.05$, Table 3).

3.3. *Soil NH_4^+-N and NO_3^--N contents*

In Kochi soil, NH_4^+-N contents increased until day 7 (K-200 and KMC-160 treatments) or 3 (the other treatments), then decreased towards day 42 (Figure 3a-c). Soil NH_4^+-N contents were almost extinct around day 21 in all treatments. The initial increment of NH_4^+-N contents was not prominent in the KMC-400 treatment, whereas the drastic reduction after day 3 was similar to the other treatments (Figure 3c).

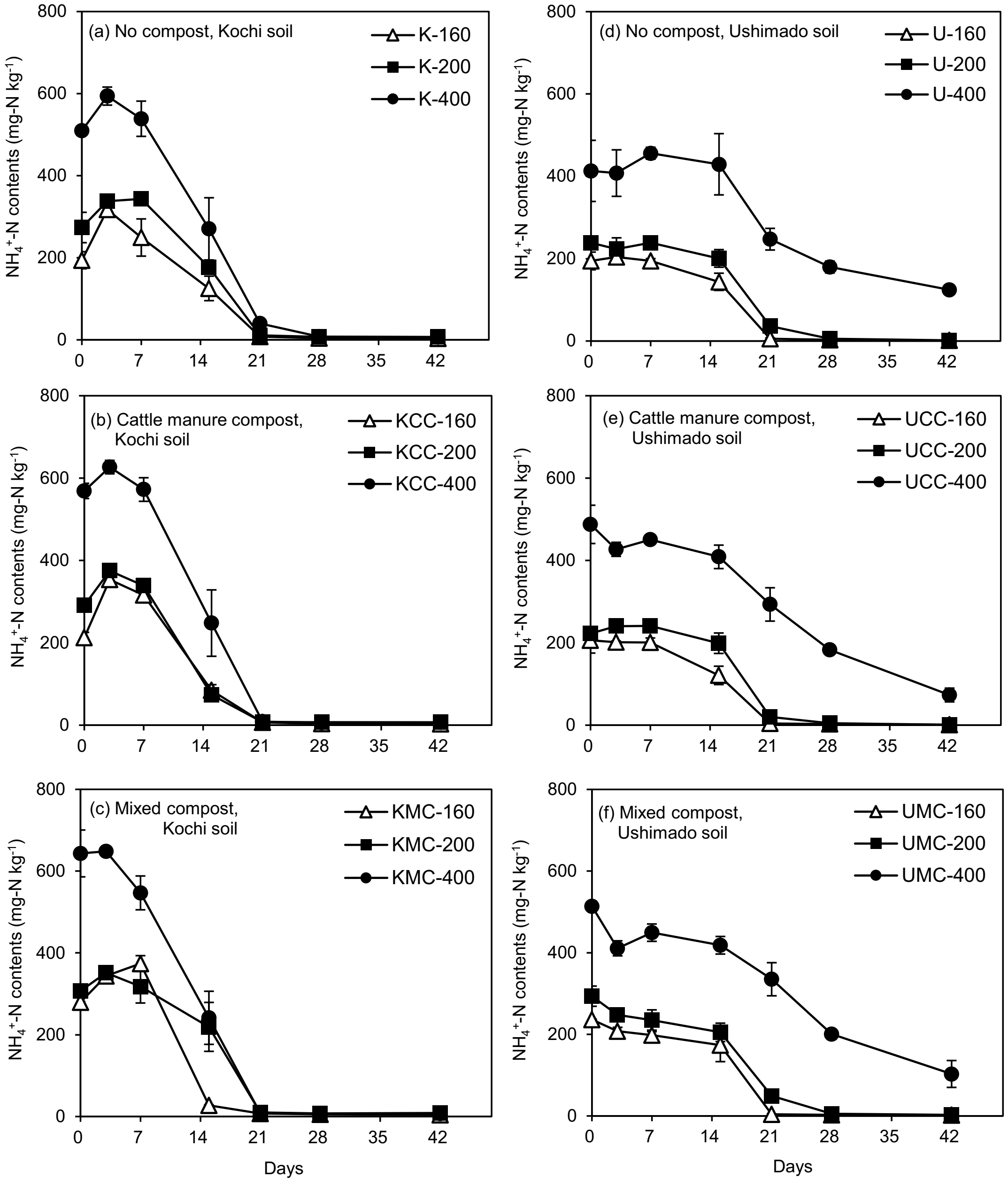

Figure 3. NH_4^+-N contents in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

In Ushimado soil, the initial increment of NH_4^+-N contents was not clear. In no compost- and CC-amended treatments, NH_4^+-N contents started decreasing on day 7 (Figure 3d, e). In MC-amended treatments, NH_4^+-N contents tended to decrease from the beginning of the incubation (Figure 3f). Soil NH_4^+-N contents decreased rapidly from day 14 to day 21 and became extinct around day 21 at NH_4^+-N rates 160 and 200 as seen in Kochi soil. At NH_4^+-N rate 400 (U-400, UCC-400, and UMC-400 treatments), NH_4^+-N contents continuously decreased and remained between 100 and 200 mg-N kg^{-1} at the end of the experiment.

In Kochi soil, NO_3^--N contents decreased until day 3 or 7, then started increasing with time in response to the reduction of NH_4^+-N contents (Figure 4a-c). In the KMC-160 treatment, the reduction in NO_3^--N content from the beginning to day 7 was more prominent than those in the other treatments. After day 21 or 28, the values became flattened. In the KMC-160 treatment, a reduction in NO_3^--N contents was observed from day 21 to 28 ($p < 0.05$, Figure 4c), indicating the possibility of denitrification during this period. The lowest NO_3^--N content among all treatments was also recorded in the KMC-160 treatment.

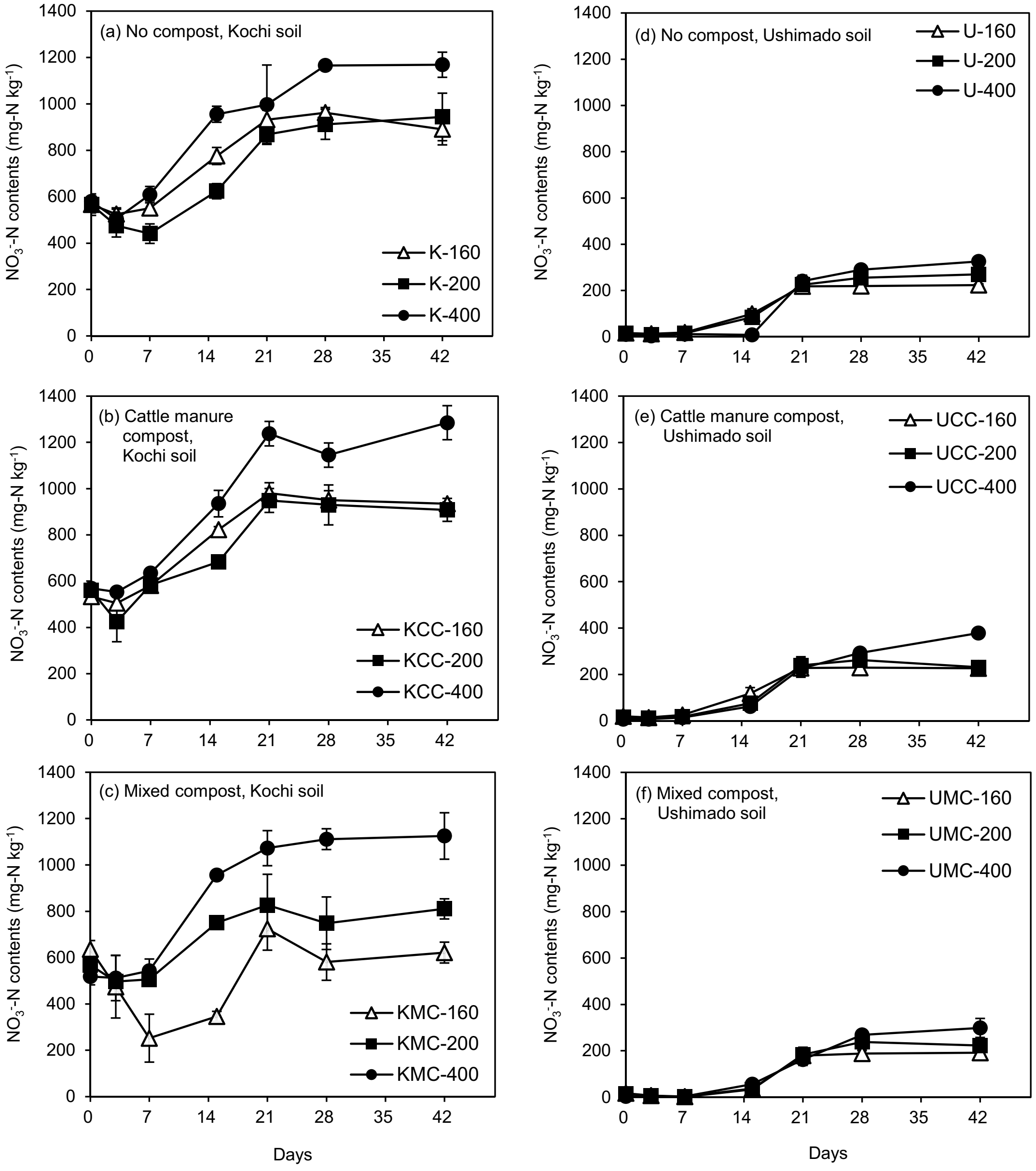

Figure 4. NO_3^--N contents in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

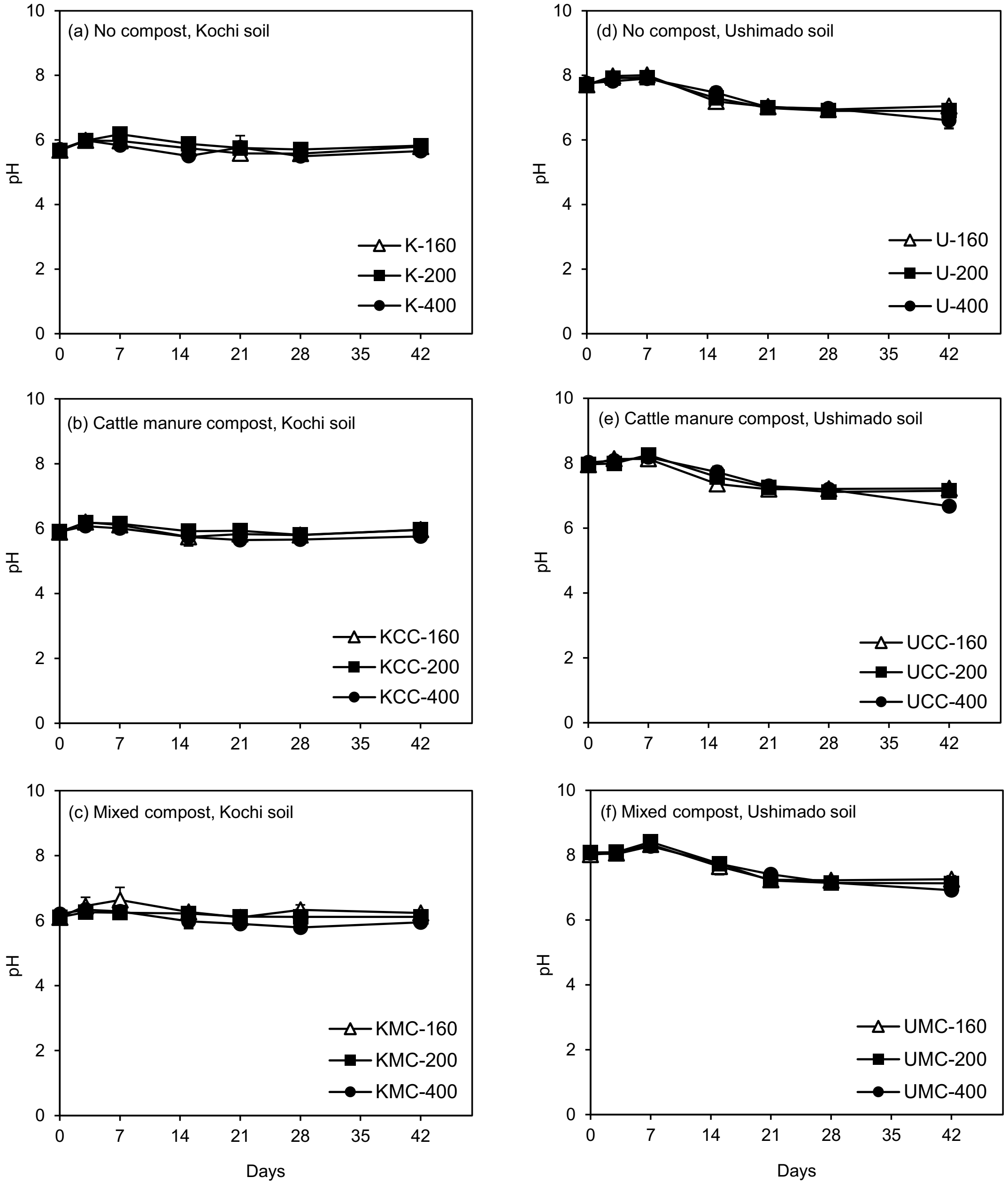

Figure 5. pH (H_2O) values in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

In Ushimado soil, NO_3^--N contents were lower and the differences among treatments were smaller than those in Kochi soil (Figure 4d-f). All treatments showed increasing NO_3^--N contents starting from day 7. At NH_4^+-N rates 160 and 200, NO_3^--N contents became flattened after day 21. Soil NO_3^--N contents at NH_4^+-N rate 400 increased to the end of the incubation (especially in the UCC-400 treatment).

3.4. Soil pH and EC

In Kochi soil, the pH range (5.5–6.6) was slightly lower than that in Ushimado soil (6.6–8.4) during the incubation (Figure 5). In both soils, pH values did not vary considerably among treatments throughout the entire period and pH slightly increased or was stable until day 7, and then continuously decreased. The slight increase of pH was probably caused by denitrification, which was corresponded to the reduction of NO_3^--N contents. The decrease of pH was more prominent in Ushimado soil (Figure 5d-f) than that in Kochi soil (Figure 5a-c). This trend corresponded to the decreased NH_4^+-N contents (Figure 3) and increased NO_3^--N contents (Figure 4) after day 7, suggesting that the decrease of pH was due to nitrification.

In Kochi soil, the EC range (0.96–1.7 dS m^{-1}) was higher than that in Ushimado soil (0.2–0.77 dS m^{-1})during the incubation (Figure 6). In Kochi soil, the highest EC values were observed at

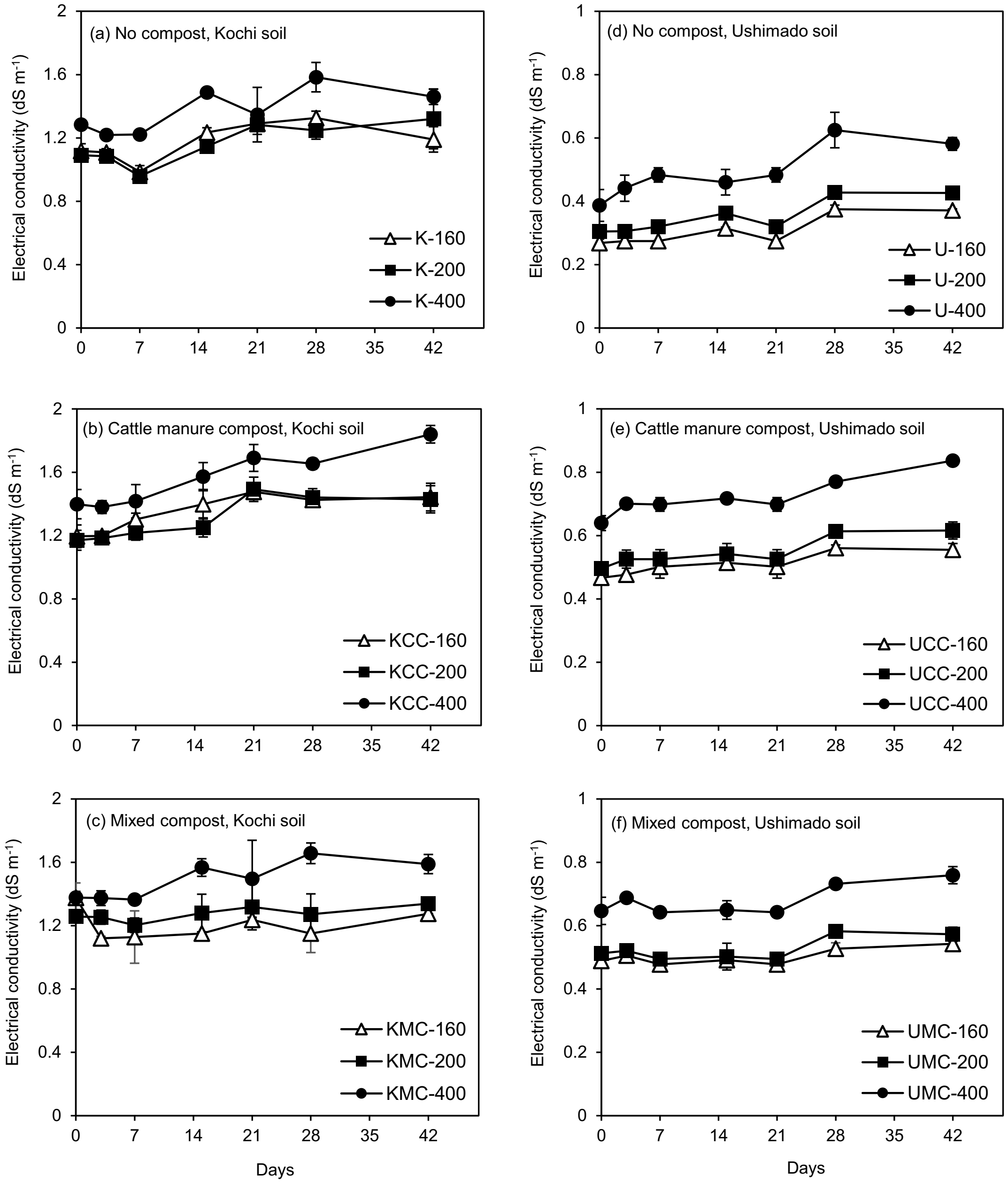

Figure 6. Electrical conductivity values in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (*n*=3).

NH_4^+-N rate 400 (K-400, KCC-400, and KMC-400 treatments, Figure 6a-c), where the values increased slightly until day 42. In no compost- and CC-amended Kochi soil, clear differences in EC among NH_4^+-N rates 160 and 200 were not seen. In MC-amended Kochi soil, the lowest EC was observed in the KMC-160 treatment.

In Ushimado soil, no compost-amended samples showed the lowest EC values in range of 0.2–0.6 dS m^{-1} (Figure 6d), while both CC- and MC-amended soils showed the higher EC values (Figure 6e, f). Among all treatments, the lowest and highest EC values were at NH_4^+-N rates 160 and 400, respectively (Figure 6d-f). At NH_4^+-N rate 400 (U-400, UCC-400, and UMC-400 treatments), EC slightly increased over time.

3.5. Relative abundances of *narG* and *nosZ* genes in soil

In Kochi and Ushimado soil treatments, *narG* and *nosZ* gene copy numbers increased relative to the original soil (see the footnote in Figure 7) during the incubation. Both no compost- and MC-amended Ushimado soil (Figure 7d, f) contained higher copy numbers of the *narG* gene than respective treatments of Kochi soil (Figure 7a, c). Even the original Ushimado soil contained more copy numbers of the *narG* gene than the original Kochi soil. In contrast, CC-amended Kochi soil (Figure 7b) had higher copy numbers of the *narG* gene than respective samples of Ushimado soil (Figure 7e). Except KMC-160 and KMC-200 treatments,

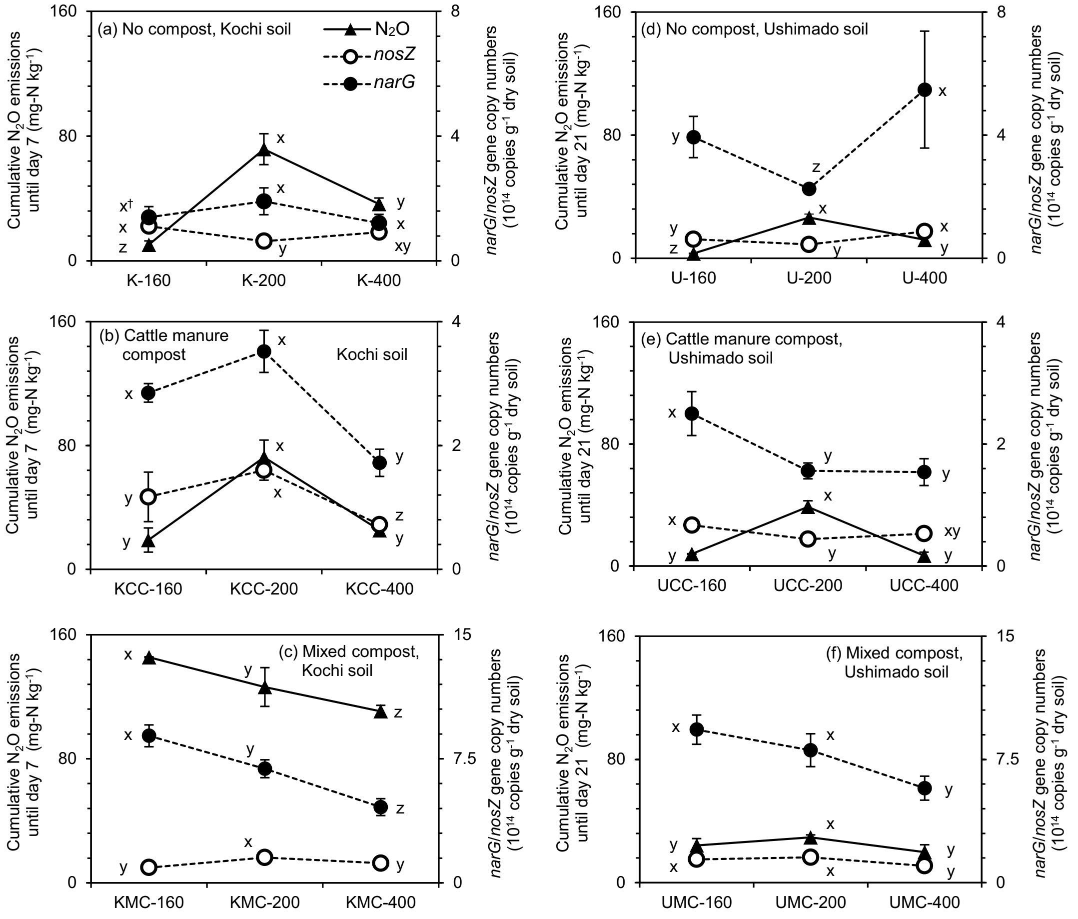

Figure 7. Cumulative N_2O emissions until day 7 or 21, *narG*, and *nosZ* gene copy numbers in the incubation experiment.The left (a)–(c) show results for Kochi soil (K) and the right (d)–(f) do for Ushimado soil (U). The upper (a) and (d) show results for no compost-, the middle (b) and (e) for cattle manure compost (CC)-, and the lower (c) and (f) for mixed compost (MC)-amended soils. The numbers (160, 200, and 400) indicate NH_4^+-N application rates to soil (mg-N kg^{-1}). Error bars indicate ± standard deviation (n=3).

[†] Means of N_2O emissions or each gene type with the same letters are not significantly different within the same treatment across NH_4^+-N rates (p >0.05).

In the original Kochi soil, *narG* and *nosZ* gene copy numbers were 0.13×10^{14} and 0.22×10^{14} copies g^{-1} dry soil, respectively. In the original Ushimado soil, *narG* and *nosZ*gene copy numbers were 0.91×10^{14} and 0.19×10^{14} copies g^{-1} dry soil, respectively.

higher copy numbers of the *nosZ* gene were found in Kochi soil than respective treatments of Ushimado soil (Figure 7).

3.6 Relationships between relative abundances of functional genes, nosZ or narG, and cumulative N_2 Oemissions untilday 7 (Kochi soil) or 21 (Ushimado soil) at different NH_4^+-N rates

In no compost-amended Kochi soil, copy numbers of the *narG* gene were the same across NH_4^+-N rates (Figure 7a). Copy numbers of the *nosZ* gene significantly decreased from NH_4^+ -N rate 160 (K-160) to 200 (K-200) (p <0.05) and those in the K-400 treatment were not different from both other treatments. Overall, cumulative N_2O emissions until day 7 were negatively correlated with copy numbers of the *nosZ* gene across NH_4^+-N rates (R^2 = 0.66, p <0.05), while they were not with those of the *narG* gene.

In CC-amended Kochi soil, copy numbers of the *narG* gene in the KCC-400 treatment were significantly lower than those in KCC-160 and KCC-200 treatments, while copy numbers of the *nosZ* gene significantly increased from NH_4^+-N rate 160 (KCC-160) to 200 (KCC-200) and decreased in the KCC-400 treatment (p <0.05, Figure 7b). Cumulative N_2O emissions until day 7 were positively correlated only with copy numbers of the *narG* gene at NH_4^+-N rates 200 and 400 (R^2 = 0.91, p <0.05).

In MC-amended Kochi soil, copy numbers of the *narG* gene continuously and significantly decreased from NH_4^+-N rate 160 (KMC-160) to 400 (KMC-400), while copy numbers of the *nosZ* gene significantly increased from NH_4^+-N rate 160 (KMC-160) to 200 (KMC-200) and decreased from NH_4^+ -N rate 200 (KMC-200) to 400 (KMC-400) (p <0.05, Figure 7c). Cumulative N_2O emissions until day 7 were positively correlated with copy numbers of the *narG* gene across NH_4^+-N rates (R^2 = 0.67, p <0.05), while they were not with those of the *nosZ* gene.

In no compost-amended Ushimado soil, copy numbers of the *narG* gene significantly decreased from NH_4^+-N rate 160 (U-160) to 200 (U-200) and increased in the U-400 treatment, while copy numbers of the *nosZ* gene were significantly higher

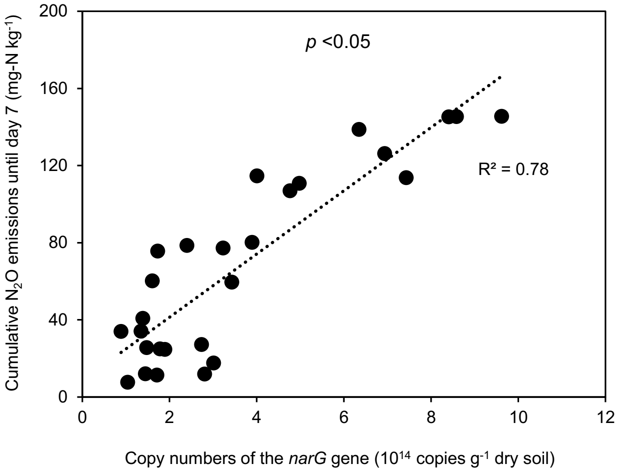

Figure 8. Relationship between cumulative N_2O emissions until day 7 and copy numbers of the *narG* gene in Kochi soil treatments.

in the U-400 treatment than those in U-160 and U-200 treatments ($p < 0.05$, Figure 7d). Cumulative N_2O emissions until day 21 were negatively correlated with copy numbers of the *nosZ* gene at NH_4^+-N rates 200 and 400 ($R^2 = 0.74$, $p < 0.05$), while they were not with those of the *narG* gene.

In CC-amended Ushimado soil, copy numbers of both *narG* and *nosZ* genes significantly decreased from NH_4^+-N rate 160 (UCC-160) to 200 (UCC-200) ($p < 0.05$), while those were not different between UCC-200 and UCC-400 treatments (Figure 7e). Cumulative N_2O emissions until day 21 and copy numbers of the *nosZ* gene at NH_4^+-N rates 160 and 200 were negatively correlated ($R^2 = 0.90$, $p < 0.05$).

In MC-amended Ushimado soil, copy numbers of both *narG* and *nosZ* genes at NH_4^+-N rate 160 (UMC-160) were not different from those at NH_4^+-N rate 200 (UMC-200), whereas a significant decrease in both genes was observed from NH_4^+-N rate 200 (UMC-200) to 400 (UMC-400) ($p < 0.05$, Figure 7f). In those samples, cumulative N_2O emissions until day 21 were not significantly correlated ($p > 0.05$) with gene copy numbers of either *narG* or *nosZ* regardless of NH_4^+-N rates.

When comparing two types of soil, although no compost- and MC-amended Ushimado soil contained higher copy numbers of the *narG* gene than respective samples of Kochi soil (Figure 7), N_2O emissions were considerably lower than those in Kochi soil (Table 2). In Kochi soil, N_2O emissions were positively correlated with copy numbers of the *narG* gene ($R^2 = 0.78$, $p < 0.05$, Figure 8), whereas they were not in Ushimado soil ($R^2 = 0.39$, $p > 0.05$).

4. Discussion

4.1. N_2O and CO_2 emissions from manure compost-amended soil

In MC-amended treatments of both soils, higher cumulative N_2O and CO_2 emissions occurred than those in respective no compost- and CC-amended samples at each NH_4^+-N rate (Table 2). Relatively higher NH_4^+-N contents in MC than those in CC (Table 1) might be a dominant contributing factor to the higher N_2O emissions. A positive relationship between daily N_2O emissions and soil NH_4^+-N contents was observed by Kim et al. (2019), because NH_4^+-N is a primary substrate of N_2O formation through nitrification (Hu et al. 2020). Furthermore, MC contained a higher NO_3^--N content (Table 1), which would have promoted denitrification to produce N_2O. High N_2O emissions can be related to soil NO_3^--N contents when soil WFPS is up to 70% because N_2O was predominantly produced by denitrification (Zanatta et al. 2010). In addition, because MC was characterized by a low C/N ratio compared with that of CC (Table 1), MC would have easily decomposed, resulting in higher N_2O and CO_2 emissions. Organic amendment with a low C/N ratio resulted in release of mineral N via mineralization, whereas those with a high C/N ratio led to temporary N immobilization (Binh and Shima 2018). Toma and Hatano (2007) observed high N_2O emissions from soil amended with plant materials having a low C/N ratio due to faster N mineralization. Mohammed-Nour et al. (2021) reported low cumulative CO_2 emission from composted cattle manure with a high C/N ratio, which agreed with our results.

Of the two types of soil, all Kochi soil treatments showed higher cumulative N_2O and CO_2 emissions than respective treatments of Ushimado soil (Table 2). Ushimado soil contained lower contents of mineral N, total N, and C than those in Kochi soil. Similar to our results of Ushimado soil, Feng et al. (2003) observed low N_2O emissions in a soil with a low organic matter content and Mazzarino et al. (1991) found that N mineralization in soil was limited with low total N contents in soil. Chiyoka et al. (2011) observed that higher N_2O emissions occurred in a soil with high clay and organic matter contents, which favored the formation of anaerobic micro-sites, promoting denitrification under aerobic conditions similar to the results of Kochi soil. The presence of readily metabolized organic matter is closely correlated to the rate of biological denitrification and hence the potential N_2O emissions from soil (Wlodarczyk, Glinski, and Kotowska 2004). Generally, nitrification is optimum within the pH range of 4.9–7.2 (Stams, Flameling, and Marnette 1990). In Kochi soil, pH values of 5.5–6.6 were favorable for nitrifying activities than those in Ushimado soil, in which pH ranged between 6.6 and 8.4. Wang et al. (2018) detected higher N_2O emissions in a soil with pH 5 than in an alkaline soil with pH 8. In all Kochi soil treatments, peak N_2O emissions appeared within the first week of incubation (Figure 1a-c). Velthof, Kuikman, and Oenema (2003) documented that metabolization of volatile fatty acids in manure occurred within a few days after the incorporation into soil by denitrification. Jager et al. (2011) reported increased N_2O emissions during a shorter period after the application of organic fertilizer because easily available organic matter fractions promoted formation of anoxic soil micro sites, triggering N_2O emissions by providing easily available substrates for nitrification and denitrification. Ushimado soil showed peak N_2O emissions comparatively later than those of Kochi soil (Figure 1d-f) probably because of the delayed growth of microorganisms in Ushimado soil due to low N and organic C availability. In addition, higher WFPS of Kochi soil (69–72%) compared with Ushimado soil (44–46%) at the same WHC has resulted in higher N_2O emissions. Davidson et al. (1991) suggested that a threshold between denitrification and nitrification existed in about 60% WFPS. These results confirmed that earlier peak and higher N_2O emissions in Kochi soil might be due to nitrification, nitrifier-denitrification, and/or denitrification presumably promoted by high contents of mineral N, total N and C, and clay, favorable levels of soil pH, and possibly because of higher WFPS than those in Ushimado soil at the same WHC. Thus, the effects of compost type and soil type on N_2O emissions were significant (p <0.001, Table 3).

4.2. Effects of different NH_4^+-N rates on N_2O emissions

In no compost- and CC-amended Kochi and Ushimado soils, the lowest and highest cumulative N_2O emissions occurred at NH_4^+-N rates 160 (K-160, KCC-160, U-160, and UCC-160 treatments) and 200 (K-200, KCC-200, U-200, and UCC-200 treatments), respectively (Table 2). According to Wang et al. (2021), NH_4^+-N content is a key factor affecting N_2O emissions because it is directly subjected to nitrification. Well et al. (2008) also found that N_2O fluxes in an aerobic soil were significantly increased by NH_4^+-N additions from 0 to 40 mg-N kg^{-1} (p <0.001) because N_2O emissions were governed by NH_4^+-N availability. In addition, higher nitrification rates lead to increased availability of NH_2OH, NO, and NO_3^-, as well as promote O_2 consumption, which can evoke denitrification and subsequent release of N_2O (Signor and Cerri 2013; Lazcano, Zhu-Barker, and Decock 2021). Thus, increased NH_4^+-N contents induced nitrification directly and denitrification indirectly to emit N_2O in the present study.

In all treatments except in MC-amended Ushimado soil, cumulative N_2O emissions at NH_4^+-N rate 400 were significantly lower than those at NH_4^+-N rate 200 (p <0.05, Table 2), which was probably due to the suppression of microbial activities by higher NH_4^+-N contents. The lower N_2O peak heights at NH_4^+-N rate 400 than those at NH_4^+-N rate 200 also hinted the suppression of microbial activities at the highest NH_4^+-N content (Figure 1). In Kochi and Ushimado soil treatments, EC at NH_4^+-N rate 400 (1.2–1.8 and 0.4–0.8 dS m^{-1}, respectively) was higher than those at NH_4^+-N rate 200 (0.9–1.4 and 0.3–0.6 dS m^{-1}, respectively, Figure 6). Smith and Doran (1996) suggested that nitrification and denitrification activities were suppressed at EC >0.6 dS m^{-1} and the EC threshold imposing microbial stress can vary due to the addition of decomposable organic amendments. Furthermore, Adviento-Borbe et al. (2006) observed decreased nitrification within the EC range of 0.5–2.0 dS m^{-1}. Meng et al. (2020) detected negative impacts of EC of 1.24 to 12.96 dS m^{-1} on denitrification. Based on these findings, the EC range in NH_4^+-N rate 400 treatments seemed to enforce more salt stress on microorganisms than that in NH_4^+-N rate 200 treatments, resulting in low N_2O emissions in our study. Deppe et al. (2017) observed inhibition of net and gross nitrification at NH_4^+-N contents higher than 450 mg-N kg^{-1} with concurrent inhibition of N_2O production. According to their experiment, nitrification was negligible at 5000 mg-N kg^{-1} NH_4^+-N content, probably due to osmotic pressure. They also suggested an inhibition effect of high NH_4^+-N contents due to pH changes in soil. However, in our experiment, considerable differences in pH values across NH_4^+-N rates were not observed (Figure 5). Muller et al. (2006) also found that bacterial growth was impaired by the addition of $(NH_4)_2SO_4$ due to an enhanced osmolarity of the medium. Tong and Xu (2012) reported that nitrification was inhibited due to the inhibition of ammonia oxidizing bacteria populations, while maintaining a constant soil pH by the addition of 300 and 400 mg-N kg^{-1} as $(NH_4)_2SO_4$. Wang et al. (2020) found that $(NH_4)_2SO_4$ did not affect soil pH but reduced net nitrification rates mainly due to the acidic nature of the fertilizer at the maximum application rate of 200 mg-N kg^{-1} soil. Hoang and Maeda (2018) observed increased cumulative N_2O emissions with increasing NH_4^+-N application rates from 0 to 800 mg-N kg^{-1} and then declined at 1200 mg-N kg^{-1} and concluded that N_2O emissions were not exponentially correlated with NH_4^+-N application rates. All these studies reported the inhibitory effect of NH_4^+-N on N_2O emissions from mineral N fertilizer added soils and the inhibitory NH_4^+-N contents were either higher or lower than those of our study. Menyailo, Stepanov, and Umarov (1997) observed a dominance of N_2O emissions in a saline soil, possibly due to the inhibition of N_2O reductase by salts, which was different from our study, in which N_2O emissions became lower at NH_4^+-N rate 400 than those at NH_4^+-N rate 200.

Kim, Lee, and Keller (2006) suggested that nitrification was inhibited by free ammonia (NH_3). At high pH, some NH_4^+-N can be converted to NH_3 (Liu et al. 2019; Mohammed-Nour, Al-Sewailem, and El-Naggar 2019). Hence, the presence of free NH_3 probably reduced N_2O emissions in Ushimado soil at NH_4^+-N 400 (U-400 and UCC-400 treatments) under high pH of 7–8.2 and high NH_4^+-N conditions. In addition to the effects of compost and soil types, NH_4^+-N rates had significant effects on N_2O emissions (p <0.05, Table 3). Our study reports the suppression of N_2O emissions at NH_4^+-N rate 400 in different soil types amended with compost for the first time to the best of our knowledge, which is a novel finding.

4.3. Effects of different NH_4^+-N rates on CO_2 emissions

Due to the suppression of cumulative CO_2 emissions by increasing NH_4^+-N contents from NH_4^+-N rate 200 to 400 in manure compost-amended Kochi and Ushimado soils (p <0.05, Table 2) except in CC-amended Ushimado soil, the effects of NH_4^+-N rates were significant (p <0.001, Table 3). In all treatments, CO_2 emissions started decreasing after peak emissions (Figure 2). The low availability of easily decomposable C is a reason for the decrease of CO_2 emissions with time. The soil C content is a crucial factor to increase CO_2 emissions from soil microbial respiration (Ray et al. 2020). Pelster et al. (2012) observed high CO_2 emissions from poultry manure-amended soil due to a higher available C content. In Kochi soil, cumulative CO_2 emissions at NH_4^+-N rate 160 were the same level as those at NH_4^+-N rate 200 and cumulative CO_2 emissions at NH_4^+-N rate 400 were significantly lower than those at NH_4^+-N rate 200 (p <0.05, Table 2). Treseder (2008) documented declining CO_2 emissions in concert with declines in microbial populations under N fertilization by induced osmotic potentials, which became toxic, owing to the additional ions from N fertilizer. In addition, nitrogenous compounds from fertilizer can condense with carbohydrates producing melanoidins, and they can increase the polymerization of polyphenols to 'brown compounds.' Both melanoidins and brown compounds are recalcitrant to decomposition, limiting C availability for microbial respiration (Cao et al. 2021). Hence, the lowest cumulative CO_2 emissions at NH_4^+-N rate 400 of Kochi soil in our experiment were probably due to osmotic stress and low C availability for microorganisms.

In Ushimado soil, significant differences in cumulative CO_2 emissions across NH_4^+-N rates were observed only when amended with MC. The cumulative CO_2 emissions in the UMC-160 treatment were higher than those in UMC-200 and UMC-400 treatments ($p < 0.05$, Table 2). In CC-amended treatments, less total C content in Ushimado soil and mineralization-resistant CC might have limited C available for microorganisms regardless of NH_4^+-N rates. In easily decomposable MC-amended Ushimado soil, cumulative CO_2 emissions were much lower at NH_4^+-N rate 400 than those at the other NH_4^+-N rates, possibly due to osmotic stress.

4.4. Relation of temporal variation of NH_4^+-N and NO_3^--N contents to N_2O emissions

In Kochi soil, NH_4^+-N contents started decreasing from day 3, except in K-200 and KMC-160 treatments, in which the reduction started on day 7 (Figure 3a-c). The reduction of NH_4^+-N contents indicated the occurrence of nitrification. The beginning date of nitrification was similar to the day of peak N_2O emissions in Kochi soil treatments (Figure 1a-c). All Kochi soil treatments those received $(NH_4)_2SO_4$ showed earlier nitrification than the KMC-160 treatment that contained only the original NH_4^+-N contents. Nitrification rates probably became faster with the addition of $(NH_4)_2SO_4$ because extra NH_4^+-N is a substrate for ammonia oxidation (Jones et al. 2007; Yin et al. 2019). In our study, the KMC-160 treatment showed a delayed start of nitrification, in which N mineralization was larger than in the other treatments because of no stress on microorganisms by the addition of NH_4^+-N.

Ushimado soil treatments also showed prominent nitrification within the first 3 weeks of incubation (Figure 3d-f), although the nitrification rate was slower than that in Kochi soil. Unlike the others, in U-400, UCC-400, and UMC-400 treatments, NH_4^+-N contents continuously decreased because of more NH_4^+-N, which remained between 100 and 200 mg-N kg^{-1} even at the end of the experiment. This indicated that NH_4^+-N in those treatments was not totally nitrified within 42 days of incubation. This was probably due to low C availability in Ushimado soil. Silva et al. (2019) and Zhang, Müller, and Cai (2015) reported the existence of heterotrophic nitrifying microorganisms. Organic C serves as energy and electron sources for heterotrophic nitrifiers (Yang et al. 2016). Hence, in Ushimado soil at NH_4^+-N rate 400, the activities of heterotrophic nitrifiers could be restricted by low C contents, resulting in reduced nitrification.

In Kochi soil, initial NO_3^--N contents decreased until day 3 or 7, on which peak N_2O emissions occurred, and then started increasing with time (Figure 4a-c), concurrently with the reduction of NH_4^+-N contents. The initial reduction of NO_3^--N suggested the occurrence of denitrification. Congreves, Phan, and Farrell (2019) suggested that at 53% to 78% WFPS, N_2O emission from soil was attributable to denitrification. Zhu et al. (2013) reported that denitrification can occur following the addition of urea or $(NH_4)_2SO_4$ to loam, clay loam, and sandy loam soils, resulting in N_2O emissions. Therefore, N_2O emissions in the first week in Kochi soil treatments (69–72% WFPS) were due to both nitrification and denitrification. The KMC-160 treatment showed the lowest NO_3^--N content among all MC-amended Kochi soil treatments (Figure 4c), revealing that at NH_{4+}-N rate 160, more NO_3^--N contents were subjected to denitrification. This can be a reason for the highest cumulative N_2O emissions in the KMC-160 treatment than those in KMC-200 and KMC-400 treatments (Table 2). With increasing NH_4^+-N rate from 200 to 400, denitrification decreased remaining high NO_3^--N contents throughout the incubation period in all Kochi soil treatments (Figure 4a-c). Similarly, Deppe et al. (2017) suggested that N_2O emissions during denitrification can be inhibited at high NH_4^+-N contents.

In Ushimado soil, NO_3^--N contents were lower than those in Kochi soil, and large differences among treatments were not observed (Figure 4d-f). Denitrification potential of Ushimado soil was lower than that of Kochi soil possibly due to low C and N availability in Ushimado soil. In addition, lower WFPS of Ushimado soil may not create favorable anaerobic conditions for high denitrifying activities than that of Kochi soil.

4.5. Relation between relative abundances of functional genes, *nosZ* or *narG*, and cumulative N_2O emissions

Both no compost- and MC-amended treatments of Ushimado soil at each NH_4^+-N rate contained higher copy numbers of the *narG* gene than respective treatments of Kochi soil (Figure 7). In contrast, N_2O emissions in Ushimado soil were considerably lower than those in Kochi soil (Figure 1). This may be due to less C and N contents in Ushimado soil (Table 1), because the activities of denitrifiers are highly affected by the supply of NO_3^--N and labile C (Lazcano, Zhu-Barker, and Decock 2021). Additionally, lower WFPS of Ushimado soil was not favorable for high denitrifying microbial activities than those of Kochi soil. This reveals that even if denitrifying genes are abundant in soil, N_2O emissions are determined by their activities, which are affected by the availability of C and N substrates. The ability of denitrifying bacteria to produce N_2O depends on abiotic conditions such as O_2 level, pH, temperature, moisture, and the availability of C and N (Yang, Zhang, and Ju 2017; Usyskin-Tonne, Hadar, and Minz 2019). Röling (2007) suggested that gene pools may not always reflect rates of N_2O emissions due to subsequent controls over gene transcription and enzyme activities. Accordingly, gene abundances may reflect only genetic potential within the cropping systems for N_2O emissions (Duan et al. 2018).

The high activities of *narG* genes in Kochi soil were confirmed by higher NO_3^--N contents available in the treatments with a substrate for bacteria with *narG* genes than those in Ushimado soil during the incubation (Figure 4). Therefore, a stronger positive linear relationship (R^2 = 0.78, Figure 8) between cumulative N_2O emissions and copy numbers of the *narG* gene was observed in Kochi soil than that in Ushimado soil (R^2 = 0.39). Yang, Zhang, and Ju (2017) observed a significant correlation between N_2O emissions and copy numbers of the *narG* gene in urea-based fertilizer and/or cattle manure-amended soil. High NH_4^+-N from urea-based fertilizers stimulated the growth of ammonia oxidizers, leading to micro-oxic or anoxic conditions, which in turn induced denitrification by heterotrophic denitrifiers, leading to high N_2O emissions. We observed a similar relationship between copy numbers of the *narG* gene and cumulative N_2O emissions in Kochi soil with high C and N contents amended with manure compost and supplemented with extra NH_4^+-N at higher WFPS than those in Ushimado soil at the same WHC.

According to the results in Figure 7, a clear inhibitory effect of high NH_4^+-N contents on both *narG* and *nosZ* genes in compost-amended Kochi and MC-amended Ushimado soils was observed due to significantly lower gene copy numbers at NH_4^+-N rate 400 than those at NH_4^+-N rate 200 (p <0.05). Dincer and Kargi (1999) reported that NaCl concentrations above 20 g L^{-1} resulted in significant reductions in performances of both nitrification and denitrification, while denitrification was more sensitive to high salt contents. Hence, the salt stress at NH_4^+-N rate 400 was a possible reason for the reduced *narG* and *nosZ* gene copy numbers.

5. Conclusions

We aimed at determining the effects of different application rates of NH_4^+-N on N_2O and CO_2 emissions from two contrasting types of manure compost-amended Kochi and Ushimado soils under aerobic conditions. Further, we analyzed the relationships between relative abundances of *narG* or *nosZ* genes and N_2O emissions from compost-amended soil. Results showed that N_2O and CO_2 emissions in this experiment were higher in both soil types when MC with a higher mineral N content and a lower C/N ratio was amended than those when CC was amended. Emissions of N_2O and CO_2 were higher in MC- or CC-amended Kochi soil because of higher mineral N, total N and C, and clay contents, favorable levels of soil pH, and greater WFPS than those of Ushimado soil at the same WHC. Additionally, in no compost- and CC-amended Kochi and Ushimado soils, raising NH_4^+-N rate from 160 to 200 increased cumulative N_2O emissions due to the stimulation of nitrification directly by the supply of NH_4^+-N. A decrease in N_2O emissions at NH_4^+-N rate 400 was observed comparing with those at NH_4^+-N rate 200 due to the suppression of microorganisms by the osmotic stress in all Kochi soil and no compost- and CC-amended Ushimado soil. A significant decrease in CO_2 emissions at NH_4^+-N rate 400 was observed in all Kochi soil and MC-amended Ushimado soil treatments comparing with those at NH_4^+-N rate 200 possibly due to osmotic stress and C limitation. Increasing copy numbers of the *narG* gene increased N_2O emissions (R^2 = 0.78) in Kochi soil with higher available N and C contents, and greater WFPS than those in Ushimado soil at the same WHC. Our study revealed that NH_4^+-N rate 400 can suppress N_2O and CO_2 emissions from compost-amended soil under aerobic conditions, suggesting that soil NH_4^+-N contents must be considered for estimation of N_2O and CO_2 emissions from manure compost-amended soil.

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