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Effect of different moisture content on nitrous oxide production in aggregated soil of Shintoku, Japan

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ABSTRACT

Agricultural soils are the major source of the potent greenhouse gas and ozone-depleting substance, N_2O. An incubation study was carried out using the volcanic ash fine textured soil of Shintoku. Soil samples used in this study were taken from managed grassland at Shintoku Experimental Livestock Farm of Hokkaido University in Southern Hokkaido, Japan (N43°05′, E142°51′). Soil aggregates were air-dried, and sieved with 4.5 mm and 2 mm and adjusted the soil moisture of 60% and 80% of field water capacity (FWC). Just after the moistening, the aggregates were incubated for 9 days under a temperature of 20°C. Just after starting the incubation, the flush of N_2O production was observed. Similar flushes of carbon dioxide (CO_2) and nitric oxide (NO) productions were also observed. All of the gas productions were higher in larger aggregates with 80% of field water capacity. The concentrations of Water Extractable Organic Carbon (WEOC), NH_4^+-N, pH and total N were significantly different before and after incubation. Shintoku soil showed a significant correlation between before and after incubation for all soil chemical properties except pH. Especially WEOC and NH_4^+-N changed immediately after the addition of water and this situation continued during the incubation. Larger aggregates showed higher amounts of NH_4^+-N and NO_3^-–N and were responsible for higher N_2O production compared to smaller aggregates. In Shintoku the results of N_2O-N/NO-N ratio in both moisture contents indicated nitrification as a main process of N_2O production. It is very well known that N_2O is produced more from the denitrification process than nitrification. Poor aeration and less diffusion of NO_3^-N and WEOC from the aerobic area to the anaerobic area reduce N_2O production in the denitrification process of fine textured Shintoku soil.

Keywords: Denitrification, Nitrification, N_2O Production, Soil Aggregate

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Introduction

Nitrous oxide (N_2O) is a long-lived greenhouse gas that contributes to global warming. Its exchange between the soil and atmosphere has received significant attention in recent decades because of its prominent role in climate change and atmospheric ozone depletion (Erik *et al.*, 2023). Agricultural activities are responsible for two-thirds of the total anthropogenic nitrous oxide (N_2O) emissions worldwide (Wang *et al.*, 2021). Nitrous oxide emissions from soils result principally from microbial nitrification and denitrification (Bouwman, 1990). These processes require mineral N (NH_4^+ and NO_3^-) as a substrate and are controlled by soil moisture content, temperature, pH and organic carbon (Bouwman, 1990; Maag and Vinther, 1996; Mosier, 1998; Yamulki *et al.*, 1997). N_2O emissions from agricultural soils vary from 0.03% to 2.7% of the total nitrogen (N) fertilizer applied (Eichner, 1990) for their very high spatial and temporal variability (Folorunso and Rolston, 1984; He´nault *et al.*, 1996). At present, no models of soil N_2O emissions exist which are universal enough to be able to predict emissions under different soil conditions and management systems (Granli and Bøckman, 1994). Thus the laboratory measurement of N_2O production under different soil and water managements is still necessary.

Methodology

Soil sampling site and location

Soil samples used in this study were taken from a managed grassland in Shintoku Experimental Livestock Farm of Hokkaido University in Southern Hokkaido, Japan (N43°05′, E142°51′) (Fig. 1). The grassland soil was covered by reed canary grass (*Phalaris arundinacea* L.) and meadow foxtail grass (*Alopecurus pratensis* L). The soil is derived from Tarumae (b) volcanic ash and is classified as Thaptic Melanudands (Soil Survey Staff, 2022; Mollic Andosol. The site is characterized by a humid continental climate with cold winters and cool summers but without apparent wet or dry seasons. The mean annual precipitation is approximately 1365 mm and the mean annual temperature is 7.9°C, with the mean monthly temperature ranging from 20.7°C in August to 3.9°C in January. The site is covered with snow from the end of December to the beginning of March (Shimizu *et al.*, 2009).

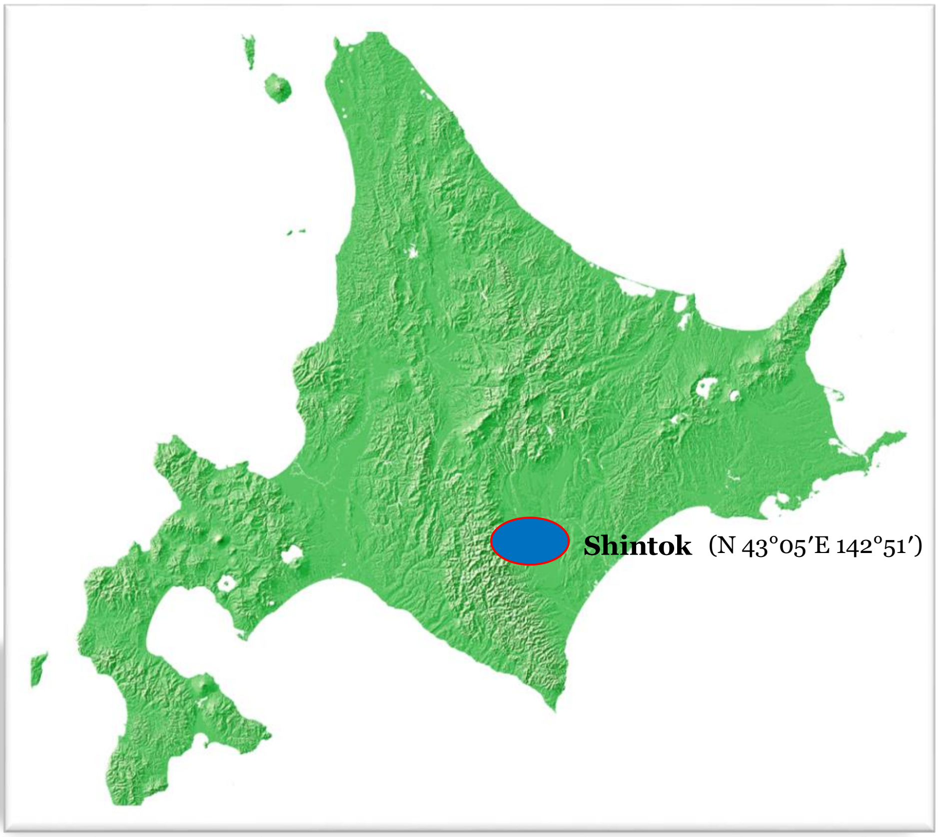

Fig. 1. Experimental site at Shintoku Experimental Livestock Farm of Hokkaido University, Japan.

The soil type of the Shintoku site is stratic fluffy ordinary andosol. The grassland was renovated 7 years before sampling. Between 1979 and 2000, the mean annual precipitation of this site was 969 mm and the mean monthly air temperature was -8.7°C for January and 18.1°C for July at the Memuro meteorological station, located 2.5 km west of NARCH (Japan Meteorological Agency, 2009).

Fertilizer

Annual average fertilizers application rate of Shintoku were 56 kg N ha^{-1}, 48 kg P_2O_5 ha^{-1}, 20 kg MgO ha^{-1}. Moreover, 277 kg ha^{-1} urine was added to the soil of Shintoku.

General properties of soil

Shintoku soil is volcanic ash light clay soil characterized by pore size distribution dominant in fine capillary pores with matric potential from 10 to 1000 kPa.

Soil sampling

In Shintoku, soils were sampled from a 3-14 cm depth on 18 August 2008 when the first harvest of grass was completed. The collected samples were brought to the laboratory and immediately stored in refrigeration at 4°C. The fresh soil samples were then spread on trays and air-dried at room temperature.

Aggregate preparation

Air-dried soil samples were gently crushed by hand to pass through a 4.5 mm sieve; roots and stones were removed from the samples by hand. The soil aggregates of <2 mm were then separated by using a 2 mm sieve. In order to measure the field water capacity (FWC), about 10 g air dried soil after aggregate preparation was saturated with water by draining loosely packed soil in a funnel of 0.55 g cm^{-3} for 24 h. The FWC of each aggregate fraction of each soil was determined to calculate the amount of distilled water that was added to achieve the desired moisture contents, which were 60% and 80% of FWC. Aggregate preparation and adjustment of moisture contents were performed at room temperature (20°C). The incubation procedure was initiated immediately after adding the distilled water to soil samples.

Incubation procedure

After adjusting the soil moisture content, 20 g dry-basis soil samples were loosely packed to a volume of 80 ml and to a bulk density of 1.9 g cm^{-3} in plastic cups and put into 1.8 L of Mason jars. The jars were sealed tightly. A jar without a soil sample was also prepared and labelled as a blank. Ambient wet air was passed through a vinyl tube connected to the jar at a rate of 0.2 ml min^{-1} for 30 min to replace the gas in the jar completely. About 250 ml of an air sample was extracted from the headspace of the blank jar into a Tedlar bag by using a 50 ml syringe for NO and CO_2 analysis, and a sub-sample of 20 ml was taken from the Tedlar bag and injected into an evacuated 10 ml vial by using a 25 ml syringe for N_2O analysis. These air samples were regarded as time 0 min. The Mason jars were left at 20°C for 24 h, and then air samples were again taken in the same manner. The jars were opened after the air sampling and were immediately closed tightly to avoid change to soil moisture by evaporation from the aggregates. Incubation was conducted for 9 days. Three replications were conducted in the experiment.

Analysis of gaseous samples

Nitrous oxide concentration was determined by gas chromatography with an electron capture detector (Model GC-14B, Shimadzu, Kyoto, Japan). Carbon dioxide concentration was analyzed with an infra-red CO_2 gas analyzer (ZFP9GC11, Fuji Electric System, Tokyo, Japan). Nitric oxide concentration was analyzed with a Chemiluminescence N Oxide Analyzer (Model 265P, Kimoto Electric, Osaka, Japan).

Gas flux calculation

Gas flux (F; µg kg^{-1} h^{-1}) was estimated using the following equation:

$$F = \rho \times V / W \times \Delta c / \Delta t \times 273 / T$$

Where, ρ is the density of gas at the standard condition (µg m^{-3}); V (m^3) is the volume of the jar; W is the dry soil weight; Δc (m^3 m^{-3}) is the gas concentration change in the jar; Δt is the incubation period (h) and T is the absolute temperature. Positive flux indicates gas emission (production) from soil into the atmosphere, and negative flux indicates gas uptake (consumption) from the atmosphere.

The cumulative gas flux during incubation (mg kg^{-1} 10-d^{-1}) was estimated using the following equation:

$$\text{Cumulative } F = \Sigma (Ri \times Di)$$

Where, Ri is the mean gas production rate of the two sampling intervals (mg kg^{-1} day^{-1}) and Di is the number of days in the sampling interval (1 day).

Soil properties analysis

Before and after the incubation, concentrations of water-extractable organic C (WEOC), NH_4^+-N and NO_3^-N and soil pH, total carbon (C) and total nitrogen (N) were analyzed. Soil microbial biomass C (MBC) before incubation and potential denitrification enzyme activity (DEA) after incubation were also analyzed. All analyses were conducted with three replications. Soil samples were extracted with distilled water for measurement of WEOC, NO_3^--N and soil pH, and with 2 mol L^{-1} KCl for measurement of NH_4^+-N about two hours before and immediately after the incubation. Soil pH, NO_3^--N and WEOC concentrations were analyzed using a combined electrode pH meter (F-8 pH meter, Horiba, Japan), ion chromatography (QIC analyzer, Dionex Japan, Osaka, Japan) and a total organic carbon analyzer (Model TOC-5000A, Shimadzu, Kyoto, Japan), respectively. The NH_4^+-N concentration was analyzed using colorimetry with indophenol-blue (Uvmini-1240, Shimadzu). Total C and N concentrations of the soil samples were measured using an N/C analyzer (Sumigraph NC-1000, Sumika Chemical Analysis Service, Ehime, Japan) after soil samples were air-dried and ground.

Microbial biomass C was analyzed before incubation by chloroform fumigation-extraction method. It was calculated by the following formula:

$$MBC = \Delta C / k$$

Where, ΔC is the difference in concentration of organic C extracted by 0.5 mol L^{-1} K_2SO_4 between fumigated and non-fumigated soil (mg kg^{-1}), and k is a factor (0.43, Martens 1995).

Denitrification Enzyme Activity (DEA)

Potential DEA was determined by the acetylene block technique, which inhibits the final conversion of N_2O to N_2 gas (Tiedje, 1994). Soil samples were incubated under an anaerobic condition at 25°C with a solution treated with chloramphenicol (1 g L^{-1}) and NO_3^--N (200 mg KNO_3-N L^{-1}). Fifteen g of wet soil was placed into a 100 ml conical flask, and 15 ml of treated solution was added to the flask. The flasks were evacuated and flushed with N_2 to ensure anaerobic conditions, and acetylene (C_2H_2) gas was added to a final concentration of 10% (10 kPa) in the headspace. The headspace gas was sampled by a syringe at 2h and 4h and denitrification rates were calculated using regression coefficients obtained from plotting N_2O concentrations against sampling time.

Judgment of denitrification and nitrification

The N_2O-N/NO-N ratio is the index of N_2O production from nitrification or denitrification (Lipschultz *et al.*, 1981). The ratio is <1 during nitrification, 1-100 during both nitrification and denitrification, and > 100 during denitrification. Using the N_2O and NO production rates during the incubation, the N_2O-N/NO-N ratio was calculated for the evaluation of denitrification and nitrification.

Statistical analysis

The values of gas flux were the mean of three replications for every sample. The pH, total N and total C, WEOC, NH_4^+-N, NO_3^--N were also presented by using the mean of replications. Statistical differences in gas fluxes and soil chemical properties due to different treatments were compared using ANOVA in SPSS 13.0 for Windows 2004 (SPSS Inc. Chicago, Illinois, USA)and simple linear regression analyses were also performed by this statistical software. The calculations of standard deviation were done using Excel.

Results and Discussion

Soil chemical properties

The soil chemical properties (WEOC, NH_4^+-N, NO_3^--N, pH, total C and total N) measured before and after incubation, MBC measured before incubation, and DEA measured after incubation are shown in Table 1. A paired t-test for differences between soil chemical properties before and after incubation showed that WEOC, NH_4^+-N, pH and total N were significantly different before and after incubation. Moreover, there was a significant correlation between soil chemical properties except pH before and after incubation. In all treatments, soil moisture content (60% or 80% of FWC) and aggregate size (<2 mm or 4.5 mm), WEOC, pH and total N decreased and NH_4^+-N increased during the incubation. But Nitrate-N concentration decreased in 60% of FWC but increased in 80% of FWC in Shintoku.

Results of ANOVA showed that all the soil chemical properties, MBC and DEA were significantly higher in 4.5 mm aggregates than in 2 mm aggregates. The WEOC, NH_4^+-N, NO_3^- -N, MBC and DEA were higher in 80% of FWC than in 60% of FWC, but pH was not influenced by soil moisture. Total C and total N did not have a clear tendency though they were significantly influenced by moisture content. Higher moisture content and larger aggregate showed higher MBC and DEA than lower moisture and smaller aggregate.

Table 1. Soil properties before and after incubation from different treatments in Shintoku (SNT).

Treat-ment	WEOC		NH_4^+-N		NO_3^-N		pH		Total C		Total N		MBC	DEA
	(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})				(g kg^{-1})		(g kg^{-1})		(mg kg^{-1})	(µg kg^{-1} h^{-1})
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Shintoku														
60% of field water capacity														
4.5 mm	743	640	57	64	32	29	6.5	6.1	72	70	5.6	4.9	4435	2.5
<2 mm	242	196	24	31	25	21	6.3	5.9	115	115	7.8	7.8	3144	1.4
80% of field water capacity														
4.5 mm	1119	992	95	95	35	37	6.1	6.1	81	80	6.0	5.3	9219	3.5
<2 mm	779	620	59	74	12	14	6.2	6.0	80	79	5.8	5.1	8505	2.1
The difference of each soil chemical properties before and after incubation (Paired t-test)														
P value	<0.001		0.01		0.391		0.001		0.505		0.007		-	-
The relationship of each soil chemical properties before and after incubation														
Slope	0.884		0.876		0.908		0.037		0.983		1.050		-	-
Intercept	-25.470		14.350		1.510		5.780		0.001		-0.001		-	-
R^2	0.977		0.912		0.865		0.007		0.953		0.770		-	-
P value	0.01		0.01		0.01		NS		0.01		0.01		-	-
P value of ANOVA														
Moisture	<0.001		<0.001		<0.001		0.521		<0.001		0.015		<0.001	<0.001
Aggregate size	<0.001		<0.001		<0.001		0.01		0.001		0.047		<0.001	<0.001

Gas production rates in Shintoku

Figure 2 shows the change in N_2O production rates during the incubation. The highest N_2O production rate was observed just after the incubation started. However, the highest N_2O production rate was different between 60% and 80% of FWC. In 60% of FWC, the N_2O production rate ranged from 0.033 (2 mm aggregate) to 0.135 (4.5 mm aggregates) µg kg^{-1} h^{-1}, while in 80% of FWC, it ranged from 0.553 µg kg^{-1} h^{-1} (2 mm aggregates) to 6.35 µg kg^{-1} h^{-1} (4.5 mm aggregates). In 60% of FWC, N_2O production rate decreased gradually after the flush but slightly increased after the fifth day of incubation. In 80% of FWC, 4.5 mm aggregate also showed a similar tendency after the fifth day of incubation, while 2 mm aggregate was almost stable during the incubation period. There was also a similar tendency that 4.5 mm aggregate showed a higher N_2O production rate than 2 mm aggregate in both soil moisture contents.

Pearson's correlation coefficients of the relationship between MBC and each of the soil chemical properties before incubation, and between DEA and each of the soil chemical properties after incubation are presented in Table 2. The MBC showed significant positive correlations only with WEOC and NH_4^+-N while DEA showed significant positive relations with all soil chemical properties except total C.

Table 2. Pearson's correlation coefficient (r) of MBC and DEA to soil chemical properties.

Gas	Incubation time	WEOC		NH_4^+-N		NO_3^--N		pH		Total C		Total N	
		r	n	r	n	r	n	r	n	r	n	r	n
MBC	Before	0.846**	12	0.812**	12	-0.134	12	-0.564	12	-0.554	12	-0.510	12
DEA	After	0.948**	12	0.897**	12	0.762**	12	0.828**	12	-0.631*	12	0.630*	12

*Three replication data are used in the analysis. MBC, microbial biomass carbon; DEA, denitrification enzyme activity; *, significant at the 0.05 level; **, significant at the 0.01 level; n, sample size*

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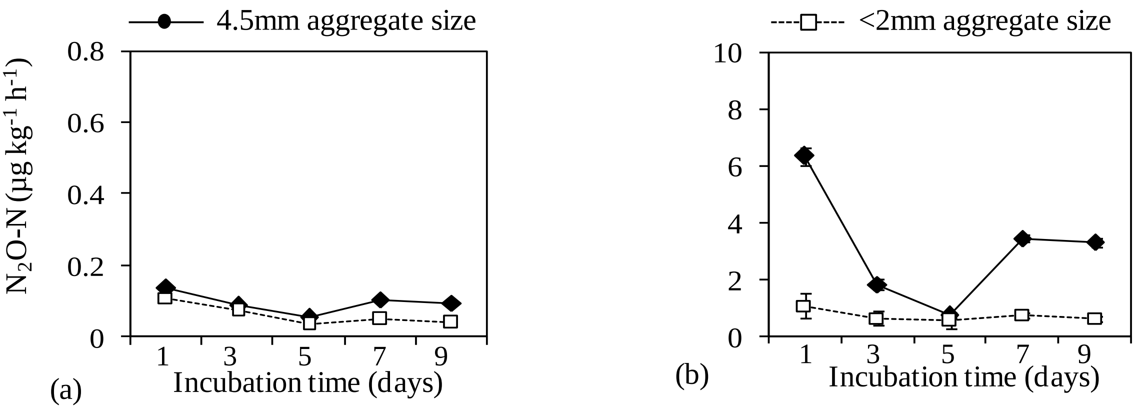

Fig. 2. N_2O production rate during incubation for the soil at (a) 60% of field water capacity (FWC) and (b) at 80% of FWC in Shintoku. Data of every treatment represents means ± standard deviation(n=3).

There was a tendency for CO_2 production rate in each soil moisture content. It was higher in 4.5 mm aggregates than in 2 mm aggregates. In 60% of FWC, the peak ranged from 3351 μg kg^{-1} h^{-1} (4.5 mm aggregate) to 2008 μg kg^{-1} h^{-1} (2 mm aggregate) until the third day after incubation started and decreased gradually to 803 to 1439 μg kg^{-1} h^{-1}. In 80% of FWC, the peak of CO_2 production rate was from 3068 μg kg^{-1} h^{-1}(4.5 mm aggregate) to 1818 μg kg^{-1} h^{-1} (2 mm aggregate) on the third day after incubation started, and decreased gradually to 898 to 1744 μg kg^{-1} h^{-1} (Fig. 3).

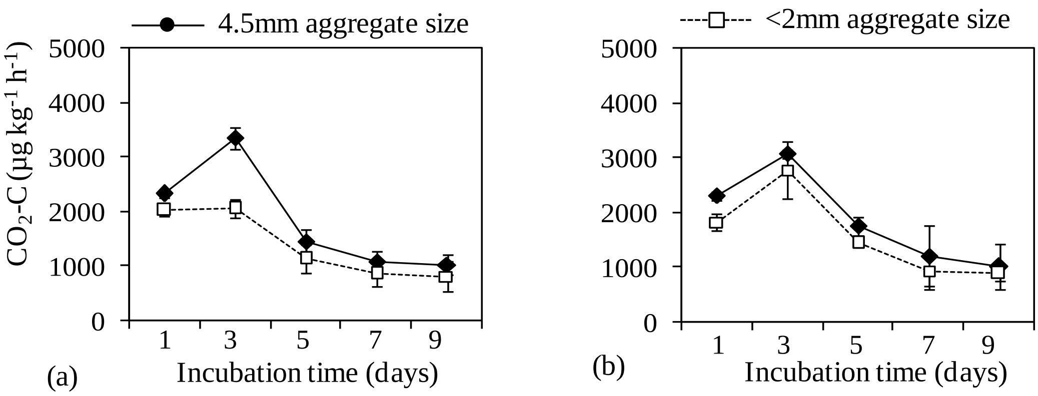

Fig. 3. CO_2 production rate during incubation for the soil at (a) 60% of field water capacity (FWC) and (b) at 80% of FWC in Shintoku. Data of every treatment represents means ± standard deviation (n=3).

Figure 4 shows the production rate of NO during the incubation. There was a flush and the peak was found just after the incubation started, which ranged from 0.051 μg kg^{-1} h^{-1} (2 mm aggregates with 80% of FWC) to 1.21 μg kg^{-1} h^{-1} (4.5 mm aggregates with 60% of FWC). The flush of NO production was larger in 60% than that in 80% of FWC. A larger aggregate showed a higher peak of NO production rate than a smaller aggregate. The NO production rate in both moisture contents decreased gradually after the flush and then after the seventh day of incubation increased slightly.

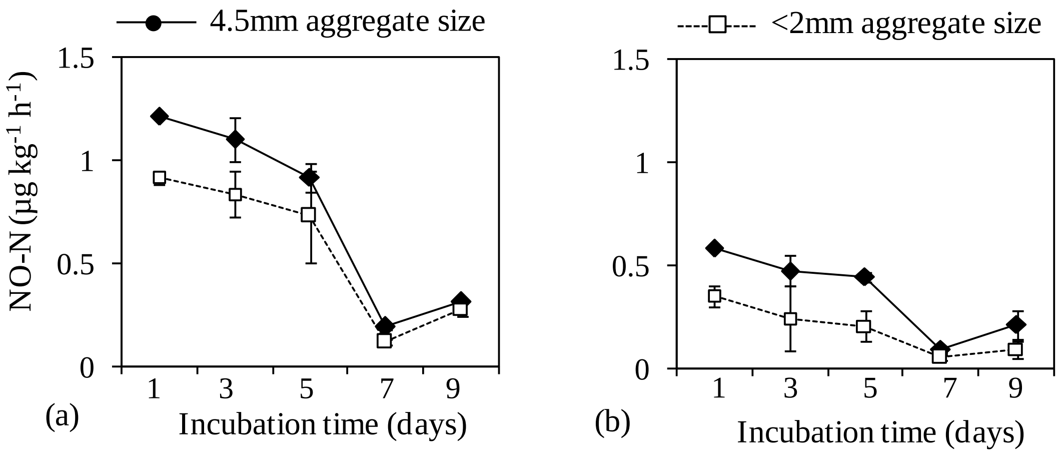

Fig. 4. NO production rate during incubation for the soil at (a) 60% of field water capacity (FWC) and (b) at 80% of FWC in Shintoku. Data of every treatment represents means ± standard deviation (n=3).

Ratio of N_2O-N: NO-N

In Shintoku, the N_2O-N/NO-N ratio mostly ranged between 0.01-1 in 60% of FWC (Fig. 5 a) and 1- 100 in 80 % of FWC (Fig. 5 b) and the N_2O production rate increased with an increase of N_2O-N/NO-N ratio from 0.01 to 1 in 60% of FWC and with a decrease of the ratio from 100 to 1. These findings indicated that the N_2O production was derived from mainly nitrification. However, in 60 % of FWC, the N_2O production rate increased with a decrease in the N_2O-N/NO-N ratio, indicating the process was mainly nitrification. However, in the 80 % FWC, the N_2O production rate increased with an increase in the N_2O-N/NO-N ratio, indicating the process was mainly denitrification.

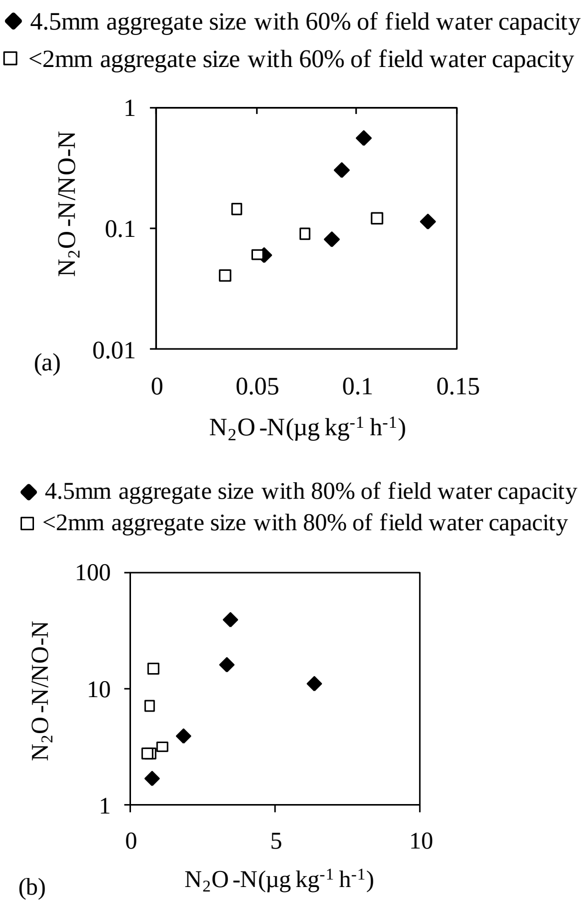

Fig. 5.The relationship between N_2O production and the N_2O-N/NO-N rate from (a) 60% of FWC (b) 80% of FWC in Shintoku.

Cumulative gas productions

Cumulative N_2O production was different between 60% of FWC and 80% of FWC (Table 3). In 60% of FWC, cumulative N_2O production was 0.012 mg kg^{-1} 9 day^{-1} for 2 mm aggregates and 0.019 mg kg^{-1} 9 day^{-1} for 4.5 mm aggregates, while it was 0.156 mg kg^{-1} 9 day^{-1} for 2 mm aggregates and 0.636 mg kg^{-1} 9 day^{-1} for 4.5 mm aggregates in 80% of FWC. The cumulative N_2O production was significantly influenced by aggregate size and soil moisture content.

Cumulative CO_2 production ranged from 294 mg kg^{-1} 9 day^{-1} (2 mm aggregates with 60% of FWC) to 406 mg kg^{-1} 9 day^{-1} (4.5 mm aggregates with 80% of FWC) (Table 3). The cumulative CO_2 production was significantly influenced by aggregate size, but not influenced by soil moisture content and there was no significant interaction among the treatments.

The values of cumulative NO production ranged from 0.039 mg kg^{-1} 9 day^{-1} (2 mm aggregates with 80% of FWC) to 0.160 mg kg^{-1} 9 day^{-1} (4.5 mm aggregates with 60% of FWC) (Table 3). The cumulative NO production was significantly influenced by soil moisture content and aggregate sizes. However, there was no significant interaction among the treatments for NO production in Shintoku Soil.

Table 3. Cumulative productions of N_2O, CO_2 and NO from different treatments in Shintoku.

Site	Moisture	Aggregate size	N_2O-N (mg kg^{-1} 9 day^{-1})		CO_2-C (mg kg^{-1} 9 day^{-1})		NO-N (mg kg^{-1} 9 day^{-1})	
			Mean	Sd	Mean	Sd	Mean	Sd
	60% of FWC	4.5 mm	0.019	0.011	401	7	0.160	0.001
Shintoku		<2 mm	0.012	0.002	294	20	0.123	0.016
(SNT)	80% of FWC	4.5 mm	0.636	0.011	406	46	0.076	0.005
		<2 mm	0.156	0.023	343	21	0.039	0.014
Sources of variation (SNT)		Df	P value		P value		P value	
Moisture		1	< 0.001		0.131		<0.001	
Aggregate size		1	< 0.001		0.001		0.001	
Moisture ×Aggregate		1	< 0.001		0.206		0.989	

Data represents ± sd. Three replication data of each treatment were used in the analysis.

Table 4 represents Pearson's correlation coefficients of the relationship between gas production and each of the soil chemical properties before and after the incubation, MBC and DEA. The results of regression analysis for cumulative N_2O production showed a significant positive correlation with WEOC and NH_4^+-N both before and after incubation. However, it was significantly correlated with NO_3^- -N after incubation, DEA and MBC. Moreover, N_2O production of Shintoku soil showed a negative relation with pH before incubation, total N and total C both before and after incubation. However cumulative CO_2 production of Shintoku soil showed a significant positive relation with WEOC and NH_4^+-N in both before and after incubation, NO_3^- -N, pH and DEA only after incubation. On the other hand, cumulative CO_2 production showed a significant and negative correlation with total C and total N before and after incubation. Cumulative NO production of Shintoku soil showed a significant positive correlation only with pH before incubation while a significant negative correlation with MBC.

Table 4. Pearson's correlation coefficient (r) of N_2O, CO_2 and NO productions to soil chemical properties, microbial biomass C (MBC) and denitrification enzyme activity (DEA).

Gas	Incubation time	WEOC		NH_4^+-N		NO_3^--N		pH		Total C		Total N		MBC		DEA	
		r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n
N_2O	Before	0.802**	12	0.854**	12	0.403	12	-0.653*	12	-0.295	12	-0.263	12	0.795**	12	-	
	After	0.910**	12	0.814**	12	0.649*	12	0.517	12	-0.284	12	-0.327	12	-		0.860**	12
CO_2	Before	0.799**	12	0.761**	12	0.519	12	0.068	12	-0.807**	12	-0.577	12	0.425	12	-	
	After	0.795**	12	0.721**	12	0.669*	12	0.895**	12	-0.779**	12	-0.734**	12	-		0.713**	12
NO	Before	-0.381	12	-0.377	12	0.544	12	0.661*	12	0.108	12	0.120	12	-.800**	12	-	
	After	-0.576	12	-0.495	12	0.330	12	0.084	12	0.084	12	0.231	12			-0.241	12

**, significant at the 0.05 level; **, significant at the 0.01 level; n, sample size*

Interaction of gases

The soil of Shintoku shows that the relationship between cumulative N_2O and CO_2 production was positive but not significant (r = 0.498, N = 12, NS) without considering the moisture content (Fig. 6a). Moreover, when the regression analysis was conducted considering the moisture content, the relationships were also positive but not significant (60% of FWC, r = 0.437, N = 6, NS and 80% of FWC, r = 0.743, N = 6, NS). The relationship between NO and CO_2 production was also positive without considering moisture content (r = 0.101, N = 12, NS) (Fig 6b). But when the analysis was conducted considering the moisture content, the relationship was significant in 80% of FWC (60% of FWC, r = 0.442, N = 6, NS and 80% of FWC, r = 0.905, N = 6, p = 0.05).

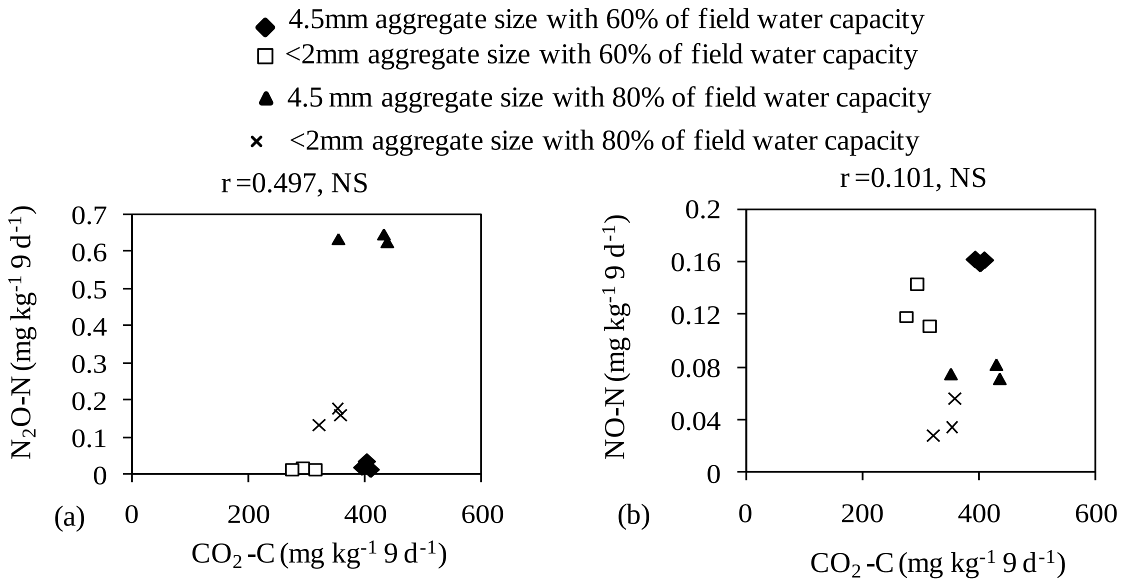

Fig. 6. Relationship between the cumulative productions of (a) CO_2 and N_2O and (b) CO_2 and NO in Shintoku. Data from three replications were used.

Discussion

Soil properties of Shintoku, especially WEOC and NH_4^+-N, changed immediately after adding the water to the air-dry soil and this situation continued throughout the incubation process (Table 1) because the added water could activate the microbial processes inside the soil. Several studies support this result and showed that WEOC concentration was higher in moist soil compared to air-dry soil (Davidson *et al.*, 1987; West *et al.*, 1992; Zsolnay, 1996). In this incubation study, in Shintoku, larger aggregates always showed higher concentrations of soil properties except for total C compared to smaller aggregates (Table 1). This result was supported by Gupta and Germida (1988) who described that macro aggregates are generally associated with larger concentrations of soil organic C, mineralizable nutrients and microbial biomass as compared with micro aggregates.

This study showed a high initial peak of N_2O production just after the addition of water to Shintoku soil. The addition of water to the dry soil can activate microbial activity by changing the aeration status. In Shintoku, cumulative N_2O production revealed a significant correlation with NH_4^+-N both before and after incubation, and NO_3^--N only after incubation (Table 4). The relationship with NH_4^+-N was stronger than NO_3^--N. These facts suggest that nitrification was a more important driver than denitrification for N_2O production, and NH_4^+-N was a reactant in this nitrification process (Lipschultz *et al.*, 1981; Heller *et al.*, 2010).

In Shintoku soil, MBC was significantly related to WEOC consumption ($r = 0.656$, $p < 0.05$, Fig. 7) and DEA ($r = 0.720$, $p < 0.01$, Fig. 8), while it was not significantly related to mineralization ($r = 0.387$, NS, Fig. 9). These facts suggest that N_2O production through the microbial activity from Shintoku soil was not effectively promoted by N mineralization ($r = 0.066$, NS) so that this soil could not develop a condition of soil mineral N suitable for high N_2O production.

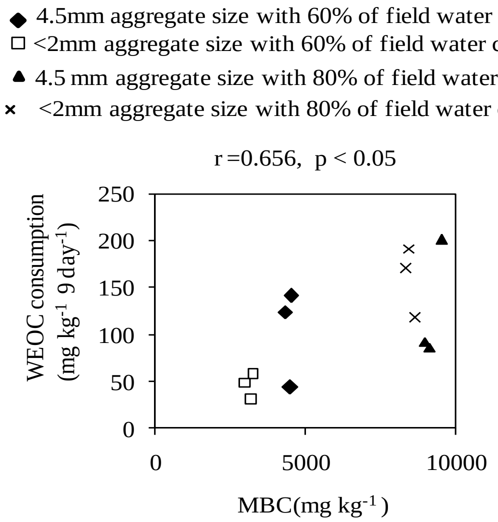

Fig. 7. Relationship between the WEOC consumption and MBC in Shintoku. Data from three replications were used.

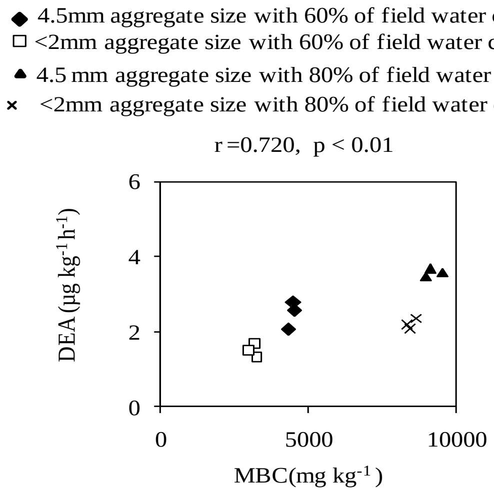

Fig. 8. Relationship between the Denitrification Enzyme Activity (DEA) and MBC in Shintoku. Data from three replications were used.

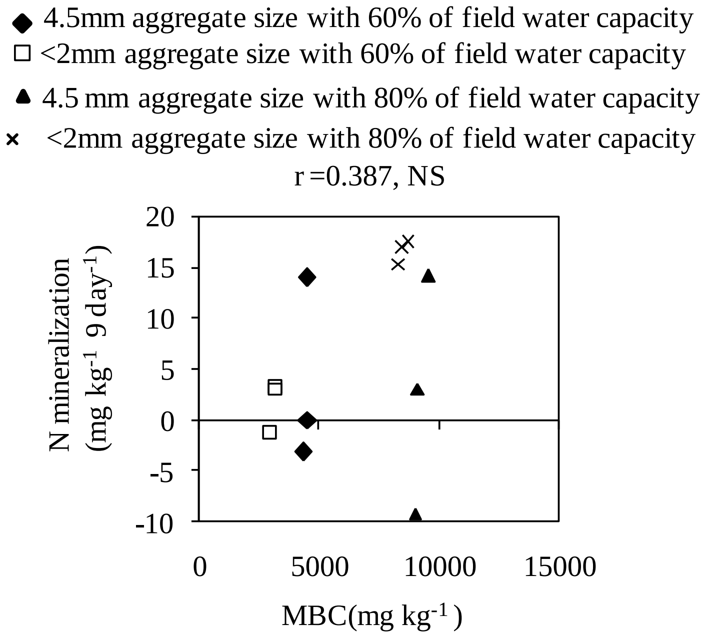

Fig. 9. Relationship between the N mineralization and MBC in Shintoku. Data from three replications were used.

This result was also supported by Connell *et al.* (1995) who found variation in N mineralization from 27 undisturbed forest soil when incubated at 20°C for 68 days at a water content near field capacity.

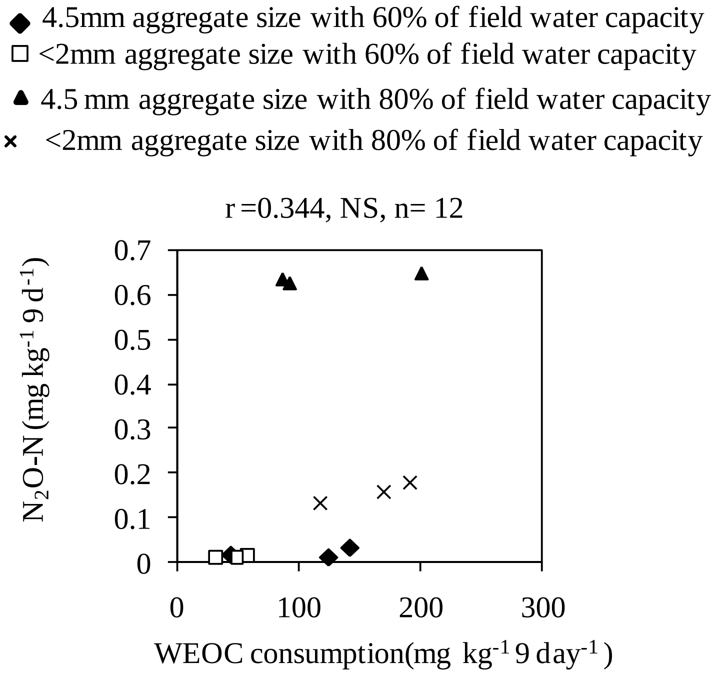

Fig. 10. Relationship between the WEOC consumption and N_2O production in Shintoku. Data from three replications were used.

A higher C/N ratio in Shintoku might result in lower N mineralization. Flush of WEOC after moistening of dry soil is a sensitive way of monitoring C mineralization in soil and is able to promote C mineralization (Merckx *et al.*, 2001). They also stated that the drying and rewetting cycle enhanced a significant increase in WEOC and later on marked decrease during moist incubation. Lower WEOC consumption of Shintoku soil indicates lower C mineralization, lower N mineralization and lower N_2O production. The WEOC consumption was significantly correlated with MBC ($r = 0.656$, $P < 0.05$). Comparing WEOC consumption and CO_2 production, all samples produced higher CO_2 than WEOC consumption (Fig. 11). This result suggests that WEOC was consumed only by decomposition.

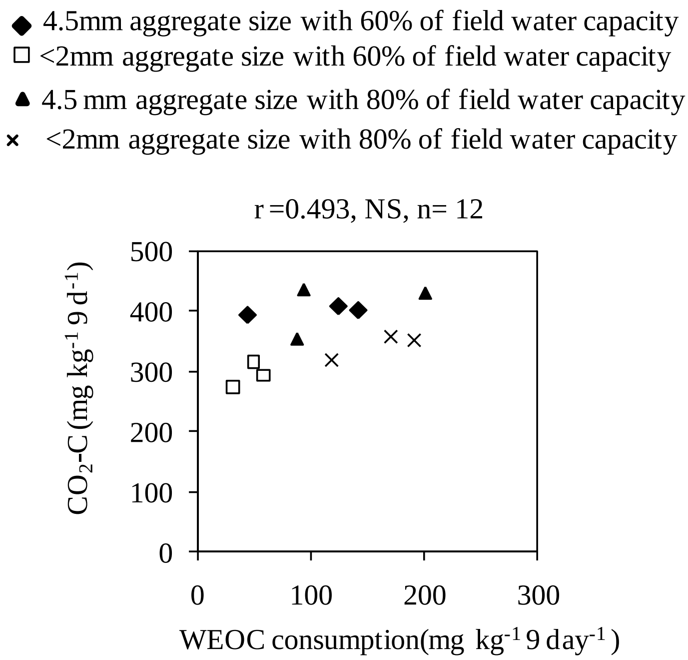

Fig. 11. Relationship between the WEOC consumption and CO_2 production in Shintoku. Data from three replications were used.

In Shintoku the ratio of N_2O-N/NO-N in 60% of FWC mostly ranged between 0.01-1 (Fig. 5a) while the N_2O production rate increased with an increase of N_2O-N/NO-N ratio from 0.01 to 1, indicating the N_2O production during incubation was derived absolutely from nitrification. In the case of 80% of FWC, the ratio of N_2O-N/NO-N of Shintoku soil mostly ranged between 1-100 (Fig. 5b) and the N_2O production rate increased with the decrease of N_2O-N/NO-N ratio from 100 to 1 indicating the N_2O production during incubation was derived mostly from nitrification.

In Shintoku larger aggregates with both moisture contents showed higher N_2O production than smaller aggregates, because of the association of higher amounts of NH_4^+-N and NO_3 -N with larger aggregates (Diba *et al.,* 2011). Lower aeration might limit the aerobic area at the surface of aggregates and reduce organic matter decomposition, N mineralization and nitrification. Anaerobic volume can develop in the center of aggregates, and diffused NO_3^- -N from the aerobic area to the anaerobic area is denitrified. However, fine texture leads to low diffusion of NO_3^- -N in the soil. The diffusion of WEOC required for denitrification also be low in fine-textured soil. Limitation of the supply of those substrates from the aerobic area to the anaerobic area for denitrification resulted in the reduction of denitrification and less N_2O production in Shintoku, Japan.

Conclusion

The effect of different moisture content and aggregate sizes on N_2O production was found in Shintoku soil. However, this study revealed that soil chemical properties and their changes through C and N mineralization are regulated by soil texture. Consequently, significant and remarkable differences were observed in terms of WEOC consumption and N mineralization which ultimately can influence nitrification and denitrification. So, it can be concluded that not only the soil water content and aggregate size but also soil type (texture) is an important factor in N_2O production in soil.

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