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The Great Cormorant (*Phalacrocorax carbo*) colony as a "hot spot" of nitrous oxide (N_2O) emission in central Japan

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ABSTRACT

Unusual high soil fluxes up to ca. 500 mg N_2O m^{-2} h^{-1} emission were associated with a continued breeding/roosting colony of Great Cormorant in central Japan. This flux is nearly two-orders of magnitude higher than those hitherto documented. The flux was markedly dependent upon the soil surface temperature, i.e., higher in April–October during the prevailing high air temperatures, as compared with November to March. Integrated input of fecal N at rearing and fledging stages of chicks followed by coupled mineralization, nitrification and subsequently denitrification processes under humid and temperate regimes is responsible for such an unusual flux. The Great Cormorant colony serves as a "hot spot" of N_2O emission of natural origin.

1. Introduction

Nitrous oxide (N_2O) is an atmospheric trace gas that at presence originates predominantly from microbial transformation of anthropogenic N (inorganic nitrogen in the form of chemical fertilizer and organic N wastes) inputs into soils. Due to its effects on the radiation balance and on stratospheric ozone, numerous studies have been conducted to identify and to quantify N_2O sources (IPCC, 1992). The anthropogenic organic N (mainly animal excreta) undergoes mineralization to produce ammonium ions, which are subjected to nitrification (microbiological oxidation of soil ammonium to nitrite and nitrate) and subsequent denitrification (microbiological reduction of soil nitrate to gaseous N compounds) where N_2O is an intermediate product of these processes. N budget and soil temperature-moisture regimes have been clearly identified among known environmental factors regulating N_2O emission from soils (Zhu et al., 2008a, b; Holst et al., 2007).

In addition to livestock related N of anthropogenic origin, distinctly high fecal N input (Kameda et al., 2006; Kolb et al., 2010) and resulting high concentration of ammonium- and nitrate-N in soils (up to several thousand mg-N kg^{-1} dry soil) affected by piscivorous avian rookeries has been documented from tropical rainforest (Fiji, South Pacific: Mizota and Naikatini, 2007) and warm-humid regions (Japan: Mizota et al., 2007; Mizota, 2009a, b). Mineralization of fecal N, nitrification and subsequent denitrification is facilitated under suitable ambient temperature and moisture conditions (Mizota et al., 2006; Mizota, 2009a, b). The process was evidenced by the use of N stable isotope ratios of ammonium – together with associated nitrate-N in soils, because $\delta^{15}N$ represents an integration of N cycles (Robinson, 2001). Based on published documents, avian colonies tend to become natural hot spots of N_2O and other gas emissions, since the flux is proportional to N input and exponentially increases when soil pH values tend to be lower (pH (H_2O) ~ 3.1; Tokuda and Hayatsu, 2001) as commonly observed for colonies of piscivorous birds such as cormorant (pH (H_2O) ~ 2.6; Hobara et al., 2005). Nevertheless, there are very few studies on N_2O emission flux from avian colonies, except for mangrove sediments (Corredor et al., 1999) and the maritime Antarctic (penguin, skua and other animals: Zhu et al., 2008a, b, 2009a, b) where ammonium-N (60 mg kg^{-1} dry soil) and nitrate-N (40 mg kg^{-1} dry soil) concentration is two-orders of magnitude lower than values observed in the tropics and warm-temperate regions (up to several grams of inorganic N per kg of dry soil: Mizota et al., 2006, 2007, 2009a, b). Measurement of N_2O flux in combination with isotopic information on both inorganic soil N is required for evaluating the dynamics of N in soils as influenced by avian colonies.

The objective of the present study is to characterize temporal changes (April to late December, 2009) in N_2O emission flux from soils under a Great Cormorant colony in Japan as affected by soil temperature and avian population. Among the known gas components emitted during the microbial transformation of fecal N from piscivorous avian, numerous studies of stable isotopes

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(Mizutani et al., 1985, 1986; Kameda et al., 2006), and chemistry (Wilson et al., 2004; Blackall et al., 2007, 2008; Kolb et al., 2010) on the NH_3 volatilization have been made. On the other hand, concomitant N_2O emission is not fully understood.

2. Materials and methods

2.1. Location of study site

An established colony of the Great Cormorant in Gyotoku, Chiba, central Japan (35°39′53″ N, 139°55′07″ E) was selected for this study. Mean annual precipitation recorded nearby Tokyo (from 1961 to 1990: 35°41′N, 139°46′E) is 1400 mm distributed throughout the year. The soils are never subjected to drying. The Great Cormorant tends to build nests on the skeletal branches of the canopy of the dominant *Pinus thunbergii*. The study site is located nearby the seashore. *P. thunbergii* at the site was planted in 1972 for native fauna conservation. Migratory settlement and subsequently breeding activity was initiated in 1995 and has been continuous until present. Annually, single breeding (January–August) of the Great Cormorant is common in southwestern Japan (Kameda et al., 2006). Nevertheless, bivoltine (breeding twice in one year) and subsequent roosting continue throughout the year at the site. First incubation starts from late December and continues up to early February, following hatching and rearing from mid February to early March and fledging up to around mid March. Successive second incubation starts from mid March and continues up to late April, following hatching and rearing from early to mid May and fledging up to late June (S. Hasuo, Private communication).

Detailed scientific description of the temporal effects of breeding activity on the floral composition of forest stand is insufficient for the site. Fragmental information has been obtained by local observations (Hasuo, S., Private communication). Expansion of the current avian population is likely the result of anthropogenic disturbance of their previous habitats. The study site is located on protected areas (Natural Reservoir of Avian and Animals; ca. 1000 × 25 m), where human disturbance is minimal during all year. The surface of the understory leaves is covered by fine whitish droppings due to high input of feces. Wilted and dead woody plants can be observed under intensive breeding/roosting areas.

2.2. Temporal changes in population dynamics of the Great Cormorant

One population of roosting Great Cormorants was censused using stereoscope from the 3rd floor of the main building of Gyotoku Observatory roughly every three days during 2002–2010. The counting was made nearby sunset, since total populations (sum of adult and chick at all stages of their development) of the Great Cormorant are then easy to identify and count.

2.3. Collection of fecal droppings

Intermittent collection of fecal deposits from the Great Cormorants was made from surface of the living tree's leaves from January to April 2009. The moist sample droppings were removed and well mixed together for measuring N isotope ratios. Two to three composite samples were prepared.

2.4. Quantitative determination of N_2O concentration in soil gas samples

According to previous studies (Ishizuka et al., 2002; Dittert et al., 2005; Arai et al., 2008), soil gas samples were collected using a static gas chamber in the morning. Stainless steel chambers (14.4 cm upper diameter, 23.5 cm high) were inserted into the soils to a depth of 2 cm before sampling. After sealing the chambers with lids containing a sampling port and an air bag to equilibrate the inside pressure to atmospheric pressure, we took 50-ml gas samples with a syringe after 0, 10, 20, and 30 min. The gas samples were ejected into previously evacuated 20-ml glass vials with butyl rubber stoppers. These glass vials were analyzed in the laboratory (Faculty of Agriculture, Meiji University) for the concentrations of N_2O with a gas chromatography (6890N, Agilent Technologies, Tokyo, Japan, Ltd.) equipped with an electron capture detector. The measurement was made within three hours after gas collection. The increase in gas concentration in the chamber during this sampling period appeared linear, therefore we calculated the gas flux by linear regression.

N_2O emissions from soils can show a high degree of a spatial heterogeneity (Folorunso and Rolson, 1984; Laville et al., 1999; Rover et al., 1999; Choudhary et al., 2002; Zhu et al., 2008). Prior to monthly collection and measurement of N_2O flux from soils, suitable numbers of gas chambers were assessed to establish the protocol. Nine chambers were installed on soils under an active breeding colony of the Great Cormorant, and soil gas samples were collected on 23 May 2009. The site was selected to represent a mid-point of actively breeding–roosting areas where living understory grasses are absent or nearly absent. Gas samples were also collected from a control site, about 20 m away from the forest where nesting and roosting activities were not observed.

Basing on the evaluation of spatial variation of N_2O flux, time (limited duration of entry in the natural reserve area) and labor cost, three chambers for triplicate measurements were installed on the soil surface with a spatial interval of 40 cm. Fresh litter was removed during gas collection. Collection of soil gas samples continued nearly monthly from early April to late December (2009), in parallel with soil temperature measurements. Sampling of soil gas was made only after 3 days of fine weather to equilibrate soil moisture conditions. Continued daily measurement was made from 12 to 18 November (2009) to examine the immediate effects of rainfall events on the flux. Surface soil temperature of the site was determined by a digital thermometer.

2.5. Chemical and isotopic analysis of soil samples

Composite soil samples were collected from several points nearby the gas sample site at 0–5 cm depth. Samples for the control site were also collected from comparable depth on the same day. In order to use available chemicals and isotopic measurements efficiently, the number of soil sample replicates was changed from four (January 1, 2009), to three (November 22, 2008), to two (February 2 and April 5, 2009) and finally to a single sample (since July 15, 2009). Intermittent sampling of soils as influenced by breeding activity of the Great Cormorant was initiated from 22 November (2008) and ended on 3 March (2010). The composite soil samples were brought to a laboratory in the Faculty of Agriculture, Iwate University. Fresh soil samples were extracted by ten-fold 2 M KCl solution (5.0 g of moist soil with 50 ml of the solution), and determined for ammonium- and nitrate-N content using steam distillation apparatus. Using the same steam distillation technique, the aliquots containing both ammonium- and nitrate-N (equivalent to 200–300 μg of nitrogen) were converted into ammonium sulfate and dried on GF/F glass fibre (Whatman Co. Ltd.). Fine-grained Devarda's alloy was used for chemical reduction of nitrate into ammonia. $\delta^{15}N$ values of all forms of N compounds were determined using GV mass spectrometer. Nitrogen isotope ratios ($\delta^{15}N$) were express as per mill deviation relative to atmospheric N ($\delta^{15}N = 0‰$). Analytical precision of the overall process is better than $\pm 0.2‰$.

3. Results and discussion

3.1. Population dynamics of breeding/roosting Great Cormorant (2002 to early 2004)

Temporal changes in the total population of the Great Cormorants in Gyotoku Natural Reserve Chiba, central Japan is shown in Fig. 1. Monthly population tended to be lower in December to early February (lower air temperature prevails), but quickly increased from April and attended maximum in June to early October (higher air temperature prevails). Such a seasonal variability may reflect the available prey sources around the river estuary of Tokyo Bay where the Great Cormorant forages.

3.2. Nitrogen concentration and stable isotope composition of fecal droppings

Total N concentration and N stable isotope composition ($\delta^{15}N$) of representative fecal droppings are shown in Table 1. The average N content was fairly high in the range of 15.0–22.5%, reflecting the piscivorous nature of the Great Cormorant. The average $\delta^{15}N$ values of fecal samples were in a narrow range from +15.2 to +15.4‰. The isotopic values were somewhat higher than those of the fecal droppings of the Great Cormorant from the fresh water Lake Biwa, southwest Japan ($\delta^{15}N = +13.2 \pm 1.3‰$, $n = 12$: Kameda et al., 2006). The higher isotopic trend of the fecal N in Gyotoku Natural Reserve may reflect the integrated anthropogenic N sources from heavily populated urban areas around capital Tokyo.

3.3. Temporal changes in concentration and stable isotope composition of inorganic soil N

Content of inorganic N in soils under the influence of breeding/roosting activity of the Great Cormorant was clearly higher than those of the control site (Table 2). Soils under the control site contained very low NH_4^+-N (0–30 mg-N/kg dry soil) and NO_3^--N (20–80 mg-N/kg dry soil). There could be seen a temporal change in both NH_4^+ and NO_3^--N in soils as influenced by the dynamics of breeding/roosting activity. Very high content of inorganic N in the breeding/roosting site soils was observed for samples collected from 22 November (2008) to 5 April (2009). Afterwards the content clearly decreased by one order of magnitude from 15 July (2009). Such an observed trend is difficult to interpret. It is probably resulted from the special shift in input N from the Great Cormorant, or elevated soil surface temperature. Both reasons are plausible. Highly heterogeneous distribution of the Great Cormorant colony in the forest area has been shown on several locations (Mizota et al., 2007).

Table 1
Nitrogen content and stable isotope composition ($\delta^{15}N$) of fecal deposits collected from Gyotoku Natural Reserve.

Date of collection (2009)	Nitrogen content (%)		$\delta^{15}N$(‰)	
	Sample	Average	Sample	Average
6-Jan	14.3; 14.8; 15.9	15.0	+15.2; +15.6; +14.9	+15.2
20-Feb	16.5; 15.4	16.0	+16.8; +13.9	+15.4
5-Apr	17.8; 25.9; 23.9	22.5	+15.8; +15.2; +14.8	+15.3

With few exceptions, higher content of NO_3^--N, relative to that of the associated NH_4^+-N is evidence of integration of mineralization and subsequent nitrification as commonly observed for ornithogenic soils under humid and temperate climatic conditions.

In comparison with $\delta^{15}N$ values of the input fecal N (Table 1), both, NH_4^+- and NO_3^--N in soils influenced by the life history of the Great Cormorants are characterized by distinctively elevated values through integration of N cycles (Robinson, 2001). Coupled processes of mineralization of fecal N following volatilization of NH_3, nitrification of NH_4^+ and denitrification of NO_3^--N prevailed in soils in the present study, as previously documented in similar piscivorous avian colonies (Black-tailed Gulls and Heron: Mizota et al., 2007; Mizota, 2009a, b).

3.4. Temporal changes in soil surface temperature

Temporal changes in soil temperatures during flux measurement of N_2O emission are shown in Fig. 2. Since the study site is located in a temperate region of the Northern Hemisphere where the seasonal changes in solar radiation are marked, a clear temporal trend of air temperature exists, showing higher values in April/November, while lower trend in December/February. Higher temperatures prevail under the breeding/roosting colony relative to the control site during early May to Late September. The canopy of the forest under the control site is nearly closed, while direct sunlight reaches the soil surfaces under the breeding/roosting colony due to partial loss of canopy associated with intense

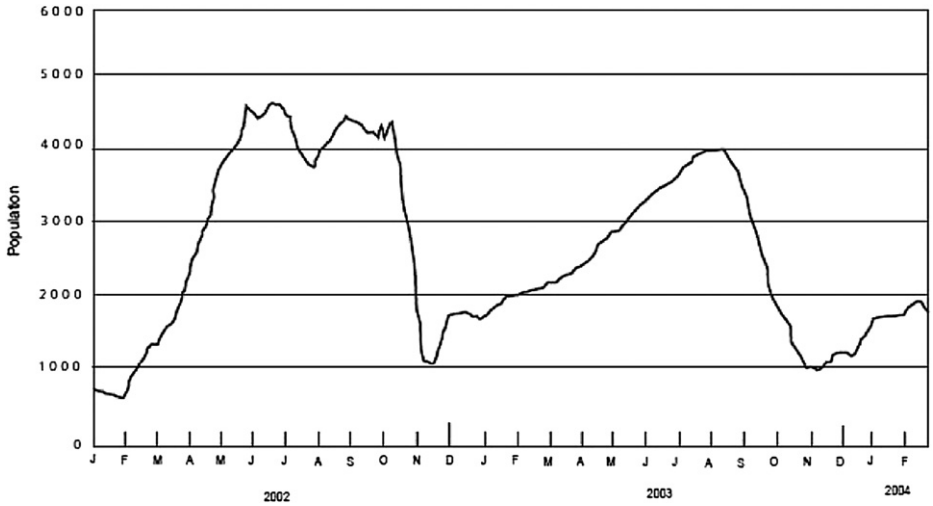

Fig. 1. Monthly changes in the population (sum of adult and chick at all development stages) of breeding/roosting Great Cormorant in Gyotoku Natural Reserve, Chiba, central Japan. The figure was reproduced from http://www.pref.chiba.lg.jp.

Table 2
Temporal changes in the content and nitrogen isotope composition ($\delta^{15}N$) of inorganic nitrogen in soils.

Date of collection (2009)	Site	Content (mg-N/kg dry soil)				$\delta^{15}N$ (‰)			
		NH_4^+		NO_3^-		NH_4^+		NO_3^-	
		Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
22 November (2008)	Breeding	2880 ($n=3$)	890	3190 ($n=3$)	1240	+46.3 ($n=3$)	5.6	+30.8 ($n=3$)	3.7
	Control	0		80		–		+6.4	
1-Jan	Breeding	9850 ($n=4$)	13,090	3530 ($n=4$)	2600	+30.7 ($n=4$)	1.9	+20.1 ($n=4$)	4.6
	Control	0		70		–		+6.5	
22-Feb	Breeding	3820 ($n=2$)		510 ($n=2$)		+31.8 ($n=2$)		+22.7 ($n=2$)	
	Control	0		40		–		+2.6	
5-Apr	Breeding	1960 ($n=2$)		3020 ($n=2$)		+42.1 ($n=2$)		+27.4 ($n=2$)	
	Control	20		40		+12.0		+1.6	
15-Jul	Breeding	280		420		+23.3		+27.6	
	Control	0		20		–		ND*	
3-Aug	Breeding	130		210		+25.8		+23.7	
	Control	30		50		ND*		ND*	
30-Oct	Breeding	70		130		+32.5		+23.7	
	Control	20		50		ND*		ND*	
16-Nov	Breeding	70		130		+38.6		+29.8	
	Control	20		50		ND*		ND*	
3 March (2010)	Breeding	290		230		+31.6		+33.3	
	Control	30		50		ND*		ND*	

ND*, not determined due to insufficient recovery of nitrogen for mass spectrometry.

destructive activities of the Great Cormorant upon the vegetation. The Great Cormorant commonly uses fresh leaves and twigs nearby the nest for their nesting materials.

3.5. Temporal changes in flux of N_2O emission from soils as affected by soil surface temperature

N_2O concentrations of gas samples (23 May, 2009) collected from nine chambers under the active breeding colony of the Great Cormorant were 23.5 mg N_2O m^{-2} h^{-1} with a standard deviation of 14.7 mg N_2O m^{-2} h^{-1}, whereas those from the control site without influence of the activity were 340 ± 320 μg N_2O m^{-2} h^{-1}, respectively (Fig. 3a, b). Data indicate highly variable N_2O flux from soils, as documented for different types of soils and sediments around the world. Such high heterogeneity mostly results from spatial variability of inorganic N in soils under piscivorous avian colonies (Mizota, 2009a, b).

Temporal changes in N_2O emission was measured from 21 February to 23 December (2009) where soil surface temperature varies from 4.5 to 28.6 °C. There is a clear temporal variation in the flux from both breeding/roosting and control sites. The flux parallel synchronizes with varying ambient temperature, but not with the temporal changes in content of inorganic nitrogen in soils (Table 2).

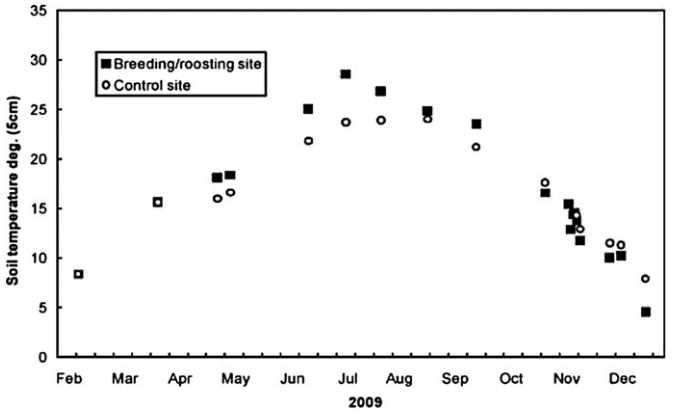

Fig. 2. Temporal changes in soil temperatures (°C) under a breeding/roosting colony of Great Cormorant (closed square) and control (open circle) sites.

The N_2O flux from the breeding/roosting site was low on 21 February (8.8 ± 8.1 mg m^{-2} h^{-1}) and on 23 December (4.0 ± 4.7 mg m^{-2} h^{-1}) when the surface soil temperatures were below 8 °C. The latter value represents the lowest flux from breeding/roosting site when the soil temperature was at 4.5 °C. Nevertheless, such values are two-orders of magnitude higher than those reported from avian colony soils from the maritime Antarctic (mean 0.09 mg m^{-2} h^{-1}; Sun et al., 2002; 0.2–1.3 mg m^{-2} h^{-1}: Zhu et al., 2008a, b).

With increasing soil temperature towards April and May, flux of the N_2O emission from the breeding/roosting site markedly increased from 34.0 ± 10.7 (5 April), 242.6 ± 41.9 (7 May) and then 412.9 ± 362.2 mg m^{-2} h^{-1} (14 May), and reached a maximum (507.3 ± 225.4 mg m^{-2} h^{-1}), when soil surface temperature was 25.0 °C on 25 June. Data also suggests that the N_2O emissions are

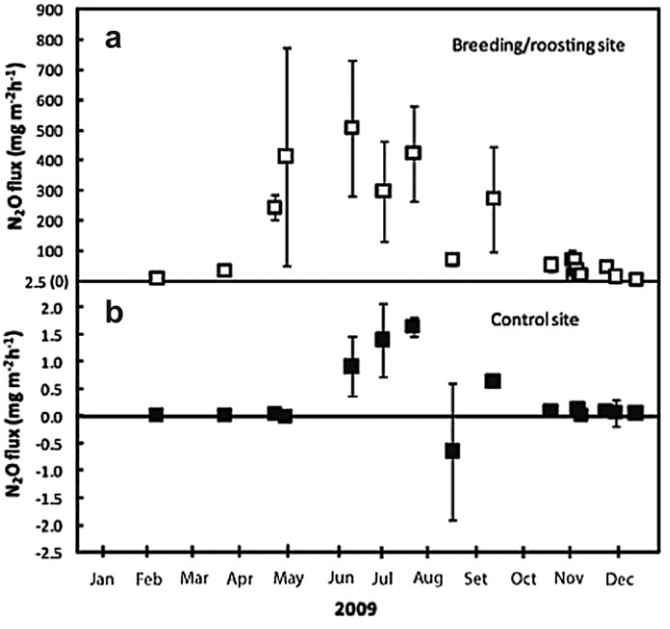

Fig. 3. Temporal changes in N_2O flux from soils under a breeding/roosting colony of the Great Cormorant (a) and under the control site (b). Vertical bars indicate the standard deviation.

linked to the input of excretion by the Great Cormorant and that as the population increases at the stages of rearing and post-fledging, the N input to the soil will increase too (Fig. 1). The flux of N_2O emission, then gradually decreased down to 14.9 ± 13.7 mg m^{-2} h^{-1} with decreasing soil temperature towards 10 December (10.2 °C). N_2O emission from 7 May (242.6 ± 41.9) to 3 August (423.3 ± 157.1) and 23 September (272.2 ± 174.1 mg m^{-2} h^{-1}) was nearly two-orders of magnitude higher than sites documented as "hot spots" from sheepfolds in northern Inner Mongolia, People's Republic of China (an average flux of 3.9 mg N_2O m^{-2} h^{-1}: Holst et al., 2007). We are unaware of an N_2O flux of such magnitude being reported for any location or emission source in the world.

Emission of nitrogenous gas components, particularly NH_3, as a result of excretal input from piscivorous avian (mainly seabirds) is documented at regional and global scale (Wilson et al., 2004; Blackall et al., 2007; Zhu et al., 2011). The source of the NH_3 emissions is mostly the same as those for N_2O emission that implies more widespread emission of N_2O from seabird colony. Since the seabird populations occur worldwide (Ellis, 2005), further data acquisition for regional and global estimation of the fluxes of N_2O emission of relevant colonies is required.

Flux of N_2O from the control site without visible sign of breeding/roosting activity of the Great Cormorant also showed a trend of soil temperature dependence (Fig. 3b). High fluxes were observed for gas samples collected on 25 June (0.9 ± 0.5), 15 July (1.4 ± 0.7), 3 August (1.7 ± 0.2) and 23 September (0.6 ± 0.1 mg N_2O m^{-2} h^{-1}), when higher soil surface temperatures prevailed (21–24 °C: Fig. 2). The observed trend also corresponds that observed for fluxes in the Great Cormorant population (Fig. 1). It is possible that there was some input of fecal N into the control site, since the control site is located ca. 20 m away from the peripheral of the breeding/roosting site, and cormorants could easily have been there. The mean N_2O flux from 25 common Japanese forest soils without input of avian N was documented 11 ± 11 μg N_2O m^{-2} h^{-1} (standard deviation) (Nishina et al., 2009) which is clearly lower than the control site.

Temporal changes in fluxes of CH_4 (methane) emission were also measured for the same gas samples. Values ranged from negative (−90 μg) to 50 μg m^{-2} h^{-1} (data not shown). Unlike N_2O, CH_4 is a very minor gas component emitted from the breeding/roosting colonies of the Great Cormorant.

3.6. Effects of rainfall events on the emission of N_2O from soils

Daily collection of gas samples from soils under the Great Cormorant breeding colony was continued from 11 November to 19 November (2009). During this period, daily rainfall was recorded 83.5 mm (11 November), 4.5 mm (13 November), 22.5 mm (17 November) and 9.0 mm (19 November), respectively. There was no correlation between the soil moisture content and N_2O flux (data not shown).

4. Conclusions

High N_2O emissions amounting to 500 mg m^{-2} h^{-1} were observed for soils under the influence of continuous breeding/roosting of the Great Cormorants in central Japan. N_2O emissions resulted from integrated fecal N input coupled with mineralization, nitrification and denitrification under suitable soil conditions of the warm and temperate climates. Such high fluxes have never been documented worldwide.

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