

Original paper**CH₄-, N₂O- and H₂- fluxes and microbial processes
in Kushiro Wetland**

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Abstract

Fluxes of radiatively active gases such as methane and nitrous oxide at different vegetations (low moor, alder forest and high moor) in Kushiro wetland, Hokkaido Japan, were measured by the chamber method in summer seasons from 1993 to 1997. Hydrogen gas fluxes were also measured simultaneously with methane fluxes by SCD (Semi-conductor detector) GC in order to understand microbial metabolism in the wetland. Distribution of several microbial groups concerning with nitrogen cycling were also studied for understanding nitrous oxide fluxes. Following results were obtained.

- (1) Methane fluxes at low moor sites were 2-3 times higher than those in another vegetation sites, and correlated loosely with average air temperature during observation period.
- (2) In contrast to methane fluxes, dissolved methane concentrations in surface water at low moor site were the lowest, and those in high moor site were the highest.
- (3) Absorption and emission fluxes of hydrogen were observed at high and low moor, respectively. These results suggest different mechanism of methane production between high and low moor sites.
- (4) Free living nitrogen fixer was dominant than the other groups of nitrogen cycling bacteria in high moor and it was not detected in low moor where aerobic heterotrophs, denitrifiers and NH₄-oxidizers were most abundant.
- (5) Surface water both in low and high moor was under-saturated with dissolved nitrous oxide. Influxes of nitrous oxide into low and high moor were sometimes observed. Absorption mechanisms of nitrous oxide in low and high moor were discussed.

Key words: methane flux, nitrous oxide flux, hydrogen flux, nitrogen cycling bacteria, Kushiro wetland.

Introduction

Wetlands are recognized as one of the largest natural source for methane, which is radiatively 21 times (in mole base) active compared with carbon dioxide. According to a report of IPCC (1995), 115 TgCH₄/yr is emitted from natural wetlands among total emission of 160 TgCH₄/yr

from natural source and 375 TgCH₄/yr from anthropogenic source including 60 TgCH₄/yr from artificial wetlands of paddy field. Whereas it is yet uncertain whether natural wetlands are sources or sinks for nitrous oxide, which is also radiatively 206 times (in mole base) active than carbon dioxide. It is important for wise use of

wetland to know or estimate the role and worth of wetland. It is uncertain but an interesting subject to be clarified whether natural wetlands contribute to recent increase in atmospheric methane and nitrous oxide concentrations or not. The purposes of this study are to measure fluxes of methane and nitrous oxide at different vegetations (high moor, low moor and alder forest moor) in Kushiro wetland by the chamber method, to discuss possible mechanisms of emission and/or absorption of these gases in a natural wetland with additional data of hydrogen gas flux and microbial population.

Study field and methods

The Kushiro wetland (43°09'N, 144°26'E) in eastern Hokkaido is the largest wetland in Japan with an area of 180 km², 77km² of which are registered in Ramsar Convention. About 70% of the area is low moor dominated by reed (*Phragmites*) and sedge (*Carex*). Some 10% is high moor dominated by sphagnum (*Sphagnum*), and 20% is low moor forested with Japanese alder (*Alnus*). These three different vegetations mingle with each other in Southwest part of Kushiro wetland in the Onnenai area, where there is a log path trail extending for 2 km. In order to measure the fluxes of methane and nitrous oxide in this wetland, we used cylindrical flux chambers, made of acrylic resin measuring 50 cm in diameter and 1 m in height, equipped with a battery driving fan to mix the chamber air before sampling. The chamber air samples were taken into evacuated glass vials through rubber septum by a gas-tight syringe with a final pressure of 1.5 atm or more. Methane fluxes were calculated from the increasing rate of methane concentration in the chamber air during 2-3 hours except for night time, when they were calculated from an accumulation rate during overnight. Although it may be possible that night time flux of methane is underestimated, it was not always lower (Fig.1). Methane and hydrogen were measured by SCD (Semiconductor detector)-GC with a charcoal column (Ohta et al. 1999) and nitrous oxide by

ECD (Electron capture detector)-GC with a GS Molesieve plot column. Atmospheric concentrations of methane and hydrogen were also monitored continuously with an automatic open-close chamber and sampling-injection device at low moor site dominated by sedge and trefoil vegetations.

Water quality and concentrations of constituents in surface and interstitial water (sampled with ceramic pore water samplers), including dissolved gases, were also monitored as background information.

Bacterial populations were measured by the viable count method with agar plate for aerobic heterotrophs and free-living nitrogen fixer, and MPN method with liquid media for denitrifiers and nitrifiers. Surface 15cm soils were sampled by a steel soil corer with 33mm diameter at 4 sites in high moor, 3 sites in alder moor and a single site in low moor. Each sampled soil was suspended and diluted in sterilized distilled water at Onnenai visitor center immediately after sampling. Diluted samples were inoculated into the following media and incubated at 20 °C: 1/100 of nutrient broth agar for aerobic heterotrophs, 1/100 nutrient broth with addition of 0.3% KNO₃ for denitrifying bacteria, inorganic nutrient media with addition of (NH₄)₂SO₄ or KNO₂ for NH₄-oxidizing or NO₂-oxidizing bacteria, respectively, and nitrogen free purified agar media for free-living nitrogen fixing bacteria.

Meteorological data were supplied from solar battery-driven meteorological observatory in the high moor by courtesy of Prof. F. Nishio at Hokkaido University of Education, Kushiro Campus.

Results and Discussions

First, we compared the background environment between the studied sites. Table 1 shows surface water qualities at different vegetation sites in three types of moor. The high moor showed 1 to 1.3 unit lower pH and 4-5 times higher DOC concentrations than low moor. It may be due to humic substance rich in high

Table 1 Comparison of the surface water quality between different vegetation sites in Kushiro wetland in August 1994.

	Temp (°C)	pH	Eh (mV)	DOC (mgC/l)	Anion		(μM)	Cation	
					Cl ⁻	NO ₃ ⁻		PO ₄ ³⁻	NH ₄ ⁺
Low moor (n=10)	20.1 (0.8)	6.4 (0.3)	287 (140)	11.5 (1.3)	22.7 (10.3)	0.81 (0.3)	1.1 (6.2)	0.3 (0.3)	0.9 (0.3)
Alder moor (n=4)	19.9 (0.2)	5.8 (0.1)	178 (76)	23.1 (7.3)	10.4 (12.4)	1.1 (0.5)	2.3 (1.3)	2.1 (1.3)	19.6 (14.1)
High moor (n=5)	19.9 (1.5)	5.1 (0.4)	281 (205)	49.4 (10.3)	16.5 (15.0)	2.0 (1.4)	3.1 (1.4)	0.4 (0.2)	8.1 (7.3)
Bog eye	11.8	7.6	276	1.8	90.9	30.9	22.2	1.0	2.4

Figures in () indicate s.d.

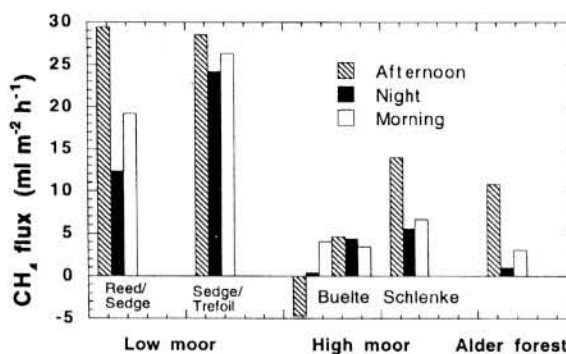
moor. The alder-forested moor showed 100mV lower Eh value and a higher concentration of NH₄⁺ than the other two moors. This nitrogen supply might be associated with co-existent N₂-fixing leguminous bacteria (*Frankia* sp) in alder root nodule. In the studied area, there is a small pond called "bog eye" which receive sprung up water from underground water system. Its surface water is completely different from the other sites: i.e. lower temperature, higher pH, lower DOC and higher Cl⁻, NO₃⁻ and SO₄²⁻ concentrations. This water is an important nutrient source for the low moor.

Secondly, we compared carbon and nitrogen content of peat soils between three moors. Although carbon contents were not so much different between three sites, nitrogen contents and C/N ratio showed clear difference between three moors (Table 2). High moor showed nitrogen limited environment characterized by the lowest nitrogen content and the highest C/N ratio. On the other hand, alder forest moor showed nitrogen rich environments indicated by

the highest nitrogen content and the lowest C/N ratio. This nitrogen rich environment is due to co-existent N₂-fixing bacteria with alder tree.

The result of methane flux measurements in 1993 summer is shown in Fig.1. Methane fluxes at two sites of low moor are higher than two sites of high moor and one alder forest site. Averaged daily fluxes (calculated from day and night fluxes) were 377, 103 and 72 mg CH₄/m²/day in low moor, high moor and alder forest, respectively.

Fig. 2 shows atmospheric methane concentration and methane fluxes monitored by an automatic open-close chamber at a low moor station during summer observation in 1996. Atmospheric methane concentrations were

**Fig.1** Methane fluxes at different vegetation sites in Kushiro wetland measured by the chamber method in different times in a day on 24-27 August, 1993.

Two sites in low moor (dominated by reed and sedge, and by sedge and trefoil) and in high moor (Buelte and Schlenke) and one site in alder forest were chosen for flux measurement station. Chamber air samples were taken 4 to 5 times during 1 to 3 hours for daytime fluxes and 1 time for overnight flux.

Table 2 Carbon and Nitrogen content and C/N ratio of sediment at three vegetation sites in Kushiro wetland in August 1995.

	High moor (4 sites)	Alder moor (3 sites)	Low moor (1 site)
N-content	12.3 (5.0)	24.8 (9.1)	18.1
C-content	358 (108)	338 (120)	376
C/N ratio	33.2 (19.2)	13.7 (0.23)	20.8

unit : mg/g dry soil Figures in () indicate s.d.

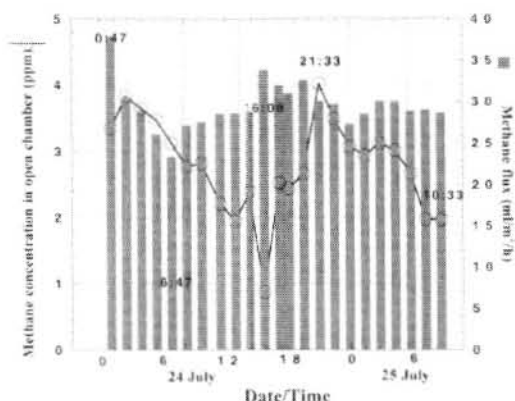


Fig.2 Atmospheric methane concentration and methane fluxes monitored by an automatic open-close chamber at a low moor station 24-25 July, 1996.

For one cycle of flux measurement, after a chamber closed, chamber air was sampled and injected into SCD-GC every 10 minutes interval for 1 hour, and the chamber was open for 30min. in order to reset chamber air to ambient atmosphere and next sampling cycle was started.

significantly higher between night to early morning (3.25 ± 0.47 ppm) compared those in daytime (2.30 ± 0.61 ppm). On the other hand, methane fluxes were not significantly different between day ($n=11$, 20.5 ± 1.9 mg $\text{CH}_4/\text{m}^2/\text{h}$) and night ($n=12$, average: 21.4 ± 2.2 mg $\text{CH}_4/\text{m}^2/\text{h}$). At the same time, methane fluxes were measured by manual handling of chamber and sampling of chamber air at a neighbor site of low moor. The result of manual measurement showed similar methane flux ($n=5$, 18.3 ± 5.5 mg $\text{CH}_4/\text{m}^2/\text{h}$) compared with that of automatic monitoring, except for higher standard deviation. Automatic methane monitoring showed very higher precision compared with manual measurement.

Methane fluxes measured by manual handling were compared between three moors during 1993-1997 summer observations (Fig.3). Differences of methane fluxes between three moors (highest flux at low moor followed by high moor and alder forest) were confirmed in every year's observations. These results are consistent with those of Shannon et al. (1996). They reported higher methane emission with vascular plant (*Scheuchzeria palustris*: 2.1 ± 0.2

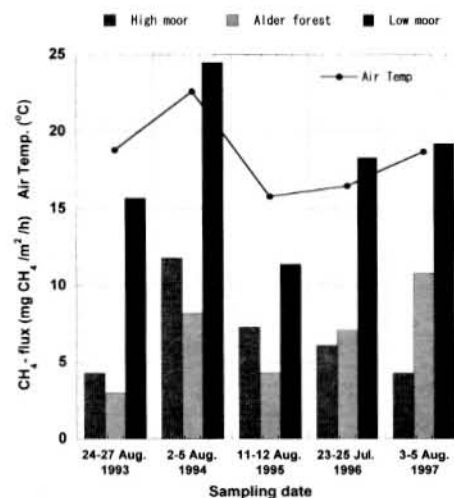


Fig.3 Compiled methane fluxes at three moors in Kushiro wetland measured by the chamber method during summer 1993-1997.

Methane fluxes in every summer were averaged for each moor and compared with each other. Averaged air temperature during experimental period monitored at high moor station was also shown.

mg CH_4/g plant/d or its contribution for methane flux was calculated as 250 ± 35 mg $\text{CH}_4/\text{m}^2/\text{d}$) and lower one with shrub (*Chamaedaphne calyculata*: 0.32 ± 0.05 mg CH_4/g plant/d) by enclosure method in southern Michigan bogs.

Methane fluxes in low moor during 1993-1997 summer were averaged to 17.8 ± 4.8 mg $\text{CH}_4/\text{m}^2/\text{h}$ (or 427 ± 115 mg $\text{CH}_4/\text{m}^2/\text{d}$). This value was rather higher compared with methane fluxes in Siberian Lowland (21-234 mg $\text{CH}_4/\text{m}^2/\text{d}$, Tohjima et al. 1995) or in Canadian Lowland (8-126 mg $\text{CH}_4/\text{m}^2/\text{d}$, Roulet et al. 1994). And also in these report, methane fluxes estimated by large scale air samplings with airplane showed rather lower methane fluxes than the chamber method.

Miyata et al. (1997) measured methane flux in the low moor near to our sampling site by an another method, the concentration gradient method. They calculated methane fluxes by using the diffusion constant estimated from wind speed and monitored methane concentration from two layers of ambient air by NDIR absorption spectra. They reported methane fluxes were almost the same in 1994 and 1995 summer (189

and 181 mg CH₄/m²/d, respectively), whereas our chamber method showed very different fluxes between two years (587 and 274 mg CH₄/m²/d, respectively). This discrepancy of methane fluxes between two methods may be due to reflection of site specific fluxes in the chamber method. So that we compared methane fluxes between one site dominated mainly by reed and another site without any vegetation (open water site) at the same low moor in 1997 summer. Methane flux was more than two times higher (27.8 mg CH₄/m²/h) in reed vegetated site than open water site (10.6 mg CH₄/m²/h). But we used the same vegetation sites for methane flux measurement every year. So that difference of methane flux from year to year is not due to such site specificity. And we monitored methane flux with an automatic open-close chamber in 1996 summer at the same vegetation site as that used by the gradient method. As mentioned above, methane fluxes measured by automatic chamber (averaged day and night flux was 506 ± 48 mg CH₄/m²/d, n=23) were 2.7 times higher than the gradient method. These results suggest that there may be some reason for lower methane flux estimation in the gradient method.

Temperature might affect on the methane flux directly or indirectly through the primary production and degradation processes of organic matter as shown by Whiting & Chanton (1992). We had unusually cold summer in 1993 in contrast to normal summer in 1995. Although air temperature during observed period was lower in 1995 than in 1993, active primary production might be expected in 1995 and it could reflect to methane production. In addition, methane production and flux in low moor might directly reflect the temperature during observed period. Different response of methane flux on peat temperature between different bog sites was reported by Shannon & White (1994), who suggested this difference due to vegetation with or without transport system.

In Kushiro wetland, methane fluxes were different not only between different vegetations but also between observed years. Methane fluxes seem to depend on temperature, especially at low moor site. Average air temperatures monitored at the meteorological station in high moor during the observation period were also shown in Fig. 3. Methane flux in low moor site at the highest temperature (1994) was more than twice as that of the lowest one (1995). From a linear regression, methane flux in low moor correlated to air temperature with the coefficient of 1.54 mg CH₄/m²/h/°C ($r=0.85$). On the other hand, in the case of high moor and alder forest, methane fluxes did not directly depend on observed air temperature. It may be due to another regulating factor(s) for methane production and flux besides temperature. In the case of high moor, where is ombrotrophic and nutrient deficient, precipitation which supply nitrogen and other nutrient might affect primary production and methane flux.

Hydrogen gas concentrations in the flux chamber were measured at the same time as methane analysis by SCD-GC, and calculated hydrogen fluxes in 1993 summer were shown in Fig. 4. Emission fluxes of hydrogen were observed at low moor and alder forest sites, while influxes of hydrogen into high moor were observed. This tendency of hydrogen fluxes were observed in the following years. Parallel

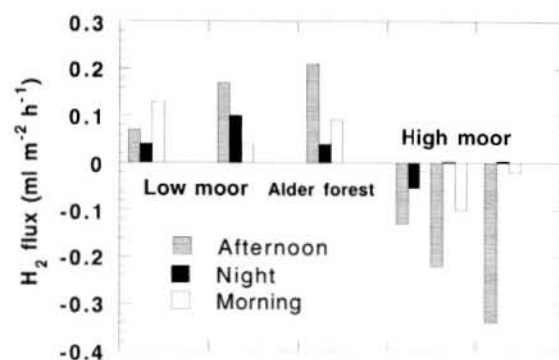


Fig.4 Hydrogen fluxes measured by the chamber method at different vegetation sites in Kushiro wetland on 24-27 August, 1993. Experimental procedure was the same as in Fig.1.

Table 3 CH₄ concentration in the surface water at different vegetation sites in Kushiro wetland in August 1994

Site	W.T (°C)	Atmospheric. CH ₄ conc. (ppmv)	Surface water CH ₄ conc. μ mol/l (A)	AEC* n mol/l (B)	ratio A/B
Low moor (n=10)	20.1 (0.8)	2.25 (0.21)	6.61 (8.44)	3.30	2,000
Alder moor (n=4)	19.9 (0.2)	2.49 (0.75)	16.61 (9.29)	3.66	4,540
High moor (n=5)	19.9 (1.5)	1.91 (0.04)	41.3 (31.2)	2.81	14,700
Bog eye	11.8	1.80	1.41	3.21	439

(): s.d., *AEC: Air Equilibrium Concentration

Table 4 N₂O concentration in the surface water at different vegetation sites in Kushiro wetland in August 1994

Site	W.T (°C)	Atmospheric. N ₂ O conc. (ppbv)	Surface water N ₂ O conc. (A**)	AEC* (B**)	ratio A/B
Low moor (n=10)	20.1 (0.8)	336 (27)	6.37 (1.61)	9.74 (0.78)	0.66 (0.18)
Alder moor (n=4)	19.9 (0.2)	352 (39)	8.41 (3.81)	10.3 (1.14)	0.80 (0.43)
High moor (n=5)	19.9 (1.5)	329 (16)	5.62 (0.80)	9.59 (0.79)	0.60 (0.08)
Bog eye	11.8	310	21.1	11.6	1.82

(): s.d., *AEC: Air Equilibrium Concentration, **concentration: nmol/l

emission fluxes of hydrogen with methane at low moor site were also confirmed by continuous monitoring with automatic chamber system. Although several measurements of dissolved hydrogen gas in fresh or saline water and some estimation of hydrogen emission fluxes were reported (Schutz et al. 1988, Butler et al. 1987, Conrad 1988), studies on direct flux measurement or influx of hydrogen into ecosystem are very limited. Difference of hydrogen flux between high and low moor may be due to difference of linkage between hydrogen and methanogenic metabolisms. Table 3 shows that dissolved methane concentrations in the surface water were higher at high moor ($41.3 \pm 31.2 \mu \text{ mol/l}$) than low moor ($6.61 \pm 8.44 \mu \text{ mol/l}$), in contrast to higher methane flux at low moor than high moor. At low moor, especially in reed site, methanogenic metabolism might proceed predominantly in rhizosphere where sufficient supply of organic matter is expected and produced

methane would be transported directly to the atmosphere via reed stem (aerenchyma) system. If hydrogen is not utilized for methanogenesis at rhizosphere, it is highly probable that methane flux might accompany efflux of excess hydrogen. On the other hand, methane fluxes at high moor were mainly through ebullition or diffusion mechanism between sediment-water and water-air interfaces. So that hydrogen might be oxidized before emission if any. Influx of hydrogen at high moor site suggest hydrogen uptake by methanogenesis. Lower level of labile organic matter supply at high moor site would facilitate hydrogen as a methanogenic substrate.

From N₂O flux measurements by the chamber method, we had obtained small but dominant influxes of N₂O into low ($-4.1 \pm 3.8 \mu \text{ l/m}^2/\text{h}$, n=6) and high moor ($-3.6 \pm 4.8 \mu \text{ l/m}^2/\text{h}$, n=9) against small efflux from alder forest ($1.5 \pm 1.9 \mu \text{ l/m}^2/\text{h}$, n=3) in August 1993.

Some influxes into high and low moors along with small effluxes from low moor were also measured in 1995 summer. As shown in Table 4, all of surface water at every sites in the moor (except for "bog eye") were under-saturated to the atmospheric equilibrium concentration of N₂O. This means the role of moor as a sink for atmospheric N₂O, as IPCC (1995) suggested the possibility of soil for N₂O sink. Reports on nitrous oxide flux at wetland as a source are very limited and reported nitrous oxide fluxes were very small (Matson and Vitousek., 1990, Paludan and Blicher-Mathiesen 1996). So that more intensive studies should be needed.

N₂O production and consumption are closely linked to microbial nitrogen transformation, such as nitrification, denitrification and nitrogen fixation. In nitrification, N₂O is produced as a by-product of NO₂⁻ production from NH₄⁺ (Bremner & Blackmer 1981). In denitrification, N₂O is produced and consumed as an intermediate from NO to N₂ (Payne 1981). In nitrogen fixation, N₂O is incorporated and reduced to N₂ or NH₄⁺ (Yamazaki et al. 1987). So that we estimated population of several kind of nitrogen cycling bacteria in high moor (4 sites), alder forest (3 sites) and low moor (1 site) in summer 1995-1998. Fig.5 shows averaged density of each kind of bacteria during observation of 4 summers.

This result shows a clear difference of microbial population between three different types of moor. Low moor showed nutrient rich environment characterized by higher population of aerobic heterotroph and no detection of free-living nitrogen fixing bacteria. On the other hand, high moor showed nitrogen limited environment characterized by higher population of free-living nitrogen fixing bacteria. These results are very consistent with the water quality and C/N ratios of peat soil as mentioned above (Table 1 and Table 2). In addition to abundance of free-living nitrogen fixing bacteria, higher nitrogen fixing activities at high moor (3.7 - 54.1 μ mole C₂H₂/m²/h) than low moor including alder forest (0 -2.3 μ mole C₂H₂/m²/h) were measured

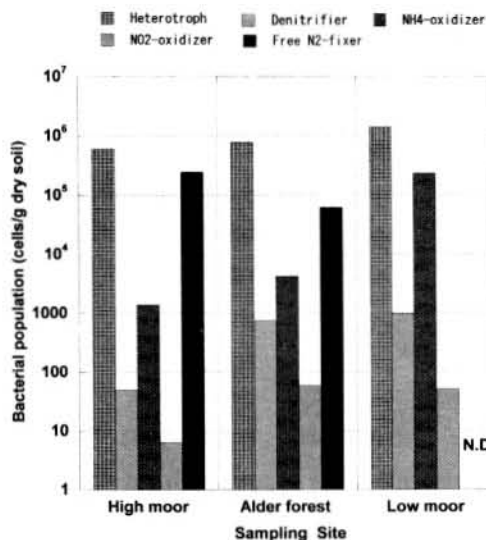


Fig.5 Averaged nitrogen cycling bacterial population at three moors in Kushiro wetland in summer 1993-1997.

Bacterial samples were taken from 4 sites in high moor, 3 sites in alder forest and 1 site in low moor. Each bacterial population was averaged in each moor. Enumeration of each bacteria is described in the text. N.D. indicate free nitrogen fixer was not detected in low moor throughout the experiment period.

by a chamber incubation method with acetylene (Yoh et al. personal communication). These results suggest functioning of a possible mechanism of N₂O uptake by nitrogen fixing activity (Yamazaki et al. 1987) for observed influxes of N₂O especially into high moor.

If it is the case, N₂O uptake flux in wetland would decrease by limitation of N₂-fixing activity and N₂O emission would increase with increasing nutrient supply. Changes of CH₄ and N₂O fluxes in natural wetland with response to environmental changes such as global warming or acid rain should be elucidated by further studies.

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タイトル：釧路湿原におけるメタン，一酸化二窒素，水素のフラックスと微生物過程

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