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Impacts of Elevated CO_2 on Algal Growth, CH_4 Oxidation and N_2O Production in Northern Peatland

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Effects of elevated carbon dioxide (CO_2) on soil microbial processes were studied in a northern peatland. Intact peat cores with surface vegetation were collected from a northern Welsh fen, and incubated either under elevated carbon dioxide (700 ppm) or ambient carbon dioxide (350 ppm) conditions for 4 months. Higher algal biomass was found under the elevated CO_2 condition, suggesting CO_2 fertilization effect on primary production. At the end of the incubation, trace gas production and consumption were analyzed using chemical inhibitors. For methane (CH_4), methyl fluoride (CH_3F) was applied to determine methane oxidation rates, while acetylene (C_2H_2) blocking method were applied to determine nitrification and denitrification rates. First, we have adopted those methods to optimize the reaction conditions for the wetland samples. Secondly, the methods were applied to the samples incubated under two levels of CO_2. The results exhibited that elevated carbon dioxide increased both methane production (210 vs. 100 ng CH_4 g^{-1} hr^{-1}) and oxidation (128 vs. 15 ng CH_4 g^{-1} hr^{-1}), resulting in no net increase in methane flux. For nitrous oxide (N_2O), elevated carbon dioxide enhanced nitrous oxide emission probably from activation of nitrification process rather than denitrification rates. All of these changes seemed to be substantially influenced by higher oxygen diffusion from enhanced algal productivity under elevated CO_2.

Key words : Greenhouse gas, Mire, Global climatic changes, Wetland, Methane oxidizer, Denitrifying bacteria

INTRODUCTION

The concentration of carbon dioxide (CO_2) in the atmosphere is increasing rapidly and is expected to double by the end of this century mainly due to anthropogenic activities such as fossil fuel burning, deforestation, and agricultural activity (IPCC, 1996). While a fertilising effect of elevated CO_2 causing stimulated primary productivity has been widely noted (Hunt *et al*., 1991), many questions are yet to be answered in relation to the below-ground microbial processes (Hu *et al*., 2001). In particular, impacts of elevated CO_2 on northern peatlands may be of great importance due to their characteristics as a source for greenhouse gases such as CH_4 and N_2O. We have proposed in a previous study that effects of elevated CO_2 in the atmosphere on peatland may speed up global warming by releasing more greenhouse gases such as CO_2 and N_2O back to the atmosphere (Kang *et al*., 2001), outweighing the CO_2 fertilization effect on primary production. Further, several studies have observed enhanced CH_4 emission from CO_2 enriched wetland soil (Dacey *et al*., 1994; Hutchin *et al*., 1995; Megonigal and

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Schlesinger, 1997; Ziska *et al*., 1998). The authors have attributed the enhanced CH_4 emission to the increased plant productivity, which may activate methanogenic bacteria by supplying more root exudates, and/or increase methane transport to the atmosphere through the plant.

In our previous paper (Kang *et al*., 2001), we have noted several changes in biogeochemical processes of the peatland cores. First, significantly higher total biomass (9.12g vs. 2.96g) as well as algal biomass (0.57g vs. 0.08g) was observed under the elevated CO_2 condition, suggesting CO_2 fertilization effect. Secondly, significantly higher DOC concentration was found in the pore-water under elevated CO_2. Finally, no significant differences in CH_4 emission were found, but N_2O emission increased significantly in the intact cores under elevated CO_2 conditions. Complex reactions are involved in the emission of those gases, each of which should be determined if we are to reveal the mechanisms of the impacts from elevated CO_2. For example, increased CH_4 emission may be caused by increased CH_4 production or/and decrease in CH_4 oxidation. Likewise, enhanced N_2O emission could be induced either by increased nitrification, increased denitrification, or both of those responses. To identify those reactions, several chemical inhibitors have been proposed and widely applied. For example, methyl fluoride (CH_3F) was described as a specific inhibitor of methyl monooxygenase (MMO), a key enzyme in methane oxidation (Oremland and Culbertson, 1992). Thus measurement of methane production with/without methyl fluoride can facilitate the determination of methane production and methane oxidation. Likewise, 10 KPa acetylene (C_2H_2) has been widely used to inhibit nitrous oxidase reductase (Ryden *et al*., 1979), which reduces N_2O to N_2 in denitrification process. It was also known that 10 Pa acetylene inhibits nitrification completely (Davidson *et al*., 1986). As such, N_2O sources from nitrification or denitrificaiton can be identified by employing different concentrations of acetylene.

In the present study, we aimed to determine the mechanisms of the findings reported in the previous report by employing chemical inhibitors. For this, we have adopted and optimised methods to determine CH_4 production/oxidation and nitrification/dentrification. Then, the samples incubated either under elevated CO_2 or ambient CO_2 were analysed using the methods adopted.

MATERIALS AND METHODS

Sampling site

The sampling site is located close to Gors-Goch in Anglesey island (N53°17′, W4°22′) of north Wales, United Kingdom. The main vegetation of the area is *Juncus* and *Festuca* spp. The hydrochemistry of the site is influenced by calcareous bedrock, and hence it contains high concentration of base cations such as Ca^{2+} (61.8∼104.7 mg L^{-1}) and Mg^{2+} (2.9∼4.9 mg L^{-1}) (Kang and Freeman, 1999).

Method optimization

Methane oxidation and nitrification/denitrification were determined by chemical inhibitors for soil systems (Schipper and Reddy, 1996; Davidson, 1986). We have modified these methodologies to apply to the peat samples. For both methods, 20 ml of peat was placed in 110 ml glass bottle capped with a lid having a hole drilled in the top, which was sealed with a rubber stopper. Through this stopper, all chemical inhibitors were injected, and accumulated methane or nitrous oxide was withdrawn into air-tight syringes. Prior to all experiments, empty glass bottles were weighed, and total dry mass was determined after the experiments by drying samples in the bottle at 105°C for 24 hours. The differences between those two values (i.e., dry mass of peat) were used to present all results.

For methane oxidation measurement, control (ambient air), 2% or 4% of methyl fluoride was added, and accumulated methane in the headspace was determined at time 0, 30 minutes, 60 minutes, and 120 minutes. For nitrification/denitrificatioin determination, control (ambient air), 10 Pa or 10 KPa of acetylene was injected, and the accumulation of N_2O gas was measured at time 0, 60 minutes, 180 minutes. For each experiment, 3 replicates were employed and differences were sought using one-way ANOVA test.

Sampling, Incubation, and Analyses

Eight soil cores of 40 cm depth were collected using OSMA™ PVC plastic piping (11 cm diameter) in July, 1998. Wetland water collected from the same site was added to the cores to keep the water level at the surface of the peat. All the

cores were incubated under ambient temperature and CO_2 concentration for 2 weeks before being transferred to two different chambers: Four of the cores were maintained under elevated CO_2 (700 ppm), and while the remaining four cores were placed under ambient CO_2 (350 ppm) in the 'Solardomes' facility located in Abergwyngregyn, north Wales. These greenhouse chambers facilitate different atmospheric CO_2 concentrations while maintaining field temperature and radiation conditions. Water collected from the wetland was added weekly to keep the water table level at the surface of the peat.

After a 4−month incubation, the peat samples were dismantled and surface peat (0 ~ 10 cm) was collected. Methane oxidation and nitrification/denitrification were determined following the methods described above. The incubation conditions for methane oxidation were under 2% methyl fluoride for 60 minutes, while those for nitrification/denitrification were under 10 Pa or 10 KPa for 180 minutes.

Methane and nitrous oxide were analysed by an Ai Cambridge model 92 Gas Chromatograph equipped with a Porapak QS column, flame ionisation detector and electron capture detector. The carrier gas was nitrogen at a flow rate of 70 cm^3 min^{-1} for N_2O, and 13 cm^3 min^{-1} for CH_4.

Differences in the processes between elevated CO_2 and ambient CO_2 conditions were determined by 2−sample t−test.

RESULTS AND DISCUSSION

Method optimization

The effects of incubation time and the concentration of methyl fluoride are presented in the

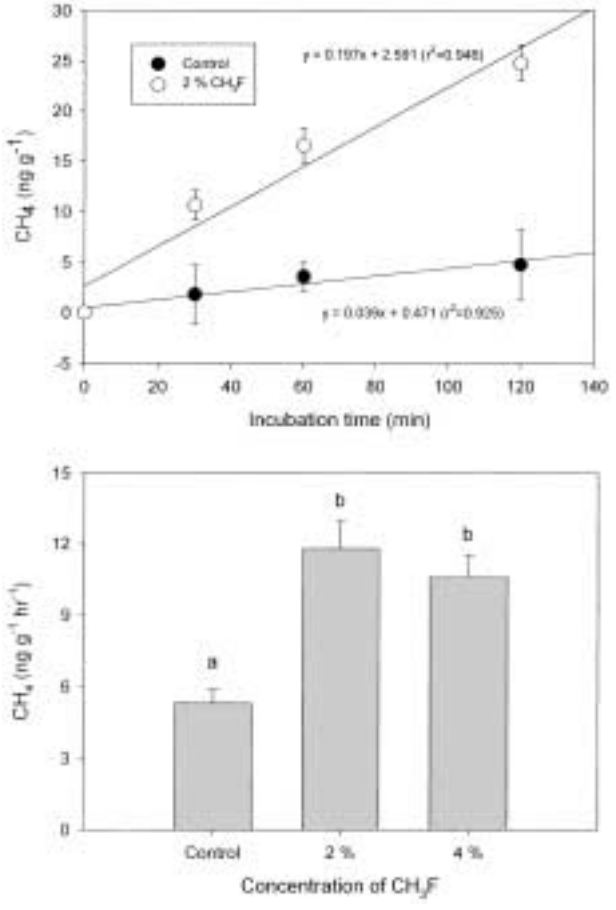

Fig. 1. Effects of incubation time (a) and the concentration of methyl fluoride (b) on methane emission. (Mean ± S.E., n = 3)

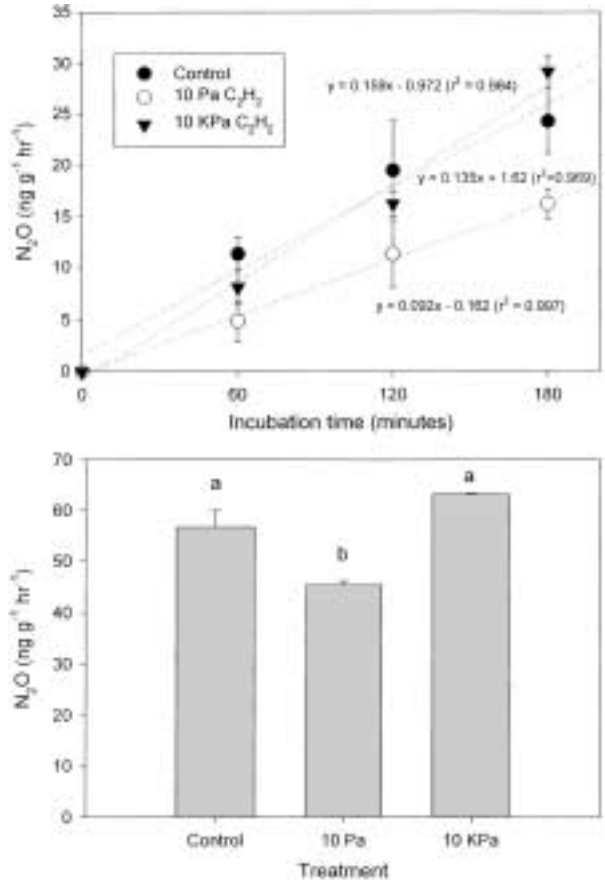

Fig. 2. Effects of incubation time (a) and the concentration of acetylene (b) on methane emission. Bars labeled with different letters are significantly different at $P < 0.05$. (Mean ± S.E., n = 3)

Fig. 1a and 1b, respectively. The methane oxidation showed linearity over 2 hours and there was no significant difference between 2% and 4%. As such, a 1-hour incubation with 2% methyl fluoride was employed for the following measurement. For nitrification/denitrification, the differences among treatments were only significant at 180 minutes, indicating fairly low production of N_2O (Fig. 2a). In the Fig. 2b, the difference between control and 10 Pa represents N_2O production from nitrification, and the difference between 10 Pa and 10 KPa represents N_2 production from denitrification (Davidson *et al*., 1986). Likewise, N_2O production from denitrification is equivalent to the N_2O production under 10 Pa treatment.

CO_2 enrichment experiment

Methane

Methane production and oxidation are presented in Fig. 3. Originally we expected higher methane emission under elevated CO_2 conditions, which was reported by other studies (Dacey *et al*., 1994; Hutchin *et al*., 1995; Megonigal and Schlesinger, 1997; Ziska *et al*., 1998). They attributed the enhanced CH_4 emission to the increased plant productivity, which may activate methanogenic bacteria by supplying more root exudates, and/or increase methane transport to the atmosphere through the plant. However, in the present study, methane oxidation increased along with augmentation of methane production under elevated CO_2 conditions, which results in no net increase in CO_2 emission. Under elevated CO_2 conditions, higher DOC input to the soils from plant root could increase the methane production. However, the higher algal biomass under the conditions (Kang *et al*., 2001) could produce oxygen which then may diffuse into soils. Such oxygenated conditions could accelerate methane oxidation by methane oxidizing bacteria, which is known to be active under oxygenated conditions.

Nitrous oxide

The results from an acetylene-blocking experiment were contradictory to our original hypothesis that higher denitrification rates under elevated CO_2 conditions may increase N_2O emission. It was observed that elevated CO_2 enhances carbon availability to microorganisms in the rhizosphere, which then may activate denitrification process since carbon availability is one of the main controlling factors for N_2O emission from soils (Davidson, 1991). For example, elevated CO_2 level has been reported to increase rhizosphere denitrifier activity in mineral soil planted with wheat (Smart *et al*., 1997), and to increase N_2O flux from calcareous grassland (Arnone and Bohlen, 1998). However, our results indicate an opposite trends, namely higher nitrification rates under the elevated CO_2 conditions (Fig. 4). Like the methane emission, oxygen diffusion from increased algal production rather than increased DOC may be the key mechanisms underlying this finding.

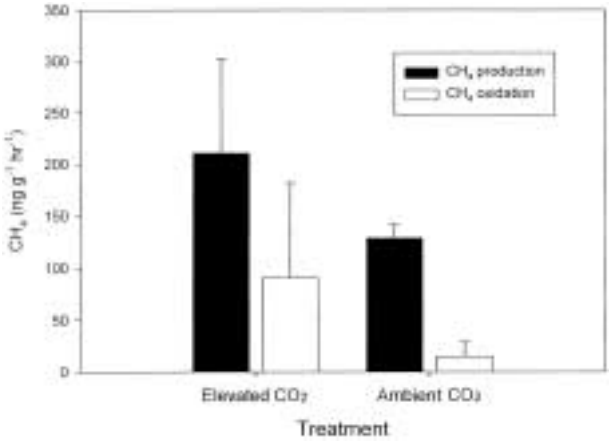

Fig. 3. Methane production and oxidation in fen peat incubated under ambient or elevated CO_2 conditions (Mean ± S.E., n = 4).

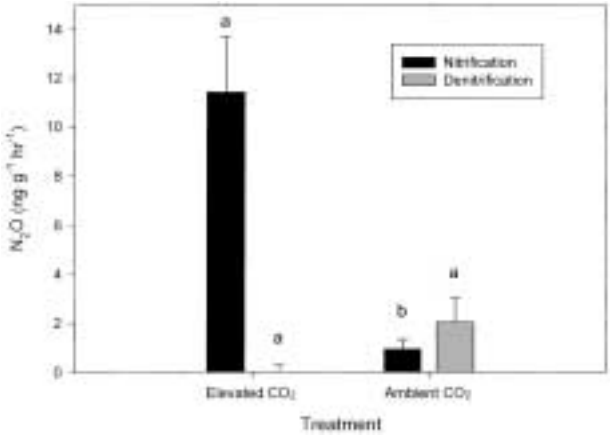

Fig. 4. N_2O production by denitrification or nitrification in fen peat incubated under ambient or elevated CO_2 conditions. Bars labeled with different letters are significantly different between the CO_2 treatments at $P < 0.05$. (Mean ± S.E., n = 4)

Most of the previous studies have focused on the CO_2 fertilization effects on primary productivity of herbaceous plant in northern peatland (Hutchin *et al*., 1995). However, the results of the present study implies that higher oxygenation by enhanced algal productivity as well as the effects from increased DOC should be considered to elucidate impacts of elevated CO_2 on fen peat biogeochemistry.

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＜국문적요＞

이탄습지에서 이산화탄소의 농도가 조류의 증식, 메탄 산화 및 아산화질소 생성에 미치는 영향

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이산화탄소 농도가 증가할 때에 북구 이탄 습지에서 나타나는 생지화학적 변화과정을 살펴보았다. 표면 식생을 포함한 온전한 코어를 북웨일스의 이탄습지로부터 채취하여, 높은 이산화탄소농도(700 ppm) 와 자연상태(350 ppm) 환경에서 4개월간 배양하였다. 배양 후, 화학적인 저해제를 이용하여 습지 토양에서 미량기체의 생성과 소비를 측정하였다. 메탄의 경우, 불화메탄(CH_3F) 를 이용하여 메탄 산화율을 결정하였고, 질산화와 탈질작용을 측정하기위해 아세틸렌(C_2H_2) 저해 방법을 적용하였다. 이를 위해, 먼저 각 측정 방법을 습지 시료에 적합하도록 최적화 시켰고, 둘째로 두 수준의 이산화탄소에서 배양한 시료에 이 방법들을 적용하였다. 높은 이산화탄소 농도는 메탄의 생성량을 증가 시켰으나(210 대 100 ng CH_4 g^{-1} hr^{-1}), 메탄 산화의 양도 증가시켜서(128 대 15 ng CH_4 g^{-1} hr^{-1}) 결국에는 순메탄 방출량에는 변화가 없었다. 아산화질소의 경우에는 증가된 발생량이 탈질 보다는 질산화 과정에서 생성된 것으로 사료된다. 이러한 변화들은 높은 이산화탄소 하에서 조류의 성장이 증가되어 야기된 것으로 추측된다.