Methane Emission from Leaves of Larch, Birch and Oak Saplings Grown at Elevated CO₂ Concentration in Northern Japan –Preliminary Study–

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Abstract

A recent study by Keppler *et al.* (2006: *Nature* **439**, 187–191) reported methane emission from leaves under aerobic conditions, and suggested that forests are major methane sources. It is therefore important to determine the contribution of temperate forest to worldwide methane gas evolution. We studied methane emission from typical tree species native to northern Japan grown under both ambient and high CO₂ concentrations ([CO₂]), and accordingly consider methane emission rates on the global scale. We used three tree species: larch (*Larix kaempferi* (Lamb.) Carrière), birch (*Betula maximowicziana* Regel) and oak (*Quercus mongolica* Fisch. Ex Ledeb. var. *crispula* (Blume) Ohashi). Fresh leaves were incubated at 35°C under illumination, and methane emission was monitored at 24 hr intervals. Methane emission was observed in leaves of all three species; specific patterns were found. Larch produced the most methane. Oak produced least methane at high CO₂ concentration. There are currently large variations in estimates of the total methane emission on the global scale. The present results suggest that further accurate methane flux studies are necessary in order to determine total methane emission rates at the global scale.

Key words: Deciduous trees, Elevated CO₂, Methane, Northern Japan, Total methane emission

1. Introduction

Methane is a greenhouse gas implicated in global warming. Its has 23 times the global warming potential of CO₂ per unit weight, at a time scale of 100 year (IPCC 2001). Its atmospheric concentration has increased by a factor of 250 since the Industrial Revolution began. Methane is generated under anaerobic conditions (Meronigal *et al.*, 2004), so that it is emitted mainly from natural sources such as wetlands, and from agricultural sources such as rice paddies. Methane emissions from these areas account

for 40–50% of total global methane emission (IPCC, 2001; Lowe, 2006).

Keppler *et al.* (2006) found, however, that methane is also generated from plant leaves under aerobic conditions. Their remarkable estimates of global methane production suggest that forests are major methane source, so that new forestation creates additional methane sources; new forestation would in that case promote global warming (Keppler *et al.*, 2006; Lowe 2006). Keppler *et al.* (2006) also found that methane emission increased with temperature and light exposure, and that pectin and lignin content in cell walls was strongly correlated with methane emission.

Cool temperate forests are distributed broadly at higher latitudes in the northern hemisphere. They play an important role in global carbon cycles and make up

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the second largest terrestrial biomass, surpassed only by tropical rain forests. Given their important role in carbon cycles and large biomass, it is important to discover the extent of temperate forest contribution to overall methane gas evolution. Several studies have reported that plant leaves grown under high CO_2 concentration ($[\text{CO}_2]$) had different chemical and anatomical characters from those grown at ambient $[\text{CO}_2]$ (e.g. higher C-N ratios, accumulation of starch) (Koike *et al.*, 1996; Tissue *et al.*, 1999; Eguchi *et al.*, 2004). We therefore expected that emission of methane from woody plants grown under elevated $[\text{CO}_2]$ would be greater than from those grown in ambient $[\text{CO}_2]$.

We investigated methane emission from typical tree species that grow in cool temperate forests at high summer temperatures, and discuss total methane emission rates at the global scale.

2. Materials and Methods

2.1. Plant materials

We chose three tree species native to northern Japan: larch (*Larix kaempferi* (Lamb.) Carrière), birch (*Betula maximowicziana* Regel) and oak (*Quercus mongolica* Fisch. Ex Ledeb. var. *crispula* (Blume) Ohashi). Samples were taken from three replications per species. For each species two kind of specimen that had been grown under two different $[\text{CO}_2]$ were used. One is 500 ppm in a Free Air CO_2 Enrichment (FACE) system (Takagi *et al.*, 2004), or at 370 ppm, which is ambient $[\text{CO}_2]$. The seedlings were 5 years old. The height of the sampled seedlings of larch and oak was approximately 2.2 m, while that of birch is 3.8 m (Eguchi *et al.*, unpublished data). Immediately after collection of fully expanded sunny shoots, the shoot ends were re-cut diagonally under water to prevent water stress. In the laboratory, the shoots were re-cut under water, and leaves were sampled for experiments, which began within one hour from the collection.

2.2. Incubation experiments

Incubation experiments were performed as described by Ishizuka *et al.* (2000). Leaf samples were placed with wetted filter papers in a 500 ml glass vial, which was then sealed with a butyl rubber stopper and silicon septum. The fresh weight of each sample was 6.0 ± 0.2 g. The headspace was filled with ambient air (approximately $1.8 \mu\text{l CH}_4 \text{ l}^{-1}$). Samples and a control blank were incubated for up to 72 hr in a growth chamber (Koito KG, Yokohama) at 35°C with PPFD of about $350 \mu\text{mol m}^{-2}\text{s}^{-1}$. After incubation, the mass

of the leaves was measured after drying at 80°C for 48 hr.

2.3. Methane measurements

10 ml of headspace air was sampled right before the closure of the glass vial and after the closure at 24 hr intervals. Methane analysis was performed on these air samples, using a gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan). The methane content in the glass vial was measured, and the accuracy of the measurement evaluated, using two standard gasses (2.02 ppm CH_4 and 9.98 ppm CH_4). The accuracy was $\pm 0.1 \text{ ppm}$.

2.4. Statistical analysis

Statistical analysis was carried out using R ver. 1.81 (The R Project in Statistical Computing, <http://www.r-projecting.org/index.html>). We analyzed differences between the treatments by the T-test with the level of significance (P) of 0.05 and 0.01.

3. Results

Methane production was observed with all three species, and specific patterns were displayed (Fig.1, 2, 3). Larch produced more CH_4 than the other tree species, but there were no clear differences between the elevated

Fig. 1. Temporal changes in methane content in a glass vials in which larch needles (*L. kaempferi*) grown in 370 ppm (open circles) or 500 ppm (closed circles) $[\text{CO}_2]$. The vertical bars indicates the standard deviation, and n.s. in the figure indicates no significant difference between two kinds of specimens. Note the difference in the scale of the vertical axis with Figs 2 and 3.

Fig. 2. Same as Fig. 1, but with birch (*B. maximowicziana*). Note the difference in the scale of the vertical axis with Fig. 1.

Fig. 3. Same as Fig. 1, but with oak (*Q. mongolica*). Note the difference in the scale of the vertical axis with Fig. 1. * and ** indicate significant difference between the two kind of specimen at the significance level (P) of 0.05 and 0.01, respectively.

[CO₂] treatment and ambient [CO₂] (Fig. 1). With birch and oak, the methane content increased from 0.3 mg CH₄ g⁻¹ at the start to a maximum of around 0.8 mg CH₄ g⁻¹ at 72 hr. There were no significant differences between elevated [CO₂] treatments and ambient [CO₂] with birch (Fig. 2). However, oak trees grown under the

Fig. 4. Rates of methane emission for three northern Japanese deciduous tree species. The clear bars indicate the ambient [CO₂] concentration. The letters a and b indicate a significant difference at the $p < 0.01$ level and n.s. in the figure indicates no significant difference between two kinds of specimens. Methane emission rates were calculated from the initial value and values after 72 hr incubation.

elevated [CO₂] treatment gave a slightly lower methane concentration than the ambient [CO₂] specimens after 72 hours incubation (Fig. 3).

With larch, methane emission rates were 29.5 ng CH₄ g⁻¹ h⁻¹ under the elevated [CO₂] treatment, and 31.3 ng CH₄ g⁻¹ h⁻¹ at ambient [CO₂] (Fig. 4). With birch, methane emission rates per unit dry mass were 7.6 ng CH₄ g⁻¹ h⁻¹ under the elevated [CO₂] treatment, and 10.0 ng CH₄ g⁻¹ h⁻¹ in ambient [CO₂] conditions (Fig. 4). For oak, methane emission rates were 5.6 ng CH₄ g⁻¹ h⁻¹ under elevated [CO₂] and 9.1 ng CH₄ g⁻¹ h⁻¹ at ambient [CO₂] (Fig. 4).

4. Discussion

Methane emissions were found with each of the representative deciduous tree species in northern Japan, and the rates of emission are similar to previous reports (Table 1). With larch, the rapid increase suggested that methane emission is influenced not by a single factor but by multiple interacting factors, including environmental factors (Ishizuka and Takahashi 2006).

We expected that elevated [CO₂] would induce greater methane emission, because the leaves that had experienced the elevated [CO₂] have different chemical

Table 1. Comparison of methane emission rates of tree species from warm and cool temperate forests.

Methane emission rates (ng CH ₄ g ⁻¹ h ⁻¹)	
Japanese ceda ¹⁾ (<i>Cryptomeria japonica</i>)	39.1
Japanese larch ³⁾ (<i>Larix kaempferi</i>)	31.3
Japanese white oak ¹⁾ (<i>Quercus myrsinaefolia</i>)	22.6
European beech ²⁾ (<i>Fagus sylvatica</i>)	21.6
Monarch birch ³⁾ (<i>Betula maximowicziana</i>)	10.0
European ash ²⁾ (<i>Fraxinus excelsior</i>)	9.1
Japanese oak ³⁾ (<i>Quercus mongolica</i>)	9.1

¹⁾ Ishizuka and Takahashi (2006).²⁾ Keppler *et al.* (2006).³⁾ This study.

and anatomical characters (e.g. higher C–N ratios, accumulation of starch) (Koike *et al.*, 1996; Tissue *et al.*, 1999; Eguchi *et al.*, 2004). However, birch and larch yielded no significant differences in methane emission between the elevated and the ambient [CO₂]. Moreover, with oak, the elevated [CO₂] specimen resulted in significantly lower methane emission than the ambient [CO₂] specimen. Why did oak emit lower methane at elevated [CO₂] concentration than at ambient concentration?

Oak is recognized as a tree species that emits isoprene (Sharkey *et al.*, 1991; Niinemets *et al.*, 2002). Red oak (*Q. rubra*) in particular, greater isoprene emissions, were found in individual plants grown at elevated [CO₂] (Sharkey *et al.*, 1991). Previous studies suggest that isoprene emission is involved in protecting photosynthetic membranes from heat stress (Heldt, 2004; Sharkey, 2005); leaves that emit more isoprene under elevated [CO₂] than ambient are better able to alleviate heat stress. Sensitivity to temperature is different in leaves grown under different [CO₂], therefore, oak leaves grown with elevated [CO₂] emitted less methane than that of grown under ambient [CO₂].

Our findings are similar to the methane emission rates determined by Keppler *et al.* (2006) (Table 1). Our result however, does not necessarily indicate that cool temperate forests are significant methane source. There are variable estimates of methane

Table 2. Comparison of total methane emission estimates from the terrestrial plant community.

Total methane emission (Tg CH ₄ y ⁻¹)	
Keppler <i>et al.</i> (2006) ¹⁾	62-236
Megonigal (2006) ²⁾	27-43
Kirschbaum <i>et al.</i> (2006) ³⁾	10-62
Guenther (2006) ²⁾	34-56

¹⁾ Keppler *et al.* (2006).²⁾ Megonigal (2006).³⁾ Kirschbaum *et al.* (2006).

emissions from terrestrial plant communities (Table 2). Megonigal (2006) and Kirschbaum *et al.* (2006) suggested that Keppler's calculations overestimate the total methane production. The IPCC (2001) estimated that terrestrial soil "absorbs" 30 Tg CH₄ per year. This soil "absorption" is one of the most important methane sinks, following photodecomposition. If total terrestrial plant communities emit less methane than 30 Tg CH₄ per year, terrestrial plant communities would not be a net atmospheric methane source. Accurate evaluations of methane flux are therefore necessary to determine whether forests are net methane sources. In particular, further studies are needed to monitor diurnal and seasonal changes in methane emission and absorption.

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References

- Eguchi, N., Fukatsu, E., Funada, R., Tobita, H., Kitao, M., Maruyama, Y., and Koike, T., 2004: Changes in morphology, anatomy, and photosynthetic capacity of needles of Japanese larch (*Larix kaempferi*) seedlings grown in high CO₂ concentrations. *Photosynthetica*, **42**, 173–178.
- Heldt, H. W., 2004: *Plant Biochemistry*. 3rd edition, Elsevier Academic Press, California, 630 pp.
- IPCC, 2001: *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (ed. by Houghton, J. T., Ding,

- Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C. A.). Cambridge University Press, Cambridge, 881 pp.
- Ishizuka, S., and Takahashi, M., 2006: Do forest ecosystems accelerate global warming, or prevent it? —Additional experiments of methane emissions from terrestrial plants under aerobic condition—. *Shinrin Kagaku*, **47**, 67–69 (in Japanese).
- Ishizuka, S., Sakata, T., and Ishizuka, K., 2000: Methane oxidation in Japanese forest soils. *Soil Biol. Biochem.*, **32**, 769–777.
- Keppler, F., Hamilton, J. T. G., Braß, M., and Röckmann, T., 2006: Methane emissions from terrestrial plants under aerobic conditions. *Nature*, **439**, 187–191.
- Kirschbaum, M. U. F., Bruhn, D., Etheridge, D. M., Evans, J. R., Farquhar, G. D., Gifford, R. M., Paul K. I., and Winters, A. J., 2006: A comment on the quantitative significance of aerobic methane release by plants. *Funct. Plant Biol.*, **33**, 521–530.
- Koike, T., Lei, T. T., Maximov, T. C., Tabuchi, R., Takahashi, K., and Ivanov, B. I., 1996: Comparison of the photosynthetic capacity of Siberian and Japanese birch seedlings grown in elevated CO₂ and temperature. *Tree Physiol.*, **16**, 381–385.
- Lowe, D. C., 2006: A green source of surprise. *Nature*, **439**, 148.
- Megonigal, J. P., 2006: Methane Cycling in upland forests: New findings and implications for forest–climate. *Abstract of IUFRO canopy processes working group workshop: regional forest responses to environmental change*, 52.
- Megonigal, J. P., Hines, M. E., and Visscher, P. T., 2004: Anaerobic metabolism: linkages to trace gases and aerobic processes. In *Biogeochemistry* (ed. by Schlesinger, W. H.). Elsevier-Pergamon, Oxford, pp. 317–424.
- Niinemets, Ü., Hauff, K., Bertin, N., Tenhunen, J. D., Steinbrecher, R., and Seufert, G., 2002: Monoterpene emissions in relation to foliar photosynthetic and structural variables in Mediterranean evergreen *Quercus* species. *New Phytol.*, **153**, 243–256.
- Sharkey, T. D., 2005: Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, Rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.*, **28**, 269–277.
- Sharkey, T. D., Loreto, F., and Delwiche, C. F., 1991: High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant Cell Environ.*, **14**, 333–338.
- Takagi, K., Eguchi, N., Ueda, T., Sasa, K., and Koike, T., 2004: CO₂ control in a FACE system for tree saplings. *J. Agric. Meteorol. Hokkaido*, **56**, 9–16 (in Japanese).
- Tissue, D. T., Griffin, K. L., and Ball, T., 1999: Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO₂. *Tree Physiol.*, **19**, 221–228.

高 CO₂ 条件で育成した北海道の主要落葉樹からのメタン放出 に関する予備実験

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要 約

Keppler *et al.* (2006: *Nature* **439**, 187–191)は植物が好気条件でメタンを放出することを発見し、さらに陸上植物群落のメタン収支を算出することで、森林がメタンの放出源になっている可能性を指摘した。そこで本研究では、1)冷温帯落葉樹からもメタンが放出されているのか、2)放出されているとすれば、どの程度放出されているのか、3)将来予想される高CO₂環境が植物体からのメタン放出にどのような影響を与えているのか、評価することを目的とし、さらに陸上植物群落のメタン収支について議論した。高CO₂条件と大気条件で栽培した北海道を代表する落葉樹3種(カラマツ, ウダイカンバ, ミズナラ)の葉からのメタン放出速度を調べた。

その結果、3種すべての、葉からメタンの放出が確認され、その放出パターンには樹種による違いが見られた。乾燥重量当りのメタン発生速度を計算した結果、放出量

はカラマツで最も多く、ミズナラとウダイカンバで同程度となった。高CO₂の条件では、ウダイカンバやカラマツでは高CO₂の条件と大気条件の間の放出速度に有意差が見られず、ミズナラでは高CO₂の条件の方が放出速度は少ないことが解った。

地球上の植物群落からのメタン収支に関する算出結果について文献調査を行ったところ、その結果には大きなばらつきがみられ、森林がメタンの放出源になっているとは断言できないことがわかった。

以上のことから今後、葉群からのメタン放出機構について明らかにすると同時に、森林土壌のメタン吸収に関しての知見を蓄積することで、より高い精度で森林のメタン収支を予測する必要があると考える。

キーワード : 高CO₂, 北海道, メタン, メタン収支, 落葉樹