森林立地55(2), 139～143 2013
Jpn J For Environ 55 (2), 139–143, 2013

Responses of microbial community to soil warming in warm-temperate evergreen broad-leaved forests

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To elucidate the effect of climate warming on the soil heterotrophic microbial community in warm-temperate, evergreen broad-leaved forests, we conducted a soil-warming experiment in a secondary forest located in the city of Higashi-Hiroshima in western Japan. We established ten trench plots (1 m × 1 m) with root barriers to prevent root regrowth in the forest. The plots were divided into warming and control treatments. Infrared heaters were used to increase the soil temperature of the warming plots by about 2.5℃ for three years. We used phospholipid fatty acid (PLFA) analysis to examine the composition of the soil heterotrophic microbial community. There were no significant differences in the total content of PLFAs (TotPLFAs) and fungal PLFAs (FungPLFAs) between the warming and control plots. However, warming caused an increase in the amount of bacterial PLFAs (BactPLFAs), the result being a lower ratio of FungPLFAs to BactPLFAs (F/B ratio) in the warming plots. In addition, PLFAs characteristic of gram-negative bacteria increased in the warming plots. The results indicated that the soil heterotrophic microbial community in this warm-temperate, evergreen broad-leaved forest was sensitive to climate warming.

Key Words : evergreen broad-leaved forest, microbial community composition, phospholipid fatty acid (PLFA), soil warming, warm-temperate zone

王　新・中坪孝之・佐々木晶子・吉竹晋平・梁　乃申・中根周歩：暖温帯常緑広葉樹林における土壌加温に対する微生物群集の反応．森林立地55：139–143，2013.

1. Introduction

The global mean temperature increased by about 0.6℃ during the 20th century as a result of climate warming and is expected to increase by another 1.1–6.4℃ by the end of the 21st century (IPCC, 2007). Terrestrial ecosystems contain almost three times the amount of carbon found in the atmosphere, with forests comprising by far the largest fraction (Schlesinger, 1997; IPCC, 2001). Because soils contain more than two-thirds of the carbon in forest ecosystems (IPCC, 2001), understanding the effect of climate warming on the functionality of terrestrial ecosystems requires knowledge about how forest soil processes respond to soil warming. Soil warming-induced changes in the composition and biomass of soil microbial communities have the potential to cause changes in soil carbon flow and are therefore likely to affect the global carbon cycle (Schimel and Gulledge, 1998; Bardgett *et al*., 2008).

To date there have been several experimental studies of the effects of soil warming on the biomass and composition of the soil microbial community in different types of forests (Schindlbacher *et al*., 2011; Frey *et al*., 2008; Allison and Treseder, 2008; Kuffner *et al*., 2012). The results of these studies have varied widely: no change in microbial biomss and composition in a spruce forest (Schindlbacher *et al*., 2011); reduction in fungal biomass and shift in the microbial community in a mixed deciduous forest (Frey *et al*., 2008); and increases in bacterial and fungal biomass in a conifer forest (Allison and Treseder, 2008). These results suggest that the effects of future climate warming on the the biomass and composition of the soil microbial community will differ among forest ecosystems with various tree species, locations and climates.

Evergreen broad-leaved forests are the dominant natural forest type in the warm-temperate zone of the east coasts and adjacent islands of Asia (Kira, 1978). This type of forest has a much higher carbon cycling rate than forests in cool climates, such as cool-temperate and boreal forests (Suzuki, 1982). Wang *et al*. (2012) recently used open-top chambers to examine the effects of climate change on soil respiration in experimental stands of *Quercus glauca* and reported that elevated temperature treatments caused a significant change

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(Received 09 July 2013, accepted 08 August 2013)

in the temperature sensitivity (Q_{10}) of heterotrophic respiration, a result that might have been caused by a change in the structure of the microbial community. However, there is little available information relevant to the potential impact of climate warming on soil microbial communities in warm-temperate, broad-leaved forests.

In this study we conducted a field experiment to examine the effect of soil warming on the soil heterotrophic microbial community in a warm-temperate, evergreen broad-leaved forest. We used infrared heaters to increase the soil temperature of experimental plots in a forest stand for three years and used phospholipid fatty acid (PLFA) analysis to examine changes in the heterotrophic microbial community.

2. Materials and methods

The experiment was conducted in a 25-year-old secondary evergreen oak forest following the pine tree death event occured in 1980s in the central part of the city of Higashi-Hiroshima in western Japan (34°41′N, 132°72′E; 335 m a.s.l). The forest area is approximately 1.6 km^2. The forest is dominated by *Quercus glauca*, interspersed with *Symplocos kuroki*, and *Ilex pedunculosa*. In 2007, the tree density and canopy height being 1,450 stems ha^{-1} and about 10 m, respectively. The soils are classified as coarse-textured, residual, immature soils with a parent rock of granite (Japan National Land Survey Division, Land and Water Bureau).

Ten plots on the forest floor within a circular area with a diameter of 40 m were randomly chosen. In order to prevent root regrowth into the plots, all the plots were treated by the trenching method developed by Liang *et al*. (2010). Ten 1 × 1 m root exclusion plots were established. Trenches with a width of 0.5 to 1 cm were dug down to 50 cm along the plot boundaries using a root-cutting chainsaw (CSVN671AG, Kioritz Co. Ltd., Tokyo, Japan) and then polyvinyl chloride (PVC) sheets (4 mm thick) were installed in the trenches to a depth of 50 cm to prevent root penetration. The plots were randomly allocated to two groups, five of which, the warming plots, were warmed continuously for 24 hours with infrared heaters beginning in October 2007. The other five served as controls. In each warming plot a 58-cm-long, 800-W infrared heating lamps were suspended in the middle of the plot at 1.6 m above the ground (Aguilos *et al*., 2013). The soil temperature and moisture content of each plot were measured continuously at soil depths of 5 and 15 cm. Data were stored on data-loggers at 10 s intervals (Liang *et al*., 2010).

Soil samples for PLFA analysis were collected from the mineral soil (0−5 cm) of the warming and control plots in October 2010. Three cores (5 cm diameter × 5 cm deep) were taken from each plot. One core was taken from the central point, and the other two cores were taken from the 1/4 point and 3/4 point of the diagonal line in each 1 × 1 m plot. We mixed the three cores from each plot to obtain one composite sample. The samples were returned to the laboratory, freeze-dried, sieved (< 2 mm) to remove gravel and dead roots, and stored at −80℃ until PLFA analysis.

Lipids were extracted by using a Bligh and Dyer (1959) extraction, as modified by White *et al*. (1979) and Frostegård *et al*. (1993). Briefly, an aliquot of 1−2 g (dry weight) of soil was extracted with a chloroform-methanol-citrate buffer mixture (1:2:0.8). The lipids were separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column (Sep-Pak™ plus silica; Waters Corp., Milford, MA, USA) (Arao *et al*., 2001). The phospholipids were esterified with a HCl-Methanol Reagent (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) (Stoffel *et al*., 1959). The resulting fatty acid methyl esters were separated with a gas chromatograph (GC-2014; Shimadzu Corp., Kyoto, Japan) equipped with a capillary column (30 m DB-5 ms, Phenyl-Methyl/Silicone; J&W Scientific Inc., Folsom, CA, USA). Helium was used as a carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard. The fatty acid nomenclature described by Frostegård *et al*. (1993) was used. The total content of PLFAs (TotPLFAs) was used as a metric of total microbial biomass (Frostegård *et al*., 1993). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, 18:1ω7c, and cy19:0 were chosen to represent bacterial PLFAs (BactPLFAs) (Frostegård *et al*., 1993; Federle, 1986). The amount of 18:2ω6,9 was used as a metric of fungal biomass (FungPLFA) (Federle, 1986; Frostegård and Bååth, 1996; Xue *et al*., 2008).

PLFAs are classifiable into several categories according to their molecular structure (Yoshitake *et al*., 2006). In this study PLFAs were classified into five categories (straight chain saturated fatty acids, branched chain fatty acids, unsaturated fatty acids, cyclopropyl fatty acids (cy17:0, cy19:0 and 18:2ω6,9) and summed their mole percents. Cyclopropyl fatty acids and branched chain fatty acids are known to be characteristic of gram-negative bacteria and gram-positive bacteria, respectively (Federle, 1986). Previous studies have shown that this classification is useful for detection of shifts in microbial community structure (Yoshitake *et al*., 2006).

Student's *t*-test was used to test for significant differences in soil properties and PLFA content between the warming and control plots. All statistical analyses were carried out with SPSS software (v. 19.0; SPSS Inc., Chicago, IL). We considered differences to be significant if the type I error rate (P) was 5% or less.

3. Results and discussion

Warming consistently increased soil temperatures at 5 cm depth by about 2.5℃ throughout the three-year experimental period. The ranges of daily average soil temperature were 4.1−22.6℃ and 7.1−25.8℃ in the control and warming plots, respectively. The corresponding ranges of soil moisture content were 20.5−37.6% and 20−36.5%, respectively. The soil moisture content of the warming and control plots did not differ significantly (Student's *t*-test, $P > 0.05$) during the three-year experimental period.

The TotPLFA content was not significantly affected by the three years of experimental soil warming (Student's *t*-test, $P > 0.05$, Table 1). A similar insensitivity of microbial

Table 1. Fatty acid content and the proportion of fatty acids in the microbial communities from the warming and control plots after three years. Mean and standard deviation (in parentheses, n = 5) are given.

	Warming	Control	*P*
Fatty acid content (nmol g^{-1})			
TotalPLFA	376.2 (86.6)	378.7 (84.6)	0.965
BactPLFA	115.4 (17.7)	90.0 (7.0)	0.017*
FungalPLFA	21.6 (8.9)	25.7 (7.9)	0.467
F/B ratio	0.2 (0.06)	0.3 (0.07)	0.040*
Fatty acid Proportions (mole percent)			
Straight chain saturated fatty acids	25.3% (5%)	23.9% (2%)	0.560
Branched chain fatty acids	28.4% (6%)	26.1% (2%)	0.442
Cyclopropyl fatty acids	12% (6%)	5.5% (2%)	0.047*
Unsaturated fatty acids	37.7% (3%)	40% (6%)	0.439
18:2ω6,9	5.7% (2%)	6.8% (1%)	0.309

Significant differences between the warming and control estimated by Student's *t*-test.
＊, $P < 0.05$

biomass to soil warming has been reported in several previous studies (Schindlbacher *et al*., 2011; Zhang *et al*., 2005; Zogg *et al*., 1997), although Petersen and Klug (1994) reported some change in soil microbial biomass in response to soil warming.

Bacterial and fungal biomass responed differently to soil warming: a significant increase in BactPLFA content occurred in the warming plots (Student's *t*-test, $P < 0.05$, Table 1), whereas FungPLFA content was not significantly affected by the three years of soil warming. As a result, the ratio of fungal to bacterial biomass decreased significantly (Table 1), an indication of a significant shift in the composition of the microbial community.

Differences in the relative abundance of cyclopropyl fatty acids, which are characteristic of gram-negative bacteria (Harwood and Russell, 1984), provided further evidence of a temperature-induced shift in community composition (Student's *t*-test, $P < 0.05$, Table 1). In addition, some new fatty acids (15:0, 17:0, 17:1ω7, i18:0, 20:4ω6, and 20:5ω3) were detected in the warming plots, whereas the fatty acid 16:1 became undetectable (Fig. 1), a further indication of changes in the composition of the microbial community.

Results of previous soil-warming field studies of microbial communities in forest ecosystems have been rather inconsistent though bacterial biomass was generally unaffected by elevated temperatures (Table 2). Schindlbacher *et al*. (2011) reported that a 4℃ increase in soil temperature during the snow-free season had no influence on the microbial community composition in a temperate, mountain forest soil. In contrast, Frey *et al*. (2008) reported a significant reduction in total microbial biomass and a shift in microbial community composition in a mixed deciduous forest after 12 years of soil warming of 5℃ above the ambient temperature. In a study of an Alaskan boreal forest, Allison and Treseder (2008) reported that bacterial and fungal abundance declined by more than 50% in a closed-top greenhouse treatment. However, because their warming treatment caused drying of the soil, the observed change in microbial biomass may have been partly due to the change in moisture content. Arnold *et al*. (1999) indicated that soil microbial response to experimental soil warming was most evident when soil moisture was not limiting (range between 20% and 120%). In this study the soil moisture content did not differ significantly between treatments, and the observed shift in the microbial community can therefore be attributed solely to the effect of warming. Considering the relatively short duration (3 years) of our study and the lower warming temperature (+ 2.5℃), we conclude that the soil bacterial community in this warm-temperate, evergreen broad-leaved forest was remarkably sensitive to warming effects.

It has been suggested that bacterial community composition influnces heterotrophic respiration and can potentially influence soil carbon storage (Cleveland *et al*., 2007). Although studies have yet to be carried out of the impact of climate change on members of the soil microbial community that are symbiotic with plants, these results suggest that changes in the heterotrophic micorobial community are among the mechanisms thorough which climatic warming affects the carbon cycle of warm temperate evergreen broad-leaved forest.

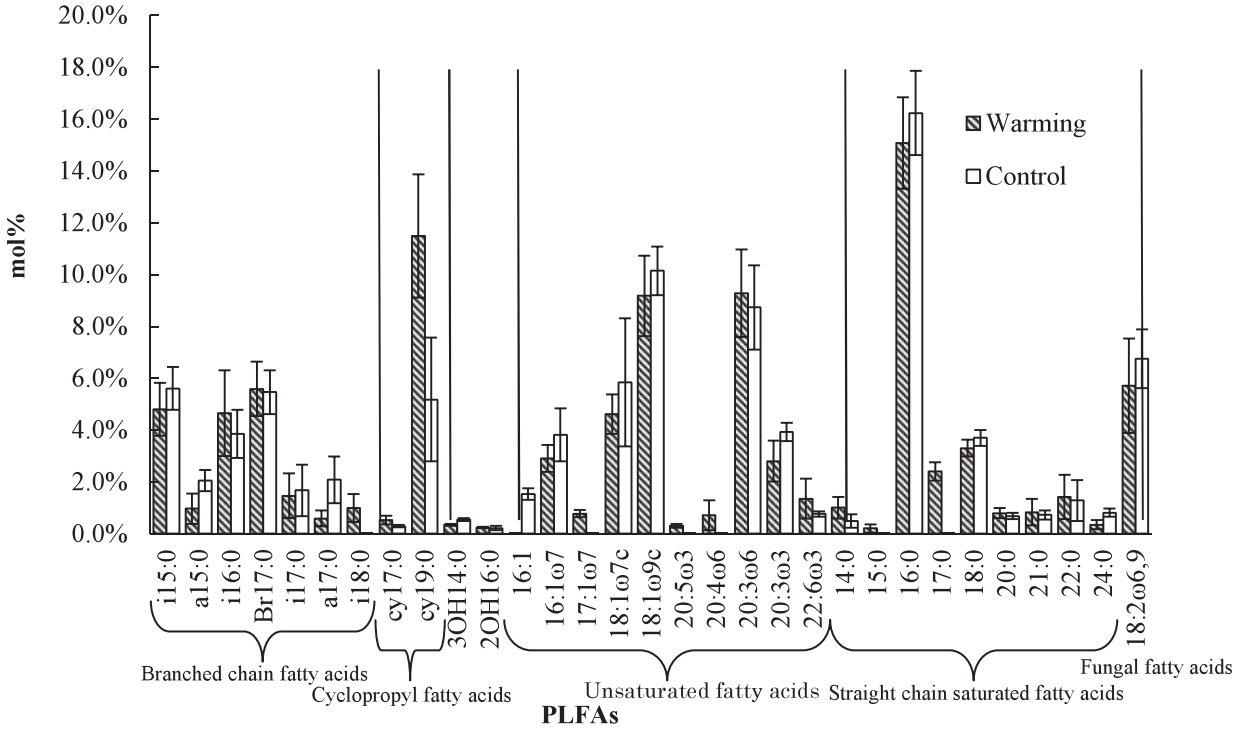

Fig. 1. Changes in the mole fraction of selected phospholipid fatty acids (PLFAs) extracted from the soils of the control and warming plots. Error bars are one standard error of the mean of five plots. W: warming; C: control.

Table 2. Effects of elevated temperature on soil microbial communities in experimental forest stands

Species	Temperature increase (℃)	Exposure period	Biomass			Reference
			Total	Bacteria	Fungi	
Conifer forest						
Picea mariana	+0.5 (Soil T)			-	-	Allison and Treseder (2008)
Picea mariana	+0.4–0.9	3 years		ns	ns	Bergner *et al*. (2004)
spruce-fir	+5		-			Arnold *et al*.(1999)
Deciduous broad-leaved forest						
Acer saccharum	5,15,25	16 weeks	-			Zogg *et al*. (1997)
Quercus velutina	+5	12 years	-		-	Frey *et al*. (2008)
Acer rubrum	+5	12 years	-		-	Frey *et al*. (2008)
Betula papyrifera	+5	12 years	-		-	Frey *et al*. (2008)
Acer pensylvanicum	+5	12 years	-		-	Frey *et al*. (2008)
Mixed forest (deciduous broad-leaved trees and conifers)						
Picea abies	+4	6 years	ns	ns	ns	Schindlbacher *et al*. (2011)
Fagus sylvatica	+4	6 years	ns	ns	ns	Schindlbacher *et al*. (2011)
Abies alba	+4	6 years	ns	ns	ns	Schindlbacher *et al*. (2011)
Picea abies	+4	4 years		ns		Kuffner *et al*. (2012)
Fagus sylvatica	+4	4 years		ns		Kuffner *et al*. (2012)
Abies alba	+4	4 years		ns		Kuffner *et al*. (2012)
Evergreen broad-leaved forest						
Quercus gluaca	+2.5	3 years	ns	+	ns	**Present study**

+ increased; − decreased; ns not significant

Acknowledgements

This work was financially supported by the Global Environment Research Fund (B-073) from Ministry of the Environment of Japan.

References

Aguilos, M., Takagi, K., Liang, N., Watanabe, Y., Teramoto, M., Goto, S., Takahashi, Y., Mukai, H., and Sasa, K. (2013) Sustained large stimulation of soil heterotrophic respiration rate and its temperature sensitivity by soil warming in a cool-temperate forested peatland. Tellus B (*in press*)

Allison, S.D. and Treseder, K.K. (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Chang Biology 14:2898−2909.

Arao, T., Okano, S. and Nishio, T. (2001) Comparison of bacterial and fungal biomass determined by phospholipid fatty acid and direct microscopical analysis in 4 types of upland soils. Soil Microorganisms 55:29−36.

Arnold, S.S., Fernandez I.J., Rustad L.E. and Zibilske L.M. (1999) Microbial response of an acid forest soil to experimental soil warming. Biology Fertility of Soils 30:239−244.

Bardgett, R.D., Freeman, C. and Ostle, N.J. (2008) Microbial contributions to climate change through carbon cycle feedbacks. The ISME Journal 2:2805−2814.

Bergner, B., Johnstone, J. and Treseder, K.K. (2004) Experimental warming and burn severity alter soil CO_2 flux and soil functional groups in a recently burned boreal forest. Global Change Biology 10:1996−04.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911−917.

Cleveland, C.C., Nemergut, D.R., Schmidt, S.K. and Townsend, A.R. (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82:229−240.

Frey, S.D., Drijber, R., Smith, H. and Melillo, J. (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology and Biochemistry 40:2904−2907.

Federle, T.W. (1986) Microbial distribution in soil_new techniques. Perspectives in Microbial Ecology, ed. by F. Megusar and M. Gantar. Ljubljana. Slovene Society for Microbiology 493−498.

Frostegård, A., Bååth, E. and Tunlid, A. (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25:723−730.

Frostegård, A. and Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22:59−65.

Harwood, J.L. and Russell, N.J. (1984) Lipids in Plants and Microbes. George Allen and Unwin, London. 162 pp.

Intergovernmental Panel on Climate Change (IPCC). (2001) The Scientific Basis. Cambridge University Press, Cambridge. 873pp.

Intergovernmental Panel on Climate Change (IPCC). (2007) Synthesis Report. Contribution of Working Group I, Cambridge University Press, Geneva. 73pp.

Kira, T. (1978) Vegetation and Physical Environment, *In* Kira, T., Ono, Y. and Hosokawa, T. (ed.) Biological Production in a Warm-Temperate Evergreen Oak Forest of Japan. JIBP Synthesis, University of Tokyo Press, Tokyo. pp. 61−127.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A. and Sessitsch, A. (2012) Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. Microbial Ecology 82:551−562.

Liang, N., Hirano, T., Zheng, Z.-M., Tang, J. and Fujinuma, Y. (2010) Soil CO_2 efflux of a larch forest in northern Japan. Biogeosciences 7:3447−3457.

Petersen, S.O. and Klug, M.J. (1994) Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profiles of a soil microbial community. Applied and Environmental Microbiology 60:2421−2430.

Schlesinger, W.H. (1997) Biogeochemistry: an analysis of global change, 2nd edn., Academic Press, San Diego. 432 pp.

Schimel, J.P. and Gulledge, J. (1998) Microbial community structure and global trace gases, Global Change Biology 4:745−758.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, M., Sessitsch, B. and Zechmeister-Bolternstern, S. (2011) Experimental warming effects on the microbial community of a temperate mountain forest soil. Soil Biology and Biochemistry 43:1417−1425.

Stoffel, W., Chu, F. and Ahrens, E.H. (1959) Analysis of long-chain fatty acids by gas-liquid chromatography. Analytical Chemistry 31:307.

Suzuki, K. (1982) Distribution of evergreen and summer green broadleaved forest in Japan. Bulletin, Institute of Environmental Science and Technology, Yokohama National University 8:151−163.

Wang, X., Nakatsubo, T. and Nakane, K., (2012) Impacts of elevated CO_2 and temperature on soil respiration in warm temperate evergreen *Quercus glauca* stands: an open-top chamber experiment. Ecological Research 27:595−602.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D. and Bobbie, R.J. (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51−62.

Xue, D., Yao, H.Y., Ge, D.Y. and Huang, C.Y. (2008) Soil microbial community structure in diverse land use systems: A comparative study using Biolog, DGGE, and PLFA analyses. Pedosphere 18 (5):653−663.

Yoshitake, S., Uchida, M., Nakatsubo, T. and Kanda, H. (2006) Characterization of soil microflora on a successional glacier foreland in the high Arctic on Ellesmere Island, Nunavnt, Canada, using phospholipid fatty acid analysis. Polar Bioscience 19:73−84.

Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L. and Hu, S. (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biology 11:266−277.

Zogg, G.P., Zak, D.R., Ringleberg, D.B., MacDonald, N.W., Pregitzer, K.S. and White, D.C. (1997) Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal 61:475−481.