Above- and belowground CH_4 fluxes from boreal forest shrubs and Scots pine

E. Halmeenmäki[1], J. Heinonsalo[2], M. Santalahti[1,2], H. Fritze[3], M. Pihlatie[1,2]

[1] Division of Atmospheric Sciences, Department of Physics, University of Helsinki, Finland

[2] Department of Food and Environmental Sciences, University of Helsinki, Finland

[3] Natural Resources Institute Finland, Vantaa, Finland

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INTRODUCTION

Traditionally, boreal upland forests are considered as important sinks for the greenhouse gas methane (CH_4) due to CH_4 oxidizing bacteria (methanotrophs) in the soil. Recent evidence, however, suggests that boreal forest ecosystems may act as occasional sources of CH_4 (Peltola *et al.* 2012; Shoemaker *et al.* 2014). Furthermore, over the last decade there has been growing evidence that vegetation can act as a significant source of CH_4 (Keppler *et al.* 2006; Mukhin & Voronin 2011; Covey *et al.* 2012), although the mechanisms of the emissions are still mostly unknown. Also, the majority of CH_4 flux studies has been conducted with soil chamber method, thus not considering the role of aboveground and belowground parts of the vegetation.

Since the first findings of Keppler *et al.* (2006) there has been growing interest about CH_4 emissions from plants. Emissions of CH_4 have been detected both from leaf material of grasses (Keppler *et al.* 2006; Vigano *et al.* 2008; Bruhn *et al.* 2009) and from tree stems (Mukhin & Voronin 2011; Covey *et al.* 2012; Machacova *et al.* 2014). Leaf CH_4 emissions from terrestrial plants under aerobic conditions have been demonstrated to be induced by UV radiation (Vigano *et al.* 2008; Bruhn *et al.* 2009) and estimated to be produced chemically from pectine (Bruhn *et al.* 2009) or leaf wax (Bruhn *et al.* 2014). Elevated CH_4 concentrations within and CH_4 emissions measured from stems of trees have been proposed to originate from methanogenic activity in the wood (Mukhin & Voronin 2011; Covey *et al.* 2012).

Moreover, methanogens have been discovered from the roots and mycorrhizae of Scots pine, silver birch, and Norway spruce seedlings, indicating that CH_4 can also be produced in the rhizosphere of upland forest soils (Bomberg *et al.* 2011). Thus, CH_4 emissions detected from above the tree canopy might originate from aboveground vegetation or from belowground parts, or soil. These findings underline the importance to assess the potential CH_4 production in different compartments of forest ecosystems, and to evaluate the role of methanogens in the CH_4 dynamics.

METHODS

We measured CH_4 flux from three common boreal forest shrubs and Scots pine growing in microcosms (Pumpanen *et al.* 2009) in laboratory. The studied species were bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), heather (*Calluna vulgaris*), and Scots pine (*Pinus sylvestris*). The studied plants were grown during 2012–2013 from seeds under laboratory conditions and replanted into the microcosms as small seedlings. The microcosms consist of a plate including the soil and the roots (if a plant is involved), which can be closed airtight, and of a separate chamber that can be attached to the shoot of a plant. The measurements of CH_4 flux were performed between November 2013 and January 2014 separately for above- and belowground parts of each plant, and compared to fluxes of control microcosms containing only humus soil without a plant. For each species there were eight plant individuals, and in total 11 soil microcosms, which were treated the same way as those with a seedling throughout the experiment. In addition to the flux measurements, DNA was extracted from the plant material (shoots and roots) and soil and stored for further analysis of methanogen presence by quantitative PCR (qPCR) using methanogen specific primer pair (Steinberg and Regan 2008).

RESULTS & CONCLUSIONS

The CH_4 fluxes from belowground parts of the studied shrubs and pine seedlings showed mainly uptake of CH_4, while the bare soil emitted CH_4 (Fig. 1a). The shoot fluxes of heather and Scots pine indicated mainly CH_4 emissions, while the fluxes from bilberry and lingonberry were close to zero (Fig. 1b). However, the differences in fluxes between different compartments of a plant or between the plant species were not statistically significant, except when lingonberry root and soil microcosms were compared ($t=-3.7$, $df=11$, $p<0.01$). The CH_4 fluxes from the shoot compartments had in general large ranges and flux values (Fig. 1b) compared to the root systems (Fig. 1a).

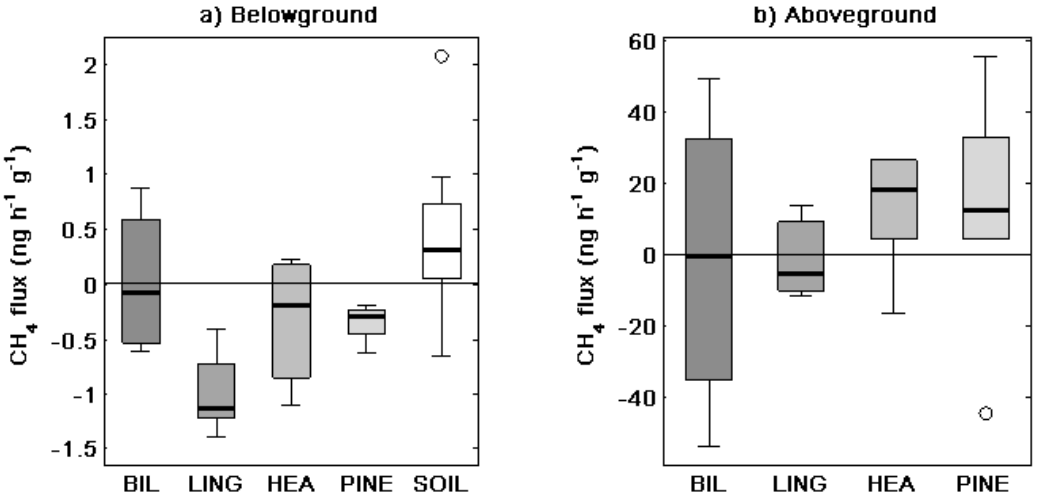

Figure 1. Boxplots representing CH_4 fluxes in ng h^{-1} g^{-1} (of dry weight of soil/aboveground plant material) from the belowground (a) and aboveground (b) compartments of different plants (BIL=bilberry, LING=lingonberry, HEA=heather, PINE=Scots pine) and soil (SOIL) (a). Negative flux values indicate uptake and positive fluxes indicate emission. The bottom and the top of the box represent 25th and 75th percentiles, respectively, the thick line inside the box shows the median, whiskers represent the range from the lowest to the highest value, and outliers (values further from the box than 1.5-times the height of the box) are represented with circles. Note that the plots a and b have different scales.

To support the results of the flux measurements, the mean CH_4 concentrations in the gas samples taken from the aboveground chambers (enclosing shoots of the plants) were compared to the mean CH_4 concentrations in the samples from the belowground chambers (enclosing roots and soil). The mean CH_4 concentrations in the shoot compartments were higher than those in the root compartments for all the plants measured, apart from one bilberry shoot, and the differences were statistically significant ($p<0.0001$) (Fig. 2). Furthermore, the mean CH_4 concentrations of the root compartments of all the plant species were significantly lower than the mean of the soil compartments ($p<0.0001$).

The results of the flux measurements suggest that roots of the studied plant species consume CH_4 compared to bare humus soil. Also, the results indicate that the shoots of heather and Scots pine emit CH_4 while the roots consume it. Additionally, for all the plant species the mean concentrations of CH_4 in samples from aboveground chambers were significantly higher than those from below ground and those from bare soil. Further analysis of methanogens within the plant-soil systems may reveal whether the origin of the produced CH_4 is of microbial origin.

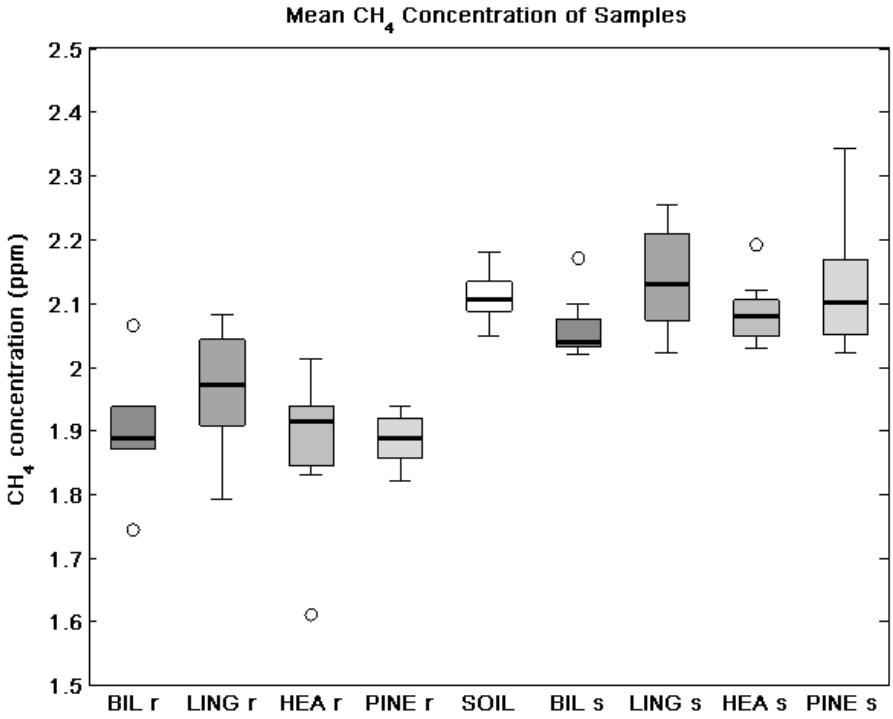

Figure 1. Boxplots representing the mean CH_4 concentrations (ppm) of the samples from each compartment of the microcosms. The plant species are marked with abbreviations (BIL=bilberry, LING=lingonberry, HEA=heather, PINE=Scots pine), and SOIL is for control, followed by 'r' or 's' for root and shoot compartments, respectively. The bottom and the top of the box represent 25th and 75th percentiles, respectively, the thick line inside the box shows the median, whiskers represent the range from the lowest to the highest value, and outliers (values further from the box than 1.5-times the height of the box) are represented with circles.

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