Are methanogens involved in methane emissions in boreal upland forest?

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INTRODUCTION

Boreal upland forests are considered as a sink for the greenhouse gas methane (CH_4) due to methanotrophic microbes that oxidize CH_4 in soils. Recently, number of studies have suggested that the ecosystem can occasionally overcome the sink strength of the soil and the forest may in total act as a source of CH_4 (Mikkelsen *et al*., 2011; Peltola *et al*., 2012; Shoemaker *et al*., 2014), and that the vegetation can act as a significant source of CH_4 (Keppler *et al*. 2006; Mukhin & Voronin 2011; Covey *et al*. 2012). However, the origin and the production mechanisms of CH_4 emitted from vegetation still remains controversial (Keppler *et al*., 2006; Bloom *et al*., 2010; Covey *et al*., 2012). The unknown role of vegetation, and the unspecified processes behind the CH_4 emissions demonstrate that our understanding of CH_4 sources in boreal forest ecosystems are not complete. Especially it is unclear whether the plant-emitted CH_4 originates from biotic or abiotic processes.

In the METAFOR project (*Revealing sources of biological methane production in boreal upland forests*), one of our aim is to evaluate whether methane producing microbes (methanogens) could be responsible for CH_4 emissions in boreal upland forest ecosystem. In order to answer this question, we screen and quantify methanogens from different compartments (soil, ground vegetation and trees) of a boreal upland forest ecosystem at the SMEAR II station in Hyytiälä, southern Finland. Finally, we relate the information of methanogens to the CH_4 fluxes measured from the same compartments of the forest.

METHODS

The study site is a boreal upland forest dominated by ~60 year old Scots pine (*Pinus sylvestris* L.) with scattered Norway spruce (*Picea abies*) and silver birch (*Betula pendula*) in the understory. To detect the abundance of the methanogenic community, samples of the most prevalent shrub (*Vaccinium vitis-idaea, Vaccinium myrtillus, Calluna vulgaris, Equisetum sylvaticum*), moss (*Sphagnum spp., Polytrichum spp., Dicranum polysetum, Pleurozium schreberi, Hylocomium splendens)* and tree (*Pinus sylvestris, Picea abies, Betula pendula, Salix spp.*) species, together with samples of soil, peat and decayed wood, were taken in June 2014 and 2015 from the study site. Five replicate samples from each sample material were divided into different compartments: shoots, stem and roots, or upper and lower layer of soil and peat. DNA was extracted manually from freeze-dried and homogenized sample material with hot-CTAB method at +65°C, modified from Salavirta *et al.* (2014), and DNA was purified with PowerClean® DNA Clean-up kit (Mo Bio Laboratories Inc,, USA). To detect and quantify the methanogenic community, quantitative PCR (qPCR) with specific primers (Steinberg and Regan 2008) targeting the α-subunit of the

methyl-coenzyme M reductase (*mcr*A) gene, was applied. To link the presence of the *mcr*A-genes to the CH_4 exchange in the field, the CH_4 fluxes were measured from different compartments of the forest (forest floor, tree stems and shoots) with static chamber method (Pihlatie *et al.*, 2013; Machacova et al., 2014). Flux measurements were conducted at minimum with monthly frequency during 2013–2015.

CONCLUSIONS

Based on our 3-year CH_4 flux measurements, for most of the year the forest floor acted as a sink of CH_4. However, from the wet spots of the forest, some emissions occurred mostly during May to July. Also, tree stems and shoots emitted small amounts of CH_4 throughout the year, with the highest emission rates coming from the trees growing on the wet locations. The qPCR analysis revealed high number of the *mcr*A-gene copies from the peat in the wet spots of the forest floor (on average $1.3*10^{10}$ and $1.5*10^{10}$ gene copies g^{-1} of peat from the upper and lower layers, respectively), while the copy numbers from drier mineral soil samples were under the detection limit. The analysis are still ongoing, but our preliminary results indicate that, in addition to the wet soil samples, the *mcr*A-gene copies are detectible also from the understory vegetation, e.g. shoots and roots of different mosses, and roots of *Equisetum sylvaticum*. These preliminary findings support our hypothesis that methanogens are involved in the CH_4 production in boreal upland forest ecosystems. However, their role in the CH_4 fluxes from boreal upland forests still needs further investigations.

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