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## Effects of the conversion of cropland to forest on the CH4 oxidation capacity in soils.

Teresa G. Bárcena (1), Ludovica D'Imperio (1), Anders Priemé (2), Per Gundersen (1), Lars Vesterdal (1), and Jesper R. Christiansen (3)

(1) University of Copenhagen, Department of Geosciences and Natural Resource Management, Frederiksberg, Denmark, (2) University of Copenhagen, Department of Biology, Copenhagen, Denmark, (3) University of British Columbia, Department of Forest Sciences, Vancouver, Canada

As the second most important greenhouse gas (GHG) in the atmosphere, methane (CH4) plays a central role in global warming. Diverse types of soil have been reported as potential CH4 sinks due to the activity of methane oxidizing bacteria (MOB), underlining the importance of this functional group of microorganisms on a global basis. Agricultural practices are known to negatively affect CH4 oxidation in soil, while afforestation of former agricultural soils has been shown to enhance CH4 oxidation over time. However, knowledge is scarce with regard to the mechanisms driving the process of CH4 oxidation in different land uses. Our aim was to study the changes in CH4 uptake capacity in soils along a land-use change gradient from cropland to forest.

We performed an incubation experiment to study the CH4 oxidation capacity of the top mineral soil (0-5 cm and 5-15 cm depth) for sites representing the transition from agriculture to afforestation based on monoculture of three tree species with different stand ages: pedunculate oak (4, 19, 42 and >200 years old), European larch (22 and 41 years old) and Norway spruce (15 and 43 years old). Main soil parameters were also measured to determine differences in soil properties between sites. Methane oxidation rates were related to the abundance of the soil methanotrophic community based on quantitative PCR (qPCR). In addition, we also estimated the abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA), in order to investigate the link between these two similar functional groups. Although present, the abundance of AOB was under detection limit. The effects and interactions among all measured variables were summarized by Principal Component Analysis (PCA).

Along the gradient, CH4 oxidation increased with increasing stand age in both soil layers (ranging from 0-1.3 nmol g-1dw d-1). However, we detected significant differences, in particular between oak and spruce, suggesting a possible tree species effect on the CH4 oxidation potential. The abundance of MOB also increased with stand age in the top layer (0-5 cm), but this trend was not clear in the 5-15 cm. On the other hand, we found a consistent decrease in the abundance of AOA with increasing stand age. This trend suggests that over time, the environmental niche shared by these microbial populations changes in favour of the MOB, most likely induced by changes in soil parameters, such as bulk density, carbon content and concentration of inorganic forms of nitrogen. In fact, results from the PCA indicated that over time, bulk density and carbon content were the variables that changed the most across the land use gradient, thereby influencing the CH4 oxidation capacity and the presence of the MOB.

Our study provides evidence for a positive impact of afforestation of former cropland on CH4 uptake capacity in soils, contributing to mitigate the climatic consequences of this strong GHG in the atmosphere.

Keywords: methane oxidation, afforestation, methane oxidizing bacteria (MOB), bulk density.