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Investigating CH4 production in an oxic plant-soil system –a new approach combining isotopic labelling (13C) and inhibitors

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Typically, aerated soil are net sinks of atmospheric methane (CH4), being highest in native ecosystems (pristine forests > managed forests > grasslands > crop fields). However, this does not exclude a simultaneous endogenic CH4 production in the plant-soil system, which cannot be detected simply via CH4 flux measurements. Methanogenic archaea producing CH4 under anoxic conditions were thought to be the only biotic source of CH4 in the soil. However, until recently a non-archaeal pathway of CH4 formation is known where CH4 is produced under oxic conditions in plants (Keppler et al. 2006) and fungi (Lenhart et al. 2012). Additionally, abiotic formation of CH4 from soil organic matter was reported (Jugold et al. 2012) and may be ubiquitous in terrestrial ecosystems.
The major goal of this project was to determine soil endogenic CH4 sources and to estimate their contribution to the endogenic CH4 production. Especially the effect of plants and fungi on soil CH4 production was investigated. Therefore, a series of experiments was carried out on field fresh soil collected in a grassland and a forest ecosystem under controlled laboratory conditions. By combining selective inhibitors and 13C labelling, CH4 production rates of several CH4 sources were quantified. The major difficulty was to detect the comparatively small flux of CH4 production against the background of the high CH4 consumption rates due to methanotrophic bacteria.
Therefore, we supplemented bare soil and soil with vegetation with selective inhibitors and 13C labelled substrates in a closed chamber system. In a first step, CH4 production was determined by the inhibition of CH4 oxidizing bacteria with Difluoromethane (DFM, 2ml l-1). In the following, a 13C labelled substrate (either CO_2, Acetate, or Methionine –S-CH3 labelled) was added in combination with a specific inhibitor –either for archaeal methanogenesis (Bromoethanesulfonate), bacteria (Streptomycin), or fungi (Captan, Cycloheximide). Gas samples were taken during the incubation for CH4 and CO_2 concentration measurements and isotope ratio mass spectrometry (CH4, CO_2).
Grassland and forest soils showed differences in CO_2 and CH4 production rates. Based on the 13C-CH4 signature we found that all substrates were metabolized to CH4, but to a different degree. Inhibitors reduced CH4 production and conversion of certain substrates to a different degree. Using the example of acetate and cycloheximide, in both soils acetate increased respiration, whereas cycloheximide reduced respiration by 56 and 62 %, respectively. For CH4 production, however, no effect was visible for the grassland soil, but in the forest soil CH4 production increased by 69 %. Cycloheximide inhibited the substrate-induced CH4 production by 63 %, indicating that fungi were responsible for this pathway. Moreover, the finding that fungi use the methyl group of acetate to produce CH4 was also verified with a sterile culture.

References

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