Geophysical Research Abstracts,
Vol. 11, EGU2009-13191, 2009
EGU General Assembly 2009

{Stable isotope probing of the physical and biological controls that influence the fate and isotopic composition of carbon derived from the terrestrial methane sink }

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Methane oxidizing bacteria (Methanotrophs) occur in every soil order, and are an important sink for atmospheric CH_4 in well aerated soils. The quantity of C cycled via methanotrophic bacteria in soils is globally significant (Le Mer *et al*., 2001) yet the fate of methane derived carbon remains largely unknown and unquantified.

There is generally good agreement regarding the magnitude of the soil CH_4 sink determined by methane flux measurements and process modeling. More poorly characterised aspects of the soil CH_4 sink include: (i) the physical and biological controls that influence the mechanism of CH_4 oxidation in soils; (ii) the fate of oxidized CH_4 carbon; (iii) the proportion of C from CH_4 oxidation that is sequestered as organic C or released as CO_2 (iv) the magnitude of kinetic isotope effects (KIEs) associated with high affinity methanotrophy in soils and the potential influence on the stable carbon isotope composition of atmospheric CH_4.

This research combines multiple stable isotope analytical approaches to investigate the magnitude, mechanism and pathways of the terrestrial methane sink. Principally $^{13}CH_4$ stable isotope labeling techniques (Stable isotope probing; SIP) have been used to characterize and quantify methanotrophic populations in a range of different soils (Maxfield *et al*., 2006). Following $^{13}CH_4$-incubations soil cores were removed for compound-specific C isotope analyses. Identification and quantification of methanotrophs was effectively achieved via the analysis of ^{13}C-labelled phospholipid fatty acids (PLFAs) to link bacterial structure and function. It was also possible to identify the predominant controls influencing the active methanotrophic populations in both grassland and woodland soils (Maxfield *et al*., 2008).

SIP can be combined with further isotopic analyses to facilitate a broader study of methanotroph C uptake and CH_4 derived C sequestration. As SIP facilitates taxonomic assignments of the soil microorganisms involved in CH_4 C cycling, associations can be made between CH_4 derived C, methanotrophic bacteria and the downstream processing of their biomass C through long-term isotope labeling incubations. The amount of ^{13}C retained within soils during the latter part of $^{13}CH_4$ incubations was monitored through bulk soil δ^{13}C analysis (total sequestered C) and isotopic analysis of soil biomarkers (C flow pathways). It was apparent that a significant proportion of the CH_4 derived C is retained within soils, as opposed to being lost from the soil as CO_2, despite significant and rapid turnover of C from methanotroph cell material.

Furthermore, SIP data can be used to evaluate in situ kinetic isotope effects (KIEs) associated with uptake of atmospheric CH_4 by high affinity methanotrophic bacteria with potential physical and biological factors that influence the mechanism of uptake including climate, site age, CH_4 oxidation rate, microbial biomass, methanotroph population size and identity. Typically soil high affinity methanotrophy KIEs appear to be largely invariant between sites. However, in situ KIEs exhibited a statistically significant relationship with methanotroph biomass and type quantified by ^{13}C stable isotope probing. This finding, albeit based upon a small dataset, suggests that ^{13}C and ^{12}C partitioning associated with oxidation of atmospheric CH_4 in soil may occur in part as a result of biological as well as physical processes as suggested by laboratory culturing experiments (Templeton *et al*., 2006).

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