Stable isotope probing reveals high activity of branched GDGT-producing microorganisms in the aerobic horizon of peat bogs

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Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are widely distributed in terrestrial and aquatic environments and are being increasingly used as temperature proxies. Nevertheless, to date, little information is known regarding the microorganisms that produce these compounds. Anaerobic horizons in peat bogs are known to contain relatively high concentrations of brGDGTs and may provide a suitable habitat for their microbial source organisms. Based on changes in lipid distributions in both laboratory and field experiments, brGDGT source microorganisms in peat were suggested to adjust their membrane lipid composition in response to soil temperature changes on timescales of 3 months to 1 year. To further investigate the metabolism and growth rate of brGDGT-producing organisms, peat samples were collected from two adjacent sites with contrasting humidity levels (hereafter called dry and wet sites) in a *Sphagnum*-dominated peatland (Jura Mountains, France) and subjected to stable isotope probing experiment,. For each site, samples from the surficial aerobic layer (acrotelm) and deeper anaerobic layer (catotelm) were collected. These samples were incubated with ^{13}C-labeled DIC and deuterium (D)-labeled water for a period of two months, under both aerobic and anaerobic conditions for the acrotelm, and only anaerobic conditions for the catotelm. The incubations were performed at 12 °C, consistent with the mean annual air temperature at the sampling site.

There was no obvious change in brGDGT distribution or abundance during the incubations. No deuterium incorporation was observed for brGDGTs isolated from anaerobically-incubated acrotelm and catotelm samples. In contrast, the D content of brGDGTs isolated from aerobically-incubated acrotelm samples from the two sites increased by 295‰ after one month of incubation, showing relatively rapid production of brGDGTs at the peat surface. Similar results were obtained from both the dry and the wet sites. However, there was no ^{13}C incorporation into brGDGTs incubated underaerobic or anaerobic incubations, indicating that brGDGT-producing bacteria are heterotrophic microorganisms .The D uptake corresponds to production rates of up to 1.4 ng brGDGT per gram of peat dry weight (g^{-1}-dw) per day (d^{-1}). The production of bacterial fatty acids on the other hand was much higher ($\delta D > 1000‰$; up to 105 ng g^{-1}-dw d^{-1}). The roughly two orders of magnitude lower production rate of brGDGTs relative to fatty acids imply that the brGDGT producers do not necessarily have to be dominant community members; the high concentrations of brGDGTs observed in many soil profiles could simply result from their selective preservation.

Taken together, our results quantify, for the first time, the rapid activity of brGDGT-producing bacteria in the aerobic acrotelm, in contrast to the catotelm, where anaerobic conditions predominate. This suggests that these microorganisms may be especially active at the peat surface, which could in turn explain the rapid adjustement of their membrane lipid composition previously observed. Consequently, the high abundance of brGDGTs observed in the catotelm may result from the accumulation of the brGDGTs actively produced in the acrotelm.