



Do variations in the $\delta^{13}\text{C}$ of soil phospholipid fatty acids indicate changes in substrate use with climate warming?

Lukas Kohl (1), Jérôme Laganière (1,2), Kate Edwards (3), Christoph Lehmeier (4,5), Kyungjin Min (4), Sharon A. Billings (4), Ford Ballantyne (4), Geert Van Biesen (1), Penny L. Morrill (1), and Susan E. Ziegler (1)

(1) Department of Earth Sciences, Memorial University, St John's, NF, Canada (lukas.kohl@mun.ca), (2) Currently at Canadian Forest Service, Laurentian Forestry Centre, Natural Resources Canada, Quebec, QC, Canada, (3) Canadian Forest Service, Atlantic Forestry Centre, Natural Resources Canada, Corner Brook, NF, Canada, (4) Department of Ecology and Evolutionary Biology, Kansas Biological Survey, University of Kansas, Lawrence, KS, United States, (5) Currently at the Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

The effect of climate warming on the microbial mineralization of soil organic carbon (SOC) remains a key uncertainty in biogeochemical models. In particular, it remains unclear whether microbial substrate use patterns change with climate. The carbon isotopic signature ($\delta^{13}\text{C}$) of soil microbial phospholipid fatty acids ($\delta^{13}\text{C}_{\text{PLFA}}$) can provide insight into substrate utilization patterns in situ without manipulations unavoidable in soil incubation experiments. The interpretation of $\delta^{13}\text{C}_{\text{PLFA}}$, however, is limited by gaps in our understanding of the isotopic fractionation associated with PLFA biosynthesis, and in particular, whether this fractionation changes with growth temperature. Characterizing the principles controlling $\delta^{13}\text{C}_{\text{PLFA}}$ under controlled laboratory conditions can help with the interpretation of field measurements of temperature effects on microbial SOC assimilation.

We measured $\delta^{13}\text{C}_{\text{PLFA}}$ from field soils in two regions along a boreal climate transect, which differ by 4.5 °C mean annual temperature. In each region, the organic (L, F, H) and mineral (B; top 10cm) soil horizons were sampled at three locations chosen for similar vegetation (balsam fir), stand age, elevation, and soil type (humo-ferric podzol).

Soils from both regions had similar bulk SOC $\delta^{13}\text{C}$ and exhibited an increase with depth from -29.5 ± 0.4 to -26.5 ± 0.6 ‰. Despite the similar $\delta^{13}\text{C}$ of SOC, PLFA in the organic horizons from the warmer region were more enriched in ^{13}C relative to those from the colder region. In a model that used region, horizon and the individual PLFA as predictors, we found that region had a subtle (0.7‰), but highly significant ($p < 0.001$) effect on $\delta^{13}\text{C}_{\text{PLFA}}$. Substantial differences existed in the regional effect on $\delta^{13}\text{C}$ individual PLFA. For example, in the H horizon regional differences were highest for PLFA 18:2 ω 6,9 (fungi; 2.3‰) and PLFA i15:0 (Gram negative bacteria; 1.3‰) and lowest for PLFA 18:1 ω 7 (Gram negative bacteria; ± 0.0 ‰). The $\delta^{13}\text{C}_{\text{PLFA}}$, however, did not differ between regions in the mineral horizons. The elevated $\delta^{13}\text{C}_{\text{PLFA}}$ in the organic horizons of the warmer region might be due to (1) the consumption of more ^{13}C -enriched (e.g. carbohydrate-rich) or less ^{13}C -depleted (lignin-derived) substrates, (2) the consumption of more processed, ^{13}C enriched SOC or (3) physiological effects impacting fatty acid biosynthesis and fractionation at a higher temperature.

To help assess whether this difference in $\delta^{13}\text{C}_{\text{PLFA}}$ between regions was due changes in substrate use or due to physiological effects of the distinct temperature regimes, we conducted a series of chemostat experiments to isolate the effect of temperature on $\delta^{13}\text{C}_{\text{PLFA}}$. We cultivated *Pseudomonas fluorescens*, a gram-negative bacterium common in soils, in continuous culture in a mineral medium amended with a single carbon substrate (cellobiose, $\delta^{13}\text{C}_{\text{cellobiose}} = -24.2$ ‰) at three temperatures (16, 21, and 26.5 °C). We found similar $\delta^{13}\text{C}_{\text{PLFA}}$ in cultures at 16 and 21 °C where the weighted mean of three PLFA were both -31.1 ± 0.1 ‰, but lower $\delta^{13}\text{C}_{\text{PLFA}}$ at 26.5 °C where the weighted mean $\delta^{13}\text{C}_{\text{PLFA}}$ was -35.1 ± 0.6 ‰. Laboratory findings from this simplified system thus contrast with those observed using more complex, environmental samples, suggesting that the climate-induced change in $\delta^{13}\text{C}_{\text{PLFA}}$ from the environmental samples is not likely dominated by a direct physiological effect of temperature on microbial metabolism. Rather our findings from intact soils suggest shifts in microbial substrate use occur with climate warming.