



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

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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 ( $\delta^{13}\text{C}_{PLFA}$ ) can provide insight into substrate utilization patterns in situ without manipulations unavoidable in soil incubation experiments. The interpretation of  $\delta^{13}\text{C}_{PLFA}$ , 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  $\delta^{13}\text{C}_{PLFA}$  under controlled laboratory conditions can help with the interpretation of field measurements of temperature effects on microbial SOC assimilation.

We measured  $\delta^{13}\text{C}_{PLFA}$  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  $-29.5 \pm 0.4$  to  $-26.5 \pm 0.6\text{‰}$ . Despite the similar  $\delta^{13}\text{C}$  of SOC, PLFA in the organic horizons from the warmer region were more enriched in  $^{13}\text{C}$  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  $\delta^{13}\text{C}_{PLFA}$ . 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 $\omega$ 6,9 (fungi; 2.3‰) and PLFA i15:0 (Gram negative bacteria; 1.3‰) and lowest for PLFA 18:1 $\omega$ 7 (Gram negative bacteria;  $\pm 0.0\text{‰}$ ). The  $\delta^{13}\text{C}_{PLFA}$ , however, did not differ between regions in the mineral horizons. The elevated  $\delta^{13}\text{C}_{PLFA}$  in the organic horizons of the warmer region might be due to (1) the consumption of more  $^{13}\text{C}$ -enriched (e.g. carbohydrate-rich) or less  $^{13}\text{C}$ -depleted (lignin-derived) substrates, (2) the consumption of more processed,  $^{13}\text{C}$  enriched SOC or (3) physiological effects impacting fatty acid biosynthesis and fractionation at a higher temperature.

To help assess whether this difference in  $\delta^{13}\text{C}_{PLFA}$  between regions was due to 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  $\delta^{13}\text{C}_{PLFA}$ . 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,  $\delta^{13}\text{C}_{cellobiose} = -24.2\text{‰}$ ) at three temperatures (16, 21, and 26.5 °C). We found similar  $\delta^{13}\text{C}_{PLFA}$  in cultures at 16 and 21 °C where the weighted mean of three PLFA were both  $-31.1 \pm 0.1\text{‰}$ , but lower  $\delta^{13}\text{C}_{PLFA}$  at 26.5 °C where the weighted mean  $\delta^{13}\text{C}_{PLFA}$  was  $-35.1 \pm 0.6\text{‰}$ . Laboratory findings from this simplified system thus contrast with those observed using more complex, environmental samples, suggesting that the climate-induced change in  $\delta^{13}\text{C}_{PLFA}$  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.