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# Microbial metabolism in soil at subzero temperatures: Adaptation mechanisms revealed by position-specific $^{13}C$ labeling

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## Abstract

© 2017 Bore, Apostel, Halicki, Kuzyakov and Dippold. Although biogeochemical models designed to simulate carbon (C) and nitrogen (N) dynamics in high-latitude ecosystems incorporate extracellular parameters, molecular and biochemical adaptations of microorganisms to freezing remain unclear. This knowledge gap hampers estimations of the C balance and ecosystem feedback in high-latitude regions. To analyze microbial metabolism at subzero temperatures, soils were incubated with isotopomers of position-specifically 13 C-labeled glucose at three temperatures: +5 (control), -5, and -20°C. 13 C was quantified in CO 2 , bulk soil, microbial biomass, and dissolved organic carbon (DOC) after 1, 3, and 10 days and also after 30 days for samples at -20°C. Compared to +5°C, CO2 decreased 3- and 10-fold at -5 and -20°C, respectively. High 13 C recovery in CO 2 from the C-1 position indicates dominance of the pentose phosphate pathway at +5°C. In contrast, increased oxidation of the C-4 position at subzero temperatures implies a switch to glycolysis. A threefold higher 13 C recovery in microbial biomass at -5 than +5°C points to synthesis of intracellular compounds such as glycerol and ethanol in response to freezing. Less than 0.4% of 13 C was recovered in DOC after 1 day, demonstrating complete glucose uptake by microorganisms even at -20°C. Consequently, we attribute the fivefold higher extracellular 13 C in soil than in microbial biomass to secreted antifreeze compounds. This suggests that with decreasing temperature, intracellular antifreeze protection is complemented by extracellular mechanisms to avoid cellular damage by crystallizing water. The knowledge of sustained metabolism at subzero temperatures will not only be useful for modeling global C dynamics in ecosystems with periodically or permanently frozen soils, but will also be important in understanding and controlling the adaptive mechanisms of food spoilage organisms.

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## Keywords

Cryoprotectants, Freeze tolerance, Metabolic pathways, Position-specific labeling, Psychrophiles

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