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Dark CO_2 fixation by soil microbes contributes to soil organic matter formation in soils from an old growth deciduous forest.

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Soils are the largest terrestrial organic carbon pool and one of the largest terrestrial sources of CO_2 in the atmosphere. However, not all CO_2 produced in soils is released into the atmosphere. Microbial CO_2 fixation has been shown to modulate CO_2 release from soils, yet studies on microbial CO_2 fixation in soils are scarce. Here, we explore microbial CO_2 fixation in soils of a temperate forest, as forests are recognized as one of the largest and most important ecosystems on earth. Our study aimed to assess the rates of microbial CO_2 fixation, the biogeochemical parameters influencing them, and the contribution of this process to soil organic matter formation, using a well characterized Eutric Cambisol soil plot in the Hainich National Park, Germany. In this study, we hypothesize that dark CO_2 fixation is mainly driven by autotrophs throughout the soil profile and that the contribution of this process to microbial biomass and soil organic matter formation increases with soil depth.
Dark CO_2 fixation was quantified via the uptake of 13C-CO_2 added to microcosms containing soils sampled from three depths. Under 2% CO_2 headspace, rates of microbial CO_2 fixation decreased with depth from 0.86 μg C gdw-1d-1 in 0 - 12 cm to 0.05 μg C gdw-1d-1 in 70 -100 cm. However, as microbial biomass also declined with depth, no significant differences were observed when rates were normalized to microbial biomass. This consistency in the normalized CO_2 fixation rates across depths presents microbial biomass as a main parameter determining CO_2 fixation rates in the soils investigated. Furthermore, an increase of the headspace CO_2 concentration enhanced microbial CO_2 fixation rates; with up to a 3.4 fold increase in fixation rate under 20% CO_2 showing that microbial CO_2 fixation can be substantial especially in soil microsites with higher CO_2 concentrations. Moreover, after 28 days of incubation, the labeled 13C fixed by microbes accounted for up to 1.1% of microbial biomass carbon and up to 0.035% of soil organic carbon. To determine if CO_2 fixation was carried out by autotrophs or heterotrophs, cbbL IA, cbbL IC and cbbM RuBisCO marker genes involved in autotrophic CO_2 fixation were quantified by qPCR. Surprisingly, the cbbL IA and cbbL IC genes together represented less than 0.1% of the bacterial 16S rRNA gene copies independent of soil depth. Likewise, a DNA stable isotope probing approach revealed no increased abundance of putative autotrophs using RuBisCO in the 13C labelled microbial community. These findings suggest that dark CO_2 fixation is an important process that contributes to microbial biomass and soil organic carbon formation across soil depths with a majority of CO_2 fixation performed by heterotrophs, and autotrophs playing only a minor role.