



Significance and driving forces of dark CO₂ fixation for organic carbon inputs in temperate forest soils

Rachael Akinyede^{1,2}, Martin Taubert¹, Marion Schruppf², Susan Trumbore², and Kirsten Küsel¹

¹Aquatic Geomicrobiology, Institute of Biodiversity, Friedrich Schiller University, Jena, Germany

²Department of Biogeochemical Processes, Max Planck Institute for Biogeochemistry, Jena, Germany

Soils are the largest terrestrial organic carbon pool and one of the largest terrestrial sources of CO₂ in the atmosphere. However, not all CO₂ produced in soils is released into the atmosphere, as dark CO₂ fixation has been shown to modulate CO₂ release from soils. Temperate forest soils store up to half of the soil organic carbon pool to 1m depth and are recognized as important components of the global carbon cycle, yet studies on dark CO₂ fixation in temperate forest soils are scarce. Using a well characterized Cambisol soil plot in the Hainich National Park (temperate forest), Germany, we explore dark CO₂ fixation with the aim to assess the CO₂ fixation rates, the influencing biogeochemical parameters, and the contribution of this process to temperate forest soil organic carbon (SOC).

Dark CO₂ fixation was quantified via the uptake of ¹³C-CO₂ added to microcosms containing soils sampled from three depths. Under 2% CO₂ headspace, rates of dark CO₂ fixation at soil level 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, accounting for up to 1.1% of microbial biomass and up to 0.035% of soil organic carbon. However, as differences in microbial biomass abundance and community profiles with depth were found, no significant difference in the rates across depth was observed at microbial level. This suggests that microbial biomass is an important driver of dark CO₂ fixation in soils. Given a global temperate forest area of 6.9 million km² and an average soil bulk density of 1 Mg/m³ dark CO₂ fixation will potentially account for the gross sequestration of 0.31 - 0.48 GtC/yr to a depth of 1 m. Furthermore, an increase in headspace CO₂ concentration enhanced CO₂ fixation rates by up to 3.4-fold under 20% v:v CO₂ showing that dark CO₂ fixation can be substantial in soils with higher CO₂ concentrations.

To validate microbial biomass as a driver of dark CO₂ fixation in soils, we made comparisons with soil plots from the Schorfheide-Chorin exploratory forest, Germany, a temperate forest characterized by vegetation-specific bacterial community structure, higher sand content and acidic pH gradients. Under these conditions, CO₂ fixation rates at microbial level were significantly different across depth suggesting that aside microbial biomass, other abiotic factors may influence dark CO₂ fixation in these soils. Of all the tested abiotic variables, water content was the main explanatory factor for the variations in dark CO₂ fixation rates in the Schorfheide-chorin soils. Additionally, based on 16S rRNA sequencing, qPCR and PICRUSt2 analysis, only a few putative

autotrophic communities were present and displayed vegetation-specific variations indicating an influence of vegetation type and input on the active community.

Our findings highlight microbial biomass, CO₂ and water content as the main drivers of dark CO₂ fixation in temperate forest soils with only a small proportion of autotrophs being present, suggesting the potential mediators of this process. We also demonstrate the significance of this process in global temperate forest SOC inputs.