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Significance of dark CO_2 fixation in arctic soils

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ABSTRACT

The occurrence of dark fixation of CO_2 by heterotrophic microorganisms in soil is generally accepted, but its importance for microbial metabolism and soil organic carbon (C) sequestration is unknown, especially under C-limiting conditions. To fill this knowledge gap, we measured dark $^{13}CO_2$ incorporation into soil organic matter and conducted a ^{13}C-labelling experiment to follow the ^{13}C incorporation into phospholipid fatty acids as microbial biomass markers across soil profiles of four tundra ecosystems in the northern circumpolar region, where net primary productivity and thus soil C inputs are low. We further determined the abundance of various carboxylase genes and identified their microbial origin with metagenomics. The microbial capacity for heterotrophic CO_2 fixation was determined by measuring the abundance of carboxylase genes and the incorporation of ^{13}C into soil C following the augmentation of bioavailable C sources. We demonstrate that dark CO_2 fixation occurred ubiquitously in arctic tundra soils, with increasing importance in deeper soil horizons, presumably due to increasing C limitation with soil depth. Dark CO_2 fixation accounted on average for 0.4, 1.0, 1.1, and 16% of net respiration in the organic, cryoturbated organic, mineral and permafrost horizons, respectively. Genes encoding anaplerotic enzymes of heterotrophic microorganisms comprised the majority of identified carboxylase genes. The genetic potential for dark CO_2 fixation was spread over a broad taxonomic range. The results suggest important regulatory function of CO_2 fixation in C limited conditions. The measurements were corroborated by modeling the long-term impact of dark CO_2 fixation on soil organic matter. Our results suggest that increasing relative CO_2 fixation rates in deeper soil horizons play an important role for soil internal C cycling and can, at least in part, explain the isotopic enrichment with soil depth.

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1. Introduction

Terrestrial ecosystems represent a major sink of CO_2 through fixation by plants but they have been shown to mitigate the rise of atmospheric CO_2 also via microbial CO_2 fixation (Ge et al., 2016; Yuan et al., 2012). Microbial CO_2 fixation has been mostly ascribed to autotrophic microorganisms (Ge et al., 2016), but fundamentally all microorganisms may use inorganic C (IC; i.e. CO_2 or bicarbonate) in their metabolism. All these fixations require energy generated by phototrophic, autotrophic or heterotrophic energy sources. IC is the main or even the only C source for chemoautotrophs and photoautotrophs, while heterotrophs and mixotrophs rely on organic C (OC) but also incorporate IC via a variety of carboxylation reactions that are part of their central or peripheral metabolic pathways (for review see Erb, 2011; Wood and Stjernholm, 1962). The importance of carboxylases in heterotrophic metabolism increases whenever microorganisms experience C limitation through a disproportion between C demand for energy generation and growth and its availability, caused by deficiency or complexity of OC sources, or fast growth (Alonso-Saez et al., 2010; Feisthauer et al., 2008; Merlin et al., 2003). Even though the occurrence of dark and largely heterotrophic CO_2 fixation in soils is generally accepted, very few studies have assessed its relevance for soil microorganisms (Miltner et al., 2004, 2005a,b; Šantrůčková et al., 2005). Estimates of the importance of soil CO_2 fixation for the C balance in certain ecosystems or within an entire soil profile are rare (Ge et al., 2016; Yuan et al., 2012) and analyses of diversity and abundance of carboxylases are missing entirely.

Soil OC becomes progressively enriched in ^{13}C with increasing soil depth (Bird et al., 2002; Gentsch et al., 2015; Nadelhoffer and Fry, 1988; Torn et al., 2002). There are several explanations but no one can fully explain the measured isotopic shift. The enrichment of soil OC with depth can be connected with decrease of $\delta\ ^{13}C$ of atmospheric CO_2 by 1.3‰ due to Suess effect (McCarroll and Loader, 2004), with preferential decomposition of different organic compounds and microbial fractionation during litter decomposition or mixing of new C input with old soil OC (Buchmann et al., 1997; Ehleringer et al., 2000; Šantrůčková et al., 2000). Another hypothesis that has been discussed but never supported experimentally states that soil microbes should be isotopically heavier as a result of carboxylation reactions (Ehleringer et al., 2000). Whenever carboxylation reactions are involved, CO_2 molecules used in the reactions likely originate from the soil atmosphere, which is isotopically heavier than the organic materials being decomposed (Cerling et al., 1991). The $^{13}CO_2$ enrichment of bulk soil atmosphere is highest in the uppermost soil horizons, where CO_2 originates mostly from the atmospheric air. In deeper horizons of the soil profile, CO_2 originates from organic matter decomposition and carries the isotopic signal of decomposed material. But still CO_2 remaining in the soil that surrounds microbes is 4.4‰ heavier than organic matter at the location due to slower diffusion of heavier $^{13}CO_2$ than lighter $^{12}CO_2$ (Cerling et al., 1991). CO_2 hydrogenation causes further enrichment of ^{13}C in HCO_3^- by 8–12‰, depending on temperature (Mook et al., 1974). HCO_3^- is accepted by many carboxylases operating in a variety of carboxylation reactions, including PEP and biotin carboxylases (Berg et al., 2010; Supplement Appendix B Table SB1), while CO_2 is used as an active species by Rubisco, the most abundant autotrophic carboxylase. Accordingly, incorporation of IC through microbial processes and accumulation of microbial products in soil theoretically might increase the isotopic signal ($\delta^{13}C$) of OC.

In arctic permafrost soils, high soil moisture, the presence of a permafrost layer and accumulation of fine particles on the interface between active and permafrost layers (Bockheim and Tarnocai, 1998; Makeev and Kerzhentsev, 1974) restrict air diffusion through the soil profile. Arctic permafrost soils are also a large reservoir of OC whose bioavailability is limited, among other factors, by the OC subduction into subsoil via cryoturbation and the subsequent formation of mineral-organic associations (Gentsch et al., 2015). High moisture content and the presence of a permafrost horizon restrict air diffusion through the soil profile, which may favor pockets and microsites with elevated CO_2 concentration. Under such conditions, CO_2 fixation might play a more important role than in well-aerated temperate soils. In addition, net primary production and soil carbon input are known to be low in northern ecosystems.

The aim of this study was to elucidate the role of dark CO_2 fixation in arctic soils. We postulated that (i) dark CO_2 fixation is a common attribute of arctic soils and occurs across the whole soil profile. We further hypothesized that (ii) various pathways of CO_2 fixation are operative in soil and distributed among different members of the soil microbial community, including heterotrophs, and (iii) CO_2 incorporation increases ^{13}C enrichment of organic carbon with soil age. To test the hypotheses, we measured isotopic signal $\delta^{13}C$ in OC, IC incorporation into OC, and abundances and taxonomic affiliations of carboxylase genes by shotgun metagenomics in soils across a range of tundra ecosystems from Eastern Siberia to Greenland, covering entire soil profiles. A simple model based on measured data was employed to elucidate a possible effect of IC incorporation on $\delta^{13}C$ of OC. In addition, ^{13}C-labelling experiments with soil from one location were performed under aerobic and anaerobic conditions and the incorporation $^{13}CO_2$ into OC was addressed by analyzing the ^{13}C incorporation into phospholipid fatty acids (PLFA) as microbial biomarkers. To gain supporting evidence of heterotrophic CO_2 fixation, CO_2 incorporation into OC, abundance of carboxylase genes and changes in microbial community composition after augmentation of bioavailable C were measured as well.

2. Material and methods

2.1. Soil sampling

We sampled soils from four different arctic tundra types (heath tundra, tussock tundra, shrub-moss tundra and graminoid tundra) that belong to the bioclimatic subzones E and D (Walker et al., 2005), also called southern tundra and typical tundra subzone in the Russian classification: (i) The heath tundra site was located in eastern Greenland close to the Zackenberg Research Station (ZK; 74° 29′ N, 20° 32′ W). (ii) The tussock tundra site was located approximately 80 km north of Cherskii (CH; 69° 26′ N, 161° 44′ E). iii) The shrubby moss tundra site was on the Taymyr peninsula in the north of central Siberia (Ari Mas, AM; 72° 30′ N, 101° 39′ E). (iiii) The graminoid (moss) tundra was also on the Taymyr peninsula, a little bit north of AM (Logata, LG; 73° 25′ N, 98° 16’ E). All areas are in the continuous permafrost zone and thaw depth during sampling reached 65–90 cm (samples were collected in late summer, close to the time of maximum active layer depth). All soils were classified as Turbic Cryosols according to World Reference Base (IUSS Working Group WRB 2007) and as Turbels according to Soil Survey Staff (2010). Two types of soil samples were used in this study, one for the general screening of dark CO_2 fixation and a second one for more detailed microbial and molecular biological analyses.

(i) Soil samples for measuring natural abundance of bulk soil ^{13}C and dark CO_2 fixation (see section 2.2) were obtained on each site from extensive soil sampling for assessment of C storage and distribution (Palmtag et al., 2015). Briefly, soil pits were excavated down to the permafrost and the active layer was sampled using a fixed volume cylinder. Samples from permafrost were collected by coring with a steel pipe (5 cm in diameter) that was hammered into the soil at 5–10 cm depth increments. Samples representative of the uppermost organic, cryoturbated organic (pockets of cryoturbated topsoil material), and adjacent active mineral layers and permafrost horizons were quickly dried in thin layers and kept at 4 °C until analyzed (in total, 149 samples from all sites). For detailed soil characteristics see Palmtag et al. (2015).

(ii) Soil samples for more detailed microbial and molecular analyses