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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

Table 1
Basic chemical (pH; total soil organic carbon – OC; total soil C/N ratio; extractable C – C_{EX}; natural abundance of soil ^{13}C – bulk $\delta^{13}C$) and biochemical characteristics (microbial biomass – C_{MB}; microbial C/N - C_{MB}/N_{MB}, net respiration; total microbial phospholipids – $PLFA_{tot}$) of three different soil horizons from Ari Mas site. Mean values and standard deviations (in brackets) are given (n = 4); different letters in superscript denote significant differences between layers (ANOVA).

soil horizon	pH_{H2O}	OC	C/N	C_{EX}	C_{MB}	C_{MB}/N_{MB}	bulk $\delta^{13}C$	net respiration	$PLFA_{tot}$	$PLFA_{tot}$
		mmol g^{-1}		μmol g^{-1}	μmol g^{-1}		‰	mmol C-CO_2 mol OC^{-1} d^{-1}	mmol C mol OC^{-1}	mol C mol C_{MB}^{-1}
upper organic	6.2	13.4(1.1)^a	20.4(0.21)^b	28.2(2.4)^a	174.1(3.2)^a	18.7(0.47)^a	27.5(0.10)^b	0.796(0.08)^a	0.339 (0.05)^a	0.022^c
cryoturbated	6.3	3.8(0.23)^b	26.2(0.32)^a	3.8(0.76)^b	10.6(0.05)^b	16.4(1.9)^a	27.5(0.17)^b	0.135 (0.10)^b	0.284(0.021)^a	0.085^b
mineral	6.7	0.8(0.14)^c	18.7(0.67)^b	1.22(0.73)^c	2.2(0.36)^c	11.7(4.3)^b	26.1(0.51)^a	0.082(0.02)^c	0.352(0.013)^a	0.106^a

were taken from 5-m long active layer pits on Cherskii, Ari Mas and Logata sites. Soil samples were taken from uppermost organic, mineral and cryoturbated horizons, as well as from the uppermost permafrost layer. One part of the samples was immediately stabilized with RNAlater and kept cold. After transporting the samples to the laboratory within 20 days, RNAlater was washed out with PBS buffer (Gittel et al., 2014) and samples were deep-frozen and later used for DNA extraction and subsequent metagenomics. From the remaining material, living roots were carefully removed and the soil was kept at 4 °C until analyzed for ^{13}C. Soils from the AM site were also used for the microbial ^{13}C incorporation and C supplementation experiments (see sections 2.3 and 2.4). For basic soil properties we kindly refer to Table 1, while details are given in Gentsch et al. (2015).

2.2. Screening of CO_2–C incorporation into OC across sampling sites

Soil (0.2 g) was moistened to 80% water holding capacity (WHC) in 10 ml vacutainers, which were covered by Parafilm and conditioned for 2 weeks at 12 °C. The vacutainers were then hermetically closed and flushed with CO_2-free air. Thereafter, the headspace was enriched with $^{13}CO_2$ (99 at% [atomic %] of ^{13}C) to a final CO_2 concentration of about 1% v/v, which is realistic for soil pores. In fact, soil CO_2 concentration can fluctuate widely, and values of 1–5% v/v CO_2 are typical, although 10% v/v and higher have also been recorded (Nobel and Palta, 1989). The soil was incubated at 12 °C for 5 days in the dark under the same conditions as in the conditioning phase. At the end of the incubation period, the CO_2 concentration in the headspace was analyzed, the soil was immediately dried at 60 °C and analyzed for total C and N and $\delta^{13}C$. All analyses and incubations were run in four replicates.

2.3. CO_2–C incorporation into microbial biomass

Soil taken from pits in the uppermost organic, mineral and cryoturbated horizons of the AM site was used in the $^{13}CO_2$ incorporation experiment. Soil moisture was adjusted to 80% WHC for incubation under aerobic conditions and to 100% WHC for incubation under anaerobic conditions. Before incubation with ^{13}C-labelled CO_2, the soil was conditioned either for 2 weeks (aerobic incubation, four replicates for each horizon, 5 g soil) or 4 weeks (anaerobic conditions, four replicates for each horizon, 5 g soil) in hermetically closed 100 mL bottles at 12 °C in the dark to allow microbial communities to stabilize. After soil conditioning, half of the samples were used for initial soil analyses (controls used for determination of natural abundance of ^{13}C in microbial biomass, extractable C and N pools, PLFA and bulk soil). The remaining bottles were flushed with CO_2-free air, and the headspace of each was enriched with $^{13}CO_2$ (99 at% of ^{13}C) to a final CO_2 concentration of about 1% v/v. The soil was incubated for 5 days under the same conditions as used for the conditioning. At the end of the incubation period, respiration was measured and the soil was used for further analyses; one part of the soil sample was immediately dried at 60 °C and used for chemical and isotopic analyses and the other part was deep-frozen and used for PLFA determination.

2.4. Effect of organic C supplement on CO_2 incorporation and carboxylase genes

As in the previous experiment, soil taken from pits in the mineral and cryoturbated horizons of the AM site was used. The soil was incubated only in aerobic conditions and conditioned in the same way as in the previous experiment. After conditioning, soils were amended with either sucrose or lipids extracted from soil (see below) as energy and C sources as follows: sucrose and lipids, respectively, were mixed with C-free silica sand and the mixture was then mixed with soil (sand/soil 1:2, w/w) to get a final concentration of the added C source of approximately 300 μg C per g dry soil. Control soil was mixed with sand only. The final soil mixture was moistened to 80% WHC with Veldkamp nutrient solution containing biotin (Veldkamp, 1970). An aliquot of the soil mixture (of all treatments) was dried to determine the natural abundance of ^{13}C before the incubation. The incubation with $^{13}CO_2$ was carried out in four replicates for each treatment as described above. After 5 days of incubation, soil respiration was measured, 1 g of soil mixture from each replicate was immediately dried (60 °C) for bulk C and ^{13}C analyses and the remaining soil was deep-frozen (−80 °C) for DNA extraction and subsequent metagenomics. The lipid mixture used for the soil C supplement had been extracted from soil slurry (equivalent of 450 g of dry soil supplemented by Veldkamp nutrient solution; Veldkamp, 1970) incubated on a shaker for 5 days. The slurry was subsequently centrifuged to remove excess of water and lipids were extracted according to Bligh and Dyer (1959). A part of the resulting extract was fractionated using SPE (Strata SI-1000mg/6 mL, Phenomenex, Torrance, CA, USA) to characterize the extracted lipids. We found that 25, 22 and 23% belonged to neutral-, glyco- and phospholipid fractions, respectively, and 30% was not held by the SPE sorbent and considered as non-lipid fraction.

2.5. Analytical methods

Microbial biomass was estimated by chloroform-fumigation and extraction with 0.5 M K_2SO_4, and calculated as the difference in soluble C between the extracts from fumigated and non-fumigated soils, using K_{EC} = 0.38 (Vance et al., 1987). Extractable organic C was analyzed on a LiquicTOC II (Elementar, Germany). Total CO_2 concentration in the headspace was measured with an HP 5890 gas chromatograph (Hewlett-Packard, East Norwalk, CT, USA), equipped with a thermal conductivity detector, at the beginning and end of the experiment (after the addition of $^{13}CO_2$ and after the incubation, respectively). The total amount of CO_2 in the bottles (totCO_2, μmol) was calculated as the sum of the amount of CO_2 in the headspace and the amount of CO_2 dissolved in the soil solution (Sparling and West, 1990). Net respiration rate was estimated as the difference between totCO_2 at the beginning and the end of the experiment divided by the number of days of incubation. Analyses of total C and N and ^{13}C contents of dried soil material were conducted with an NC Elemental analyzer (ThermoQuest, Bremen, Germany) connected to an isotope ratio mass spectrometer (IR-MS Delta X Plus, Finnigan, Bremen, Germany). Prior to carrying out the analyses, all samples were tested for their carbonate content. No carbonates were detected (data not shown).

A binary mixing model was used to estimate the amount of the pulse-derived ^{13}C immobilized in the various C pools (bulk soil, PLFA):

$$^{13}C\ (\mu g\ g^{-1}) = [(at\%_{sample} - at\%_{control}) / (99.90 - 1.10)] \times C\ pool\ size\ (\mu g\ C\ g^{-1})$$

where $at\%_{control}$ is the natural abundance in the control samples, at $\%_{sample}$ is the ^{13}C abundance in the samples after labeling, 99.9 is the pulse ^{13}C at% and 1.10 is the at% of the ambient atmosphere. All results were normalized to total C content in order to eliminate differences in C contents of the soils.

PLFA were extracted from subsamples of 0.3–2 g dry soil containing comparable amounts of OC according to Frostegård et al. (1993), with minor modifications. Purification of phospholipids was conducted on silica columns (SPE-SI Supelclean 250mg/3 mL; Supelco, PA, USA) using chloroform, acetone and methanol. Following trans-esterification (Bossio and Scow, 1998), the concentration and isotopic composition of individual PLFAs was determined on a GC-IRMS system consisting of a Trace GC coupled to a Delta V Advantage IRMS via a GC Isolink interface (Thermo Fisher Scientific, Waltham, MA, USA); see Wild et al. (2014) for a detailed description of the instrument setup. Concentration and isotopic composition of each PLFA were corrected for C added during methylation. The microbial community composition was described using PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 as markers of Gram-positive bacteria, 16:1ω9, 16:1ω7, 16:1ω5, cy17:0, 18:1ω7, cy19:0 as markers of Gram-negative bacteria and 18:1ω9, 18:2ω6,9 as markers of fungi (Frostegård and Bååth, 1996). Total bacterial biomass was calculated as the sum of general bacterial markers 15:0, 17:0, 18:1ω5 and markers for Gram-positive and negative bacteria. The PLFAs 14:0, 16:0, 16:1ω11, 18:0, 19:1ω8, and 20:0 were considered nonspecific markers (Kaiser et al., 2010).

DNA was extracted from samples of cryoturbated (4 samples), mineral (2) and top soil (4) from Logata, AriMas and Cherskiy sites using bead-beating and the phenol-chloroform method (Gittel et al., 2014; Urich et al., 2008). Total DNA was quantified using SybrGreen (Leininger et al., 2006). In the case of the incubation experiment with added substrates (sucrose or lipids), only the DNA from cryoturbated horizons (9 samples) contained a reasonable amount of DNA of high quality (Table 2). Sequencing of DNA from *in-situ* and incubation experiments was performed on an IonTorrent PGM (Thermo Fisher Scientific, Waltham, MA, USA) sequencer at the Department of Archaea Biology and Ecogenomics (University of Vienna). Barcoded, adapter-ligated DNA libraries were generated and sequenced using 200 bp sequencing chemistry and 318 chips according to the manufacturer's instructions. Sequence reads were quality-trimmed (Phred score > 20) and size-selected (> 100 bp) before further processing. For the identification of carboxylase genes, all metagenome reads were translated into all six frames, with each frame into separate open reading frames (ORFs), avoiding any '*' characters marking stop codons in a resulting ORF. All ORFs equal to 30 amino acids or larger were screened for assignable conserved protein domains using reference hidden Markov models (HMMs) of the PfamA database (Punta et al., 2012; PfamA release 25, http://Pfam.janelia.org) with HMMER tools (http://hmmer.janelia.org/). All database hits with *e*-values below a threshold of 10^{-4} were considered significant (Tveit et al., 2015). To obtain taxonomic information of reads with Pfam code, a BLASTX search implemented in diamond software (Buchfink et al., 2015) was performed (-minscore 50, -maxhits 25) and the resulting hits in sam format were analyzed by MEGAN 5.11.3 (Huson et al., 2007). Taxonomy was assigned using the last common ancestor (LCA) algorithm (LCA parameters: MinScore 50, MaxExpect = 0.01, TopPercent = 10, MinSupport = 1) implemented in MEGAN 5.11.3 software.

The calculations of carboxylase gene abundances per g soil were done by combining relative abundance of SSU rRNA genes of bacteria in the metagenomes with the number of bacterial SSU rRNA genes per g soil as determined by qPCR in the same DNA sample (Table SB2). The absolute number of carboxylase genes per g of soil was used for normalizing their abundance to soil C_{mic} and/or OC contents. The molecular data were processed as follows: metagenome sequences encoding fragments of SSU rRNA genes were extracted with the program SortMeRNA (Kopylova et al., 2012), applying default parameters and the reference databases therein. Extracted reads were compared with BLASTN (Astchul et al., 1990) against the ARB Silva SSUref database v. 119 (Quast et al., 2012) and analyzed in MEGAN 5.11.3. Carboxylase reads were recalculated to absolute number of gene copies per g of soil by combining the absolute quantity of bacterial SSU rRNA genes and the amount of bacterial SSU rRNA reads determined by the MEGAN LCA algorithm in each metagenome, using the following formula:

$$q(carboxylase) = \frac{seqs(carboxylase)}{seqs(bacterial\ \ SSU\ \ rRNA\ \ gene)} \cdot q(bacterial\ \ SSU\ \ gene)$$

where *seqs(carboxylase)* is the amount of metagenome carboxylase gene sequences (assigned by hmmer algorithm using Pfam database), *seqs (bacterial SSU rRNA gene)* is the amount of bacterial SSU rRNA gene sequences and *q(bacterial SSU rRNA gene)* is the quantity of bacterial SSU rRNA genes (gene copies per g soil) determined by qPCR.

2.6. Statistics and modeling

A general linear model, followed by Newman-Keuls post-hoc testing, was used to determine the differences in C incorporation, respiration rate, bulk C content and isotopic signal between sites and horizons at a significance level of $P \leq .05$. To compare total PLFA contents between control and $^{13}CO_2$-incubated samples in the laboratory experiment, one-way ANOVA and Newman-Keuls post-hoc test was used. Data were log-transformed in all cases except for total PLFA. Statistical evaluation of data was carried out with STATISTICA 13.

The statistical analysis of carboxylase genes was done using the statistical program R (Team, 2016). Because data were not normally distributed, generalized linear models with gamma distribution were

Table 2
DNA concentration, net respiration and inorganic carbon incorporation into soil organic carbon (OC) of cryoturbated and mineral soil horizons from Ari Mas site incubated under aerobic conditions without carbon source addition (control) and with sucrose or mixture of lipids as added carbon source. Mean values and standard deviations are given (n = 3). Different letters in superscript denote significant differences between layers (ANOVA).

soil horizon	treatment	DNA concentration	net respiration	^{13}C immobilization	
		ng DNA mol OC^{-1}	mmol C mol OC^{-1} d^{-1}	μmol ^{13}C mol OC^{-1} d^{-1}	% net respiration
cryoturbated	control	8.61(0.13)	0.168(0.074)^{b}	3.06(0.59)^{c}	2.08(1.59)^{c}
	sucrose	32.8(15.7)	0.541(0.141)^{a}	21.7(6.26)^{b}	4.32(2.16)^{b}
	Lipids	22.8(6.20)	0.106(0.032)^{b}	13.0(2.89)^{b}	13.2(4.99)^{a}
mineral	control	nd	0.144(0.027)^{c}	7.37(1.02)^{c}	5.16(0.79)^{ab}
	sucrose	nd	1.254(0.240)^{a}	46.8(4.34)^{a}	3.82(0.74)^{b}
	Lipids	nd	0.333(0.120)^{b}	18.3(2.70)^{b}	5.81(1.85)^{a}

nd – not detected.

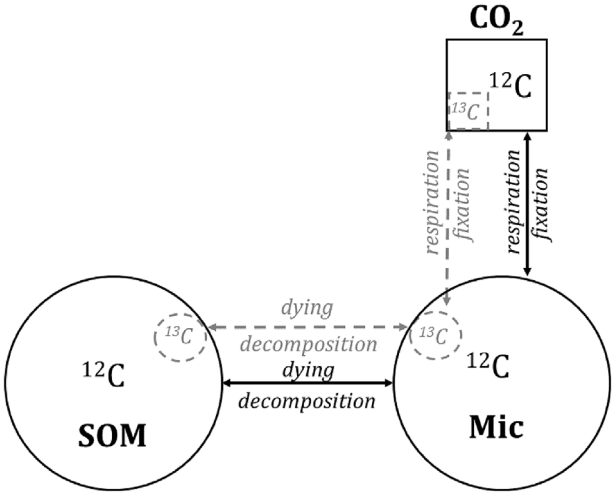

Fig. 1. Scheme of the soil organic matter (SOM) decomposition model that was used to estimate the effect of long-term CO_2 incorporation into SOM on its isotopic signal (for details of the model see Supplementary information, Appendix A).

used to test the significance of the effects of lipids or sucrose addition and soil horizon, respectively, on the abundance of carboxylase genes. Soil horizons had unequal numbers of replicated measurements of carboxylase gene abundance. Therefore, we calculated the type-II F statistic using the package car (Fox and Weisberg, 2011). Post-hoc multiple comparisons were carried out based on least-square means using the package lsmeans (Lenth, 2016).

In order to estimate tentatively the effect of CO_2 fixation on the isotopic signal of OC ($\delta^{13}C_{tot}$), we applied a simple model of microbial OC decomposition (Fig. 1; for details see Supplementary Information Appendix A). Briefly, decomposition of OC by heterotrophic soil microorganisms (Mic) is a process with first-order kinetics. Organic C from decomposing organic matter with an initial isotopic signal of −27‰ is consumed by soil microorganisms and respired or assimilated into microbial biomass. Microbial biomass is dying over time and becomes part of OC. For the sake of simplicity, we assume that no isotopic discrimination occurs during decomposition and microbial dying. Heterotrophic microorganisms largely depend on organic matter as C and energy source but under certain circumstances they use IC as additional C source (see introduction of this article for more details). We assume that microbes are capable of assimilating part of the respired CO_2 back and incorporate it into microbial biomass. Before CO_2 is fixed by soil microorganisms, an isotopic discrimination of 4‰ occurs because of the faster diffusion of $^{12}CO_2$ out of the soil. By fixing heavier CO_2, microbial biomass is becoming more enriched in ^{13}C. When this microbial biomass is dying and becomes part of OC, OC becomes enriched in ^{13}C as well. In the model, CO_2 fixation is set to be proportional to respiration. Four scenarios were modelled, with CO_2 fixation making up 0, 0.1, 1 and 5% of respiration, respectively.

3. Results

3.1. Screening for CO_2 fixation in arctic soils

Sampling site and soil horizon significantly affected soil OC content and respiration rate (Table 3) while natural abundance of ^{13}C in the soil (bulk $\delta^{13}C$) and CO_2 incorporation were affected only by the type of soil horizon. Consequently, data from the particular horizons were averaged across sampling sites (Table 3). Across all sites, average bulk $\delta^{13}C$ in the upper organic horizon was −27.3‰ at the start of incubation. In comparison with the upper organic horizon, the mineral, cryoturbated and permafrost horizons were all ^{13}C-enriched by 1.2, 1.1, and 2.7‰, respectively (Table 3). Incorporation of $^{13}CO_2$-derived C per unit total OC was lowest in the upper organic horizon (4.9 μmol ^{13}C mol $C_{tot}^{-1} d^{-1}$) and increased two-to three-fold in cryoturbated and mineral horizons (Table 3). In the permafrost horizon, ^{13}C incorporation was higher by almost two orders of magnitude (Table 3). ^{13}C incorporation reached 0.4, 1.0, 1.1 and 16% of net respiration in the organic, cryoturbated organic, mineral and permafrost horizons, respectively. When ^{13}C incorporation was expressed per mol C of soil OM, it also increased with soil depth, and it rose exponentially with the $\delta^{13}C$ value of OC ($(R^2 = 0.44, n = 149, P < .001; C_{incorp} = e^{(21.09 + 0.72\ \delta 13COC)}$).

Carboxylase genes employed in autotrophic as well as heterotrophic metabolism were detected in metagenomes of all horizons and were not affected by site. Their abundances were higher in mineral horizons than in organic and cryoturbated horizons when normalized to microbial biomass ($F = 4.2$, $df = 2$, $P = .02$; Fig. 2a). However, deeper active layer horizons (mineral and cryoturbated) had lower abundances than organic horizons when carboxylase genes were normalized to total C ($F = 4.1$, $df = 2$, $P = .02$; Fig. SB1a). Genes encoding Rubisco contributed about 5% (4.3–5.3%) to the total abundance of carboxylase genes, whilst anaplerotic carboxylase genes (pyruvate and PEP carboxylase genes) were the most abundant carboxylase genes and contributed more than 30% (31–42%).

The ^{13}C data of OC within the soil profile corresponds well with the model predictions (Fig. 3). The estimates indicate that dark fixation of C released from decomposed OC could, after 1000 years, cause an increase of the isotopic signal of OC from an initial −27‰ to −26.7 or −15.2‰ when IC incorporation represents 0.1 or 5% of net respiration, respectively (Fig. 3). C sequestration via dark CO_2 fixation of 0.1, 1 and 5% of net respiration, respectively, would add 0.1, 1.4 and 7.4 mg C per g soil, respectively, over a time frame of 250 years (Fig. SA4).

3.2. ^{13}C incorporation into microbial biomass at the AM site

Under anaerobic conditions, CO_2 fixation was below the detection limit in the cryoturbated and mineral horizons and negligible in the upper organic horizon (0.251 μmol ^{13}C mol C_{tot}^{-1} day^{-1}, corresponding to 0.1% of net respiration). The anaerobic incubation is thus not discussed any further. During aerobic incubation neither microbial functioning (respiration rate) nor microbial biomass (total PLFA) ($P > .3$ for all horizons, $df = 1$, $n = 3$) or PLFA profiles were changed (Table SB3).

Bulk ^{13}C incorporation ranged from 2.4 μmol ^{13}C mol OC^{-1} day^{-1} (cryoturbated horizon) to 7.6 μmol ^{13}C mol OC^{-1} day^{-1} (mineral horizon, Table 4), which corresponds well with a range of ^{13}C incorporation observed in the individual horizons during the screening across all study sites (Table 3). It represented less than 2% of net respiration in the organic and cryoturbated horizons but exceeded 13% in the mineral horizons. Of the total amount of ^{13}C incorporated into the soil, generally less than 1% of bulk ^{13}C was found in PLFA biomarkers (^{13}C PLFA to bulk ^{13}C) and less than 0.05% of total PLFA-C was newly incorporated ^{13}C (^{13}C PLFA to PLFA C; Table 4). All detected PLFAs were enriched in ^{13}C, but the distribution of ^{13}C among PLFA markers of individual functional groups showed that more ^{13}C was incorporated into bacterial than fungal PLFA. The ratio of ^{13}C in fungal PLFA to ^{13}C in bacterial PLFA increased from 0.20 in organic and cryoturbated horizons to 0.45 in the mineral horizons. The significantly higher fungal to bacterial PLFA ratio in the organic layers and lower ratio in mineral layers compared to the ^{13}C in fungal to ^{13}C in bacterial PLFA (Table 5) indicate, that bacteria were more active in ^{13}C incorporation per unit of biomass compared to fungi in organic layers, but less active in mineral layers. Within bacteria, Gram-negative bacteria incorporated three to six times more ^{13}C into PLFA than Gram-positive bacteria (Table 5). The ratios of ^{13}C incorporated into PLFAs of Gram-negative to PLFAs of Gram-positive bacteria were on average three times higher compared to ratios of total Gram-negative to Gram-positive PLFA contents. Therefore, the Gram-negative bacteria were not only the overall most active microbial population in the ^{13}C-CO_2 assimilation, but also specifically

Table 3
Soil organic carbon (OC), natural abundance of ^{13}C, soil respiration and CO_2 incorporation into OC pool of four different soil layers from four different arctic tundra localities (upper part of the table). The effects of locality, soil layer, and their interaction were calculated by factorial ANOVA (lower part of the table). Different letters in superscript denote significant differences between soil horizons (Newman-Keuls post-hoc test).

locality	soil horizon	number of samples	OC	^{13}C of bulk soil	net soil respiration	^{13}C -incorporation	
			mmol g^{-1}	‰	mmol CO_2 mol $C_{tot}^{-1}d^{-1}$	µmol ^{13}C mol$C_{tot}^{-1}d^{-1}$	% of respiration
Cherskii	upper organic	16	21 ± 9.2	−27.3 ± 0.85	1.3 ± 0.9	7.1 ± 5.1	0.6 ± 0.5
	cryoturbated	13	4.5 ± 2.5	−25.8 ± 0.36	1.0 ± 0.8	5.2 ± 4.0	0.6 ± 0.2
	mineral	17	1.9 ± 1.7	−26.1 ± 0.85	2.1 ± 1.5	11.5 ± 6.9	0.9 ± 0.8
	permafrost	17	1.5 ± 0.9	−24.6 ± 2.58	2.0 ± 1.4	310 ± 496	13.4 ± 20.6
Ary Mass	upper organic	4	17.8 ± 5.7	−27.8 ± 0,32	2.8 ± 1.7	3.8 ± 1.3	0.2 ± 0.2
	cryoturbated	4	1.0 ± 0.2	−26.3 ± 0.16	2.2 ± 0.6	8.7 ± 0.6	0.4 ± 0.1
	mineral	11	0.9 ± 0.4	−25.8 ± 1.02	3.8 ± 1.7	17.2 ± 15.4	1.0 ± 1.3
	permafrost	15	1.4 ± 1.6	−24.0 ± 1.67	0.9 ± 0.7	81.5 ± 42.3	14.4 ± 13.3
Logata	upper organic	3	18.6 ± 7.4	−27.3 ± 0.65	3.7 ± 1.1	5.4 ± 4.4	0.1 ± 0.1
	cryoturbated	6	3.1 ± 2.3	−27.1 ± 0.71	2.9 ± 1.2	21.3 ± 27.2	2.1 ± 4.0
	mineral	7	2.5 ± 1.4	−27.4 ± 0.18	3.9 ± 1.0	7.1 ± 2.4	0.2 ± 0.1
	permafrost	9	2.0 ± 0.3	−25.5 ± 1.50	1.3 ± 1.0	102 ± 83.9	20.1 ± 18.9
Zackenberg	upper organic	8	16.4 ± 5.8	−27.3 ± 0.75	1.9 ± 1.1	0.8 ± 0.6	0.1 ± 0.1
	cryoturbated	9	6.4 ± 1.6	−25.2 ± 0.66	0.9 ± 0.5	8.9 ± 17.5	1.3 ± 2.8
	mineral	8	2.0 ± 1.0	−25.6 ± 1.04	1.2 ± 0.4	15.8 ± 27.2	2.0 ± 4.0
	permafrost	2	0.9 ± 0.1	−26.0 ± 1.13	0.9 ± 0.6	74.0 ± 69.7	32.1 ± 31.8
all sites	upper organic	31	19.0 ± 8.18^{a}	−27.3 ± 0.78^{c}	1.88 ± 1.36^{ab}	4.86 ± 4.86^{c}	0.37 ± 0.44^{c}
	cryoturbated	32	4.33 ± 2.72^{b}	−26.2 ± 0.73^{b}	1.47 ± 1.20^{b}	9.71 ± 16.6^{c}	1.08 ± 2.38^{b}
	mineral	43	1.77 ± 1.44^{c}	−26.1 ± 1.06^{b}	2.62 ± 1.94^{a}	13.1 ± 15.4^{b}	1.00 ± 2.14^{b}
	permafrost	43	1.55 ± 1.18^{c}	−24.7 ± 2.13^{a}	1.46 ± 1.34^{b}	175.7 ± 338^{a}	16.1 ± 19.5^{a}
	effect of locality		****	n.s.	****	n.s.	n.s.
	effect of layer		****	****	****	****	****
	layer*loc		****	n.s.	****	n.s.	n.s.

per unit of microbial biomass, which was most apparent in the cryoturbated horizon (Table 5). The proportion of bulk ^{13}C built into PLFA was higher in the organic and cryoturbated horizons than in the mineral horizons. Across all horizons, ^{13}C incorporated into PLFA was closely correlated with net respiration rate RR ($R^2 = 0.89$, n = 9; $^{13}C_{(PLFA/OC)} = 7.36 + 20.5\ RR_{(CO2/OC)}$).

3.3. Effect of C addition on dark CO_2 fixation

Addition of sucrose significantly increased net respiration rate in the mineral and cryoturbated horizons while addition of lipids had a positive effect only in the mineral soil (Table 2). ^{13}C immobilization ranged from 3.1 µmol ^{13}C mol OC^{-1} day^{-1} (cryoturbated horizon; control) to 46.8 µmol ^{13}C mol OC^{-1} day^{-1} (mineral horizon; sucrose addition) and increased in both the cryoturbated and mineral horizons in the order control < lipids < sucrose. Across all treatments, IC incorporation was closely correlated with respiration rate ($R^2 = 0.91$, n = 18; $^{13}C_{(incorp/OC)} = 4.86 + 31.9\ RR_{(CO2/OC)}$). Both C substrates induced microbial growth (measured as an increase in DNA amount per gram soil; Table 5, Fig. 4) in the cryoturbated horizon. Unfortunately, the amount of extracted DNA from the mineral soil was too small to be evaluated or used for further analyses. The increase of microbial biomass in the cryoturbated horizon was accompanied by a shift in the composition of the microbial community. Analysis of the SSU rRNA gene fragments in the metagenomes showed that the relative abundance of *Beta-, Gammaproteobacteria* and *Saccharibacteria* increased due to substrate addition, whereas the relative abundance of *Alphaproteobacteria, Deltaproteobacteria and Firmicutes* rather decreased, mainly in the sucrose addition treatment (Fig. 4). The abundance of carboxylase genes normalized to microbial biomass was not affected by C source addition (Fig. 2b) and increased along with microbial biomass growth, thus a significant increase of carboxylase genes per unit of total C was observed with C source addition (Fig. SB1b). Rubisco accounted for 13–18% of total carboxylase and anaplerotic carboxylase genes (pyruvate and PEP carboxylases) accounted for about 40% (37–46%). Annotated PEP carboxylase genes were taxonomically binned (Fig. SB2). The addition of either sucrose or lipids decreased the proportion of PEP carboxylases affiliated to Alphaproteobacteria, Cyanobacteria and Actinobacteria, but increased those affiliated to Beta-, Gammaproteobacteria, Firmicutes, Chlamydiae/Verrocomicrobia group and Bacteroidetes/Chlorobi group.

4. Discussion

4.1. General relevance of dark CO_2 fixation in arctic soils

The available knowledge about dark CO_2 fixation is still very limited and can be summarized as follows: It is a relevant process in the soil mediated by soil microorganisms, it is correlated with microbial respiration rate, it can be enhanced by addition of bioavailable OC, and it is related to microbial heterotrophic activity (Miltner et al., 2004, 2005a; Šantrůčková et al., 2005). Previous studies estimated that about 2–7% of fixed CO_2 can be incorporated into microbial biomass (Miltner et al., 2004; Šantrůčková et al., 2005). In addition, this study documents that (i) IC incorporation increases with depth in the soil profile and is higher in the permafrost layers by more than one order of magnitude due to higher CO_2 fixation rate, lower OC and higher CO_2 concentrations with depth; (ii) genes encoding Rubisco, the only enzyme operating strictly in autotrophic metabolism, account for a small part of all carboxylase genes only, while anaplerotic carboxylase genes are several times more abundant; (iii) a wide spectrum of soil microorganisms contain genes encoding PEP carboxylases; (iv) IC incorporation may lead to ^{13}C enrichment of soil OC in the long-term and the increase is more pronounced in the deeper soil horizons. Across all study sites, IC incorporation expressed per unit of total C was higher in the mineral than in the organic horizons. The larger C incorporation in mineral horizons was accompanied by the highest abundance of all detected carboxylase genes per unit microbial biomass, suggesting that both the chemoautotrophic and heterotrophic pathways accounted for the larger IC incorporation in the mineral horizons as compared with the top soil. The significant proportion of anaplerotic carboxylase genes (pyruvate and PEP carboxylases) further emphasizes the importance of

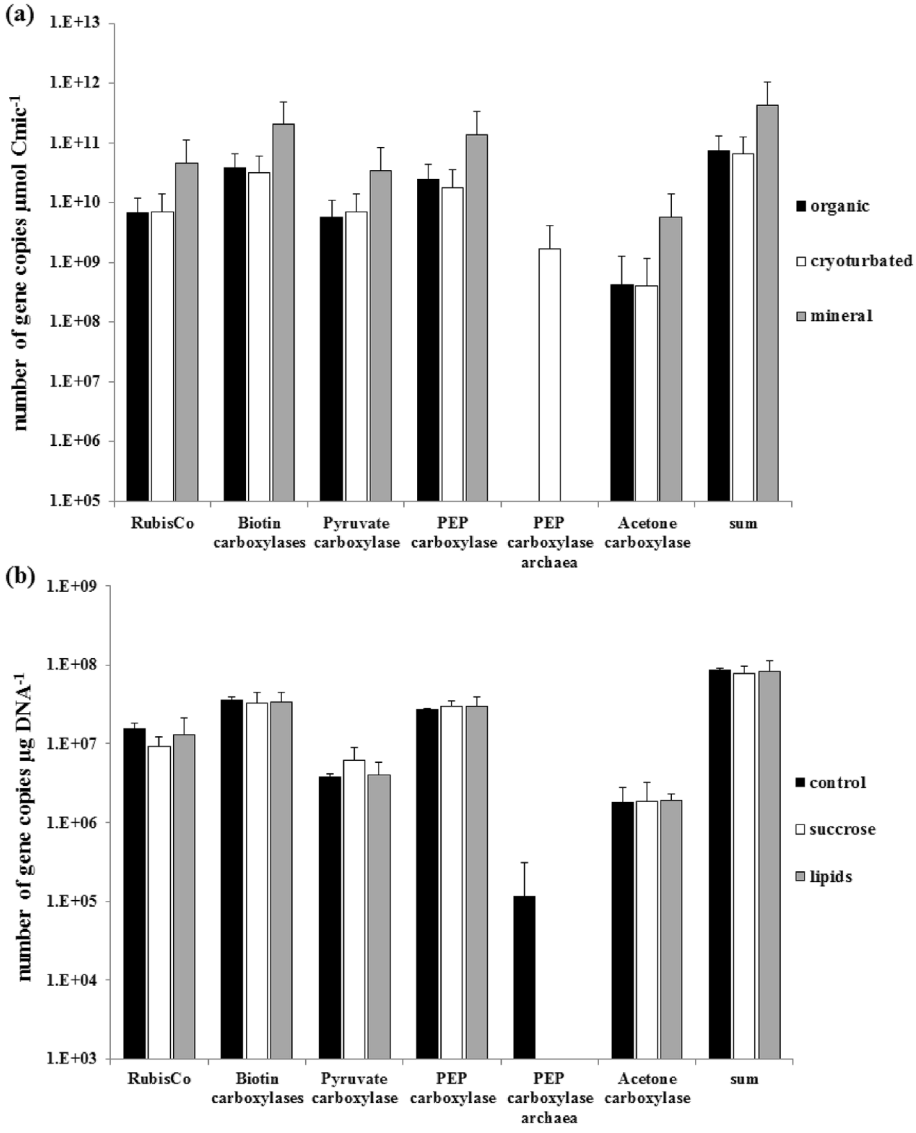

Fig. 2. Abundance of carboxylase genes normalized to microbial biomass in uppermost organic, cryoturbated and mineral soil layers from Cherskii, Ari Mas and Logata sites **(a)**, and in the cryoturbated layer from Ari Mas site, either unamended (control) or amended with organic C sources **(b)**. Note that the scale on *y* axis is logarithmic. The standard deviations of absolute carboxylase gene counts represent the difference among the localities. They were in some cases larger than average, only positive error bars are thus visualized.

reactions replenishing central metabolic pathways under conditions where microbial C demand exceeds C supply or where microorganisms catabolize complex hydrocarbons, the dominant fractions of OC in mineral horizons (Alonso-Saez et al., 2010; Feisthauer et al., 2008; Merlin et al., 2003). It implies an important regulatory function of CO_2 fixation, which may enable microbes to keep activity in harsh conditions of C limitation.

4.2. Link between dark CO_2 fixation and microbial communities

We found less than 1% of fixed C in PLFA (0.2–0.5%), which is much less than it was found in bulk microbial cells (Miltner et al., 2004; Šantrůčková et al., 2005). The low values can be explained by the fact that membranes constitute only about 4% of cellular material and, therefore, bulk microbial biomass should contain more fixed C. This suggestion is supported by Feisthauer et al. (2008) who found that the amount of fixed C in bulk microbial biomass is higher by one order of magnitude than that of fatty acids, indicating greater incorporation of fixed C into other cell components. Proteins, the most abundant component of cytoplasm, contain four to eight times more fixed C than PLFA (Miltner et al., 2004).

The variety of detected carboxylase genes, the wide spectrum of bacteria linked to PEP carboxylase genes and significant ^{13}C incorporation into all detected PLFAs including fungal markers imply a general importance of CO_2 incorporation for the microbial community. This matches existing knowledge that, apart from chemoautotrophic bacteria and archaea, which use CO_2 as the only source of C (for review see Berg et al., 2010; Saini et al., 2011) a wide spectrum of heterotrophic bacteria and fungi employ carboxylases to (i) assimilate various organic substrates such as acetone, phenolics, propionate, or leucine, (ii) replenish the citric acid cycle in anaplerotic reactions and, finally, (iii) synthesize cellular compounds (e.g. Erb, 2011; Hesselsoe et al., 2005). The significant increase in the amount of genes involved in CO_2 fixation belonging to predominantly heterotrophic genera (*Arthrobacter*, *Nocardioides* and *Pseudomonas*; Fig. SB3) after addition of sucrose or lipids indicated increased potential of heterotrophic metabolism for additional IC incorporation into the bacterial biomass.

We found a positive effect of bioavailable C addition on IC incorporation, which contradicts our expectation that the importance of heterotrophic carboxylases would increase with any imbalance between C demand and supply (Alonso-Saez et al., 2010; Feisthauer et al., 2008; Merlin et al., 2003). This apparent discrepancy might be partly explained by the increase of chemoautotrophic C fixation because of increasing CO_2 concentration in the system. A more likely explanation is that the added C initiated fast microbial growth as indicated by increased DNA concentration (Table 2), but was exhausted within days,

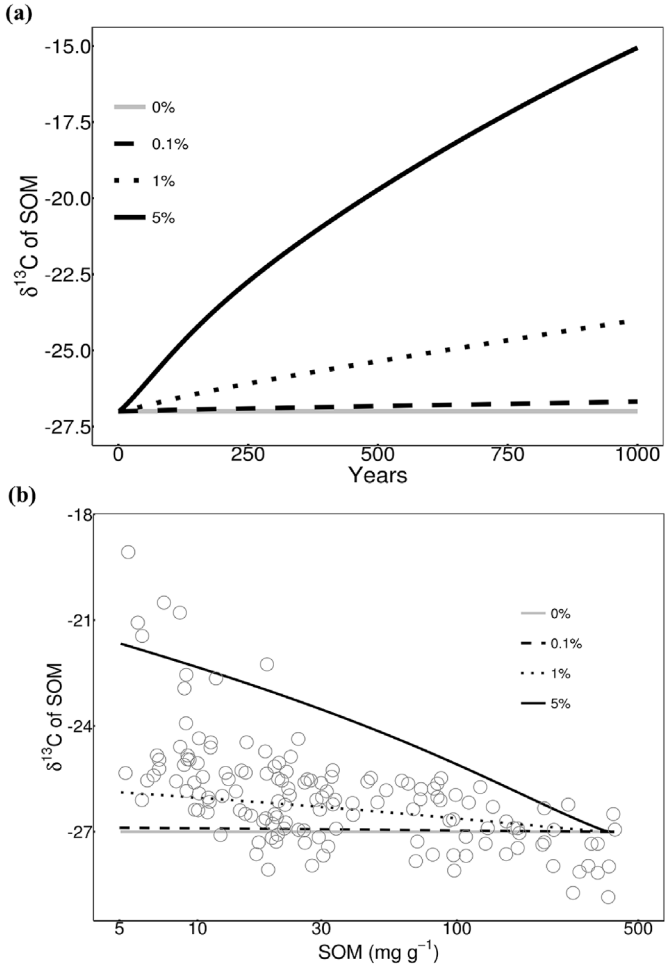

Fig. 3. The modelled shift of SOM isotopic signal over time caused by heterotrophic CO_2 fixation **(a)**, and relationship between the soil SOM concentration and δ ^{13}C ratio **(b)** (please note that x-axis is in logarithmic scale). Open circles represent measured data and lines the model predictions. The solid grey line represents control (0% - no fixation), the dashed, dotted and solid black lines represent CO_2 fixation rates equal to 0.1, 1 and 5% of net respiration rate, respectively.

microbes quickly became substrate limited and microbial C demand exceeded C supply at the end of the experiment when the analyses were performed. Therefore, IC had to replenish C for microbial metabolism.

Even though we detected genes for key enzymes in various autotrophic pathways (e.g. Calvin Benson Bassham cycle, reductive citric acid and reductive acetyl-CoA cycles, 3-hydroxypropionate cycle, 4-hydroxybutyrate cycle), we assume a negligible contribution of autotrophs to overall IC incorporation into OC. These chemoautotrophic pathways are, except for the Calvin Benson Bassham cycle and 4-hydroxybutyrate cycle, operated by strictly anaerobic prokaryotes, and in our study the anaerobic CO_2 incorporation was below detection limit. We are aware that the contribution of the chemoautotrophic IC incorporation may be higher under natural conditions, but heterotrophy should be prevalent. This is supported by the generally lower growth efficiency of chemolithotrophic compared to heterotrophic growth, because the former requires more energy to produce a unit of biomass. In addition, carboxylases have low substrate affinity and need a high concentration of IC for its effective incorporation into organic compounds (Bar-Even et al., 2012). The most kinetically favorable carboxylases are anaplerotic PEP carboxylases, the main carboxylase for replenishing the citric acid cycle, and pyruvate carboxylases (Bar-Even et al., 2012).

4.3. Importance of dark CO_2 fixation for C sequestration

We assessed the importance of dark CO_2 fixation for C sequestration in the soils from two different perspectives: (i) promotion of microbial metabolism and (ii) role in soil C balance. With respect to the promotion of the heterotrophic metabolism, IC may be used as an additional C source to replenish central metabolic cycles (anaplerotic reactions) in situations when organic carbon is limiting and primarily used for energy production. In such a case, microbes may re-fix CO_2 that has been respired for energy production and thus minimize the overall loss of carbon. Carboxylation is also a vital step of fatty acid biosynthesis in general, and it enables the utilization of acetone by nitrate reducing bacteria (Acosta et al., 2014). Inorganic C has a stimulatory effect, which is most apparent under low metabolic activity in resting aerobic and facultative cells (Harris, 1954) and in the metabolism of resource-depleted bacteria (Alonso-Saez et al., 2010). Inorganic C also serves as the only C source for a variety of obligate chemoautotrophs, either bacteria or archaea, which provide many metabolic pathways that are indispensable for soil functioning, such as oxidation of sulphur, ammonia, reduced metal ions, and methane production (Badger and Bek, 2008; Berg et al., 2007; Konneke et al., 2014). With respect to the role in the soil C balance, we consider a mean CO_2 release from tundra soil by soil respiration of about 76 g m^{-2} yr^{-1} (Fahnestock et al., 1999). Our data thus indicate that re-use of 0.1–5% of net respiration may account for dark fixation of 0.08–3.8 g C m^{-2} yr^{-1}. Above-ground net primary production (i.e. IC incorporation into plant material) ranges from 10 to 500 g C m^{-2} yr^{-1} (Gould et al., 2003). Thus microbial CO_2 fixation may correspond in the long term from 0.016 to 38% of plant C fixation.

4.4. Effect of dark CO_2 fixation on isotopic signal of OC

In general, the $\delta^{13}C$ of organic matter mirrors the $\delta^{13}C$ of plant input in the uppermost horizons. However it increases with soil depth to values 1–3‰ higher than in the uppermost organic horizon (e.g. Bird et al., 2002; Buchmann et al., 1997). This was also observed for our arctic tundra soils (Tables 1 and 2; Gentsch et al., 2015). The mechanisms behind this enrichment are still unclear and none of the following potential causes can fully explain it: isotopic change of atmospheric CO_2 and microbial processing of OC (Boström et al., 2007), mixing of new C input with old soil organic matter (SOM) and microbial fractionation during litter decomposition (Ehleringer et al., 2000), increase of leaf internal to ambient CO_2 concentration (ci/ca) due to global change during the last 40 years (Betson et al., 2007). Similarly, the causes of the ^{13}C-enrichment of microbial biomass (Dijkstra et al.,

Table 4
Bulk inorganic ^{13}C incorporation into soil organic carbon (OC) and PLFA in three different soil layers from Ari Mas site incubated under aerobic conditions. Mean values and standard deviations (in brackets) are given (n = 3). Different letters in superscript denote significant differences between horizons (Newman-Keuls post-hoc test).

soil layer	bulk ^{13}C incorporation		^{13}C incorpotration to PLFA	PLFA ^{13}C to bulk ^{13}C	PLFA ^{13}C to PLFA C
	μmol ^{13}C mol OC^{-1} d^{-1}	% respiration rate	nmol ^{13}C mol OC^{-1} d^{-1}	%	%
organic	6.13(0.62)^{b}	0.78(0.05)^{c}	19.8(2.65)^{a}	0.377(0.04)^{a}	0.041(0.004)^{a}
cryoturbated	2.40(0.21)^{b}	1.88(0.25)^{b}	11.2(0.75)^{b}	0.536(0.11)^{a}	0.028(0.002)^{b}
mineral	7.61(0.79)^{a}	13.57(3.23)^{a}	7.26(0.20)^{c}	0.153(0.02)^{b}	0.015(0.001)^{c}

Table 5
The ratios of ^{13}C in fungal PLFA to ^{13}C in bacterial PLFA, ^{13}C in Gram-negative bacterial PLFA to ^{13}C in Gram-positive bacterial PLFA, and ratios of fungi to bacterial and Gram-negative to Gram-positive bacteria C contents together with relative contribution of specific PLFAs to total ^{13}C incorporated in three different soil layers from Ari Mas site incubated under aerobic conditions. Mean values and standard deviations (in brackets) are given (n = 3). Different letters in superscript denote significant differences between horizons (Newman-Keuls post-hoc test).

soil layer	^{13}C fungi to ^{13}C bacteria	fungi to bacteria	^{13}C G- to ^{13}C G+	G- to G+	relative contribution of specific PLFAs to total ^{13}C incorporated into PLFA [%]				
					G-	G+	fungi	bacteria	nonspecific
organic	0.21(0.02)^{b}	0.44(0.02)^{a}	4.42(0.56)^{b}	2.42(0.27)^{a}	53	12	14	68	18
cryoturbated	0.22(0.004)^{b}	0.23(0.01)^{c}	6.55(1.27)^{a}	1.78(0.06)^{b}	57	9	16	67	17
mineral	0.45(0.06)^{a}	0.37(0.02)^{b}	3.69(0.44)^{b}	1.72(0.03)^{b}	39	11	24	54	22

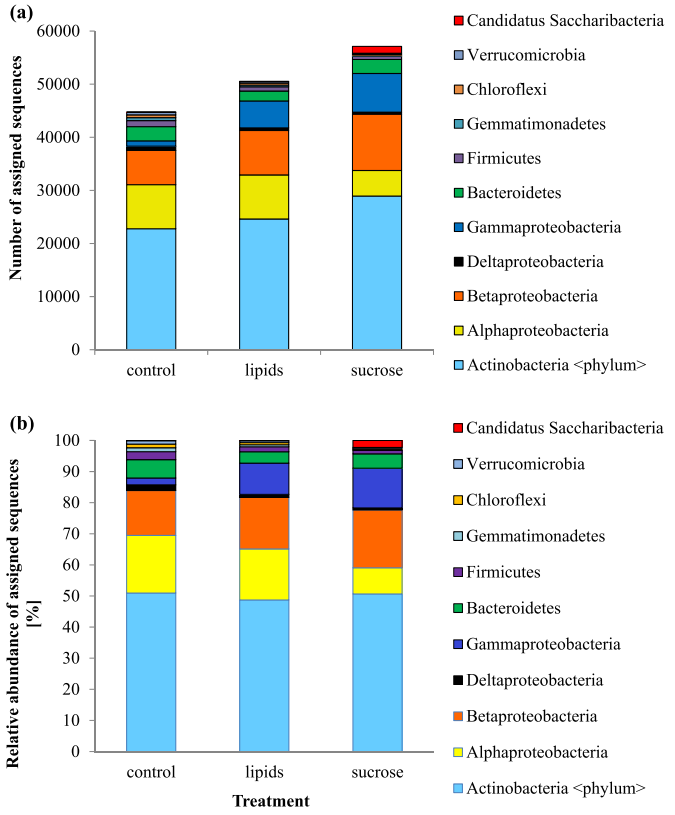

Fig. 4. Composition of the bacterial community (phylum level) based on absolute (a) and relative (b) abundance of assigned sequences in the cryoturbated layer, either unamended (control) or amended with organic C sources from Ari Mas site. Taxonomic assignment is based on LCA classifiers implemented in MEGAN software package.

2006; Šantrůčková et al., 2000) have not been satisfactorily resolved yet. Our results suggest that IC incorporation into microbial biomass and OC can contribute to soil microbial biomass and OC enrichment. Our model of CO_2 fixation, which was parameterized using data from the same locations, indicates that in the long term, IC incorporation into OC can result in similar ^{13}C-fixation as measured for bulk soils in the deep soil. The model assumes CO_2 enrichment in the soil profile corresponding to 0.1–5% of net respiration, which is within the range of measured values. Unfortunately, the uncertainty connected to changes in isotopic signal of various soil pools during decomposition process is high. Our model is therefore largely simplified and the predicted change of SOM isotopic signature is only tentative. We used several assumptions, which may affect model prediction. More specifically, we assumed that there is one pool of well mixed SOM with unique isotopic signal in soil which is decomposed at a constant rate. Including additional, more resistant SOM pool with the same isotopic signal would lead to lesser enrichment of SOM by heterotrophic CO_2 fixation in long-term. It is, however, uncertain whether chemically different pools of SOM have the same isotopic signal. The predicted change of SOM isotopic signal would largely depend on the signal of the resistant fraction at the start of decomposition process if accounted for. It might be also argued that all initial SOM is in fact decomposable in long-term and the resistant SOM fraction, represented by microbial products, builds up during decomposition. If this was the case, the enrichment of SOM would be similar as predicted but it would take longer. We further assumed that no other processes lead to isotopic discrimination. If any such process would be relevant, our predictions might be affected positively or negatively depending on the particular process, its rate and the connected discrimination.

However, four lines of evidence support our suggestion that IC incorporation can importantly contribute to ^{13}C enrichment of soil OC. **First**, across all sites, inorganic ^{13}C incorporation into OC, expressed on a total C basis, occurred throughout the soil profile and increased with soil depth. The isotopic signal of OC exponentially increased accordingly (R^2 = 0.44, n = 149). **Second**, IC available in the soil profile is enriched relative to associated SOM and plant material. Soil CO_2 may be more enriched in ^{13}C than SOM by up to 5‰ because of diffusion of lighter $^{12}CO_2$ out of the soil profile, leaving the heavier $^{13}CO_2$ behind (Cerling et al., 1991). Carbon dioxide dissolution (CO_2^*) causes only negligible fractionation (around 1‰) and CO_2^* entering carboxylation reactions should be enriched by 4‰ relative to the surrounding OC. Carbon dioxide hydrogenation comes with a huge positive discrimination, and HCO_3^- entering carboxylation reactions is enriched by 9‰ at 25 °C. The discrimination increases with decreasing temperature to 12‰ at 0 °C (equilibrium fractionation factor; Mook et al., 1974). Thus, IC entering carboxylation as HCO_3^- should be enriched relative to the surrounding organic material by 13–16‰ (4‰ plus 9‰–12‰)). Methanogenesis in anaerobic microsites and deeper parts of the soil profile can cause further CO_2 enrichment as methanogens strongly discriminate against heavier ^{13}C (difference between CO_2 and CH_4 [$\Delta_{CO2/CH4}$] from 5‰ to 93‰; Penger et al., 2012), producing relatively light CH_4 and leaving behind much heavier CO_2 (Han et al., 2007). **Third,** genes for anaplerotic, assimilatory and biosynthetic carboxylase enzymes accounted for the major part of detected carboxylase genes. The majority of those enzymes accept HCO_3^- instead of CO_2 (Table SB1). Thus the initial reactant entering the carboxylation reaction is substantially enriched relative to SOM. Although HCO_3^- entering carboxylation can originate from HCO_3^- hydrated in soil solution (see above) or from CO_2^* that is transported from soil solution into the cell, it will always be enriched relative to OC, as intracellular CO_2^* hydration is catalysed by intracellular carbonic anhydrase which prefers ^{13}C. The reaction causes an enrichment of HCO_3^- by 7‰. Carbonic anhydrase is widespread among autotrophs but also among heterotrophic eukaryota and prokaryota (Merlin et al., 2003; Nafi et al., 1990; Smith and Ferry, 2000) and is indispensable for the HCO_3^- concentrating mechanism. If there were no CO_2 leak from cells, every bicarbonate ion pulled into the cell should end up in organic compounds, and the isotopic signal of biomass would be determined by carbonic anhydrase fractionation (Hayes, 1993). Even though there is always a leakage, cells utilizing bicarbonate should be enriched relative to CO_2 (Hayes, 1993). It has been documented in plant cells that initial hydration of CO_2^* to bicarbonate and subsequent PEP carboxylation causes

enrichment of the resulting OC by 5.7‰ at 25 °C relative to gaseous CO_2. The enrichment is dependent on temperature and the amount of carbonic anhydrase present (Cousins et al., 2006; Farquhar, 1983). **Finally,** while autotrophs mostly discriminate against heavier ^{13}C and mean C discrimination of various autotrophic pathways (ΔIC/cell) ranges from zero to 26.7‰ when measured in pure cultures (House et al., 2003 and reference therein), the cells utilizing HCO_3^- will not always be depleted relative to CO_2 due to fractionation in the hydration of CO_2 to HCO_3^-. In addition, the discrimination can be decreased by limited gas diffusion in water and the soil environment (Descolasgros and Fontugne, 1990) and low cell density (House et al., 2003). The autotrophs employing Rubisco, the most common autotrophic carboxylase accepting CO_2^* and discriminating against $^{13}CO_2$ (−11 to −30‰; see Table SB1), should be depleted in ^{13}C relative to soil CO_2. The proportion of autotrophs in bulk microbial biomass is, however, generally very low. In our experiment, genes encoding Rubisco represented at most 18% of all carboxylase genes, implying that autotrophic prokaryotes should not determine the isotopic signal of total microbial biomass.

5. Conclusion

Our results demonstrate that dark CO_2 fixation is common in all arctic soils investigated and anaplerotic reactions are mainly responsible for this. Many anaplerotic pathways in heterotrophic CO_2 incorporation do not lead to any net C assimilation and biomass production (Alonso-Saez et al., 2010). Microbial biomass did not increase in soils without addition of bioavailable substrate either, which further suggests that dark IC fixation may only enable microorganisms to maintain metabolic activity even in C poor conditions. Inorganic C incorporation into OC only corresponds to a few percent of net soil respiration, but still it can play an important role in supporting microbial metabolism and organic matter transformation. We further demonstrate a positive impact of bioavailable soil organic compounds on inorganic C incorporation, implying that increases in plant litter decomposition induced by projected warming and input of root-derived compounds may also enhance C incorporation via dark C fixation.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.12.021.

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