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Assimilation of microbial and plant carbon by active prokaryotic and fungal populations in glacial forefields

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Abstract

Microbial communities and soil carbon (C) have been shown to vary in response to increasing vegetation cover during soil development after deglaciation. However, little is known about the ability of microorganisms to utilize various C sources in glacier forefield soils. We supplied ecologically relevant ^{13}C-labeled C sources (*Chlorella*, *Penicillium* and *Festuca*) to three distinct environments (supraglacial sediments, barren soils and vegetated soils) of the Damma glacier area to monitor ^{13}CO$_2$ production. We identified prokaryotic and fungal populations able to utilize these sources by using DNA-stable isotope probing coupled with Illumina MiSeq sequencing of ribosomal markers. A high initial ^{13}CO$_2$ pulse indicated that ^{13}C-labeled microbial and plant material were consumed. The ^{13}C-enriched DNA results indicated that betaproteobacterial taxa affiliated to the families *Oxalobacteraceae* and *Comamonadaceae* were important players in C utilization from different sources and present in all environments. In contrast, different fungal taxa played different roles in C degradation depending on the soil environment. Overall, our findings reveal that C utilization is driven by similar prokaryotic populations along a glacier forefield, while the distribution of active fungal populations are more influenced by environmental factors.

Highlights

- C utilization in successive stages of soil development formed after glacier retreat.

- DNA-SIP coupled with Illumina MiSeq sequencing to characterize microbial C utilizers.

- Prokaryotic C utilizers are copiotroph and similar among stages of soil development.

- Fungal C utilizers are specialist and differ among stages of soil development.

Keywords

Illumina MiSeq sequencing, DNA-stable isotope probing, glacier forefield, microbiota

Introduction

Environmental changes in polar and alpine ecosystems have been occurring at unprecedented rates (Ernakovich et al., 2014) leading to glacier disappearance (Zemp et al., 2009). After glacier retreat, the exposed mineral debris are rapidly colonized by microorganisms that trigger soil formation and development thereby influencing soil carbon (C) dynamics (Bernasconi et al., 2011; Bajerski and Wagner, 2013).

Several studies have focused on soil C dynamics under climate change (Sistla et al., 2013; van Groenigen et al., 2014), but the importance of the different sources of C to C dynamics during soil development in glacial forefields still lacks quantification. Recently deglaciated soils depleted in C receive organic C from autochthonous microbial production (Freeman et al., 2009), from allochthonous sources (wind-deposited pollen and fungal spores as well as plant and faunal debris) (Ohtonen et al., 1999; Hodkinson et al., 2003) and from ancient ice-trapped organic matter released with glacier melting (Bardgett et al., 2007). Microbial necromass also accumulates as a non-negligible C pool in initial soils (Schurig et al., 2013) and can be utilized by other microorganisms (Zumsteg et al., 2013b). After plant establishment, soil C stocks increase substantially (Egli et al., 2010; Kabala and Zapart, 2012; Smittenberg et al., 2012) and C quality changes (Dümig et al., 2011) with the accumulation of organic C from plant litter leading to higher C fluxes (Guelland et al., 2013). Such changes in resource quantity and quality are known to influence microbial communities in soils (Goldfarb et al., 2011). Despite the close relationship between microorganisms and the soil C cycle (Schimel and Schaeffer, 2012), only a few studies have investigated the utilization of different C sources by microbial communities thriving in distinct stages of soil development (SSD) of a glacier forefield. Some pioneering work along this line has been done in the Damma glacier forefield. Esperschütz et al. (2011) incubated ^{13}C-labeled plant litter in different SSD in the Damma glacier forefield and observed only slight changes in ^{13}C-enriched phospholipid fatty acid (PLFA) patterns,

suggesting that similar microbial communities are actively involved in the C dynamics throughout the forefield. However, the phylogenetic resolution of PLFA is not sufficient to identify the main players in the utilization of C sources (Dumont and Murrell, 2005). By using a DNA-stable isotope probing (SIP) approach, Zumsteg et al. (2013b) observed that algal and fungal cell fragments were crucial C sources in C-depleted barren soils of the Damma glacier forefield. These studies yielded novel information about C assimilation in developing soils, but the methods used were limited in phylogenetic resolution and breadth, and therefore they could not identify bacterial and fungal taxa utilizing C from different organic sources during soil development.

In order to advance our understanding of C acquisition during soil development in glacial forefields, we aimed at characterizing the microbial key players involved in these processes by using DNA high-throughput sequencing coupled to stable isotope probing with different relevant C sources. This approach enabled us to identify the main players within the microbial community directly responsible for C utilization at different SSD. We incubated under controlled conditions three distinct SSD (supraglacial sediments, barren soils and vegetated soils) to which we supplied three ^{13}C-labeled C sources naturally present in the Damma glacier forefield (*Chlorella*, *Penicillium* and *Festuca*). The utilization of these C sources was monitored by measuring CO_2 emission rates and δ^{13}C signatures. The prokaryotic and fungal communities able to utilize ^{13}C-labeled organic compounds were characterized with paired-end MiSeq Illumina sequencing of ribosomal markers in distinct fractions (light ^{12}C-DNA and heavy ^{13}C-DNA) retrieved from a cesium chloride (CsCl) gradient after ultracentrifugation. We incubated soil surfaces of the different SSD because this depth harbors the most active microbial communities as determined by leucine incorporation and ergosterol content (Rime et al., 2015) ensuring a sufficient incorporation of isotopes into microbial DNA used for isopycnic fractionation. Our study specifically addressed the following questions. 1) Does CO_2 released during incubation

and its $\delta^{13}C$ signature vary among the different C sources supplied and in the distinct SSD investigated? 2) What are the main prokaryotic and fungal taxa that utilize the different C sources in pristine glacier forefield environments? 3) Do prokaryotic and fungal C utilizers differ among distinct SSD?

Experimental procedure

Site description

Soil samples (surface soils down to 2 cm depth) from three different stages of soil development (referred to hereafter as SSD: supraglacial sediments, barren soils and vegetated soils) were collected in the Damma glacier area in July during the growing season. The Damma glacier area is located in the Swiss Central Alps and has a typical alpine climate with large seasonal temperature fluctuations (from -2°C to 8°C, on yearly average) as well as a high precipitation regime (2300 mm per year). The Damma glacier forefield has been described extensively in previous studies characterizing the dynamics of this ecosystem (Bernasconi et al., 2011; 2008; Brunner et al., 2011; Göransson et al., 2011; Zumsteg et al., 2012; Frey et al., 2013), which provides a wealthy database of information that can be compared with this study. The different SSD used in this study were selected to represent different soil ecosystems typical of the Damma glacier area. Supraglacial sediments were selected to represent the initial SSD in the Damma glacier area while barren and vegetated soils were investigated because they represent two distinct stages of the soil chronosequence along the Damma glacier forefield. The different SSD differ in vegetation and in microbial community compositions (Rime et al., 2015). Soil texture was determined with the hydrometer technique and gravimetric soil moisture was calculated by measuring differences in weight after oven-drying at 105°C overnight. Dried soils were used to measure water-holding capacity and 2 g of homogenized dried soil were

milled to measure total C and N contents with a CN analyzer (Shimadzu, Tokyo, Japan). Dried soil was extracted with milliQ water (1:10 m/v) with an overhead shaker overnight to measure soil pH with a FEP20-FiveEasy pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland), dissolved organic C and N with a TOC-v analyzer (Shimadzu), nitrate, sulfate and phosphate concentrations by ion chromatography with an IC:DX-120 chromatograph (Dionex Corp., Sunnyvale, CA, USA) and ammonium concentration with an FIAS 300 (Perkin-Elmer, Waltham, MA, USA), as previously described (Rime et al., 2015).

Experimental design

We conducted a stable isotope probing (SIP) microcosm experiment under controlled conditions (temperature: 10°C, no light) for 21 days. The microcosms consisted of approximately 10 g dry soil equivalent previously homogenized through a 2 mm sieve and placed in 100 mL polypropylene pots (Sarstedt AG & Co., Nümbrecht, Germany). Samples were previously equilibrated at 10°C for three days which represents the average temperature in the Damma glacier forefield (Zumsteg al., 2013a). Samples of each SSD received either no additional C (no C, control treatment) or were supplied with 20 mg of three distinct C sources separately, each of which was labeled with either ^{12}C- or ^{13}C (*Chlorella* sp. cells, *Penicillium* sp. mycelium, or *Festuca* sp. leaves; referred hereafter simply as *Chlorella*, *Penicillium* and *Festuca*). These sources of C were selected because these organisms had previously been isolated in the Damma glacier area (*Chlorella*: identified by Frey et al. (2010) and experimentally used by Zumsteg et al. (2013b); *Penicillium*: identified by Brunner et al. (2011) and experimentally used by Zumsteg et al. (2013b); *Festuca*: Bernasconi et al. (2011)). The added material was homogenized through a mill with a sieve size of 250 μm prior to mixing with the soils to enable homogenous mixing and to obtain organic fragments with a particle size greater than 250 μm. Such a particle size ensures that most of the cells were still intact and represents realistic

fragments found in these soils (Schurig et al., 2013) and utilized by microorganisms after the initial degradation by macro- and microfauna. ^{13}C-labeled *Chlorella* and *Festuca* were produced by IsoLife bv (% ^{13}C atom > 97%, Wageningen, The Netherlands) while ^{13}C-labeled *Penicillium* was produced in-house with ^{13}C-glucose (% ^{13}C atom > 99%, Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) according to the method described by Zumsteg et al. (2013b). Since ^{13}C-labeled *Penicillium* material was only produced with uniformly and highly labeled ^{13}C-glucose, we assume that this C source was highly labeled with ^{13}C (% ^{13}C atom > 90%). The soils incubated were, on the other hand, characterized by negative δ^{13}C values (-22.5 ‰). The C and N contents of the C sources were measured with a CN analyzer. C contents of the added C sources were similar (48%) while the N contents were higher in *Chlorella* (6.5%) than in *Penicillium* (1.9%) and *Festuca* (2.0%). During the incubation, the microcosms were watered by spraying distilled water every two days to compensate loss by evaporation and keep the gravimetric soil moisture constant. The microcosms were destructively sampled 2, 6 and 21 days after the additional C was supplied and kept frozen at -80°C until DNA extraction. Overall, we set up 189 microcosms (3 incubation times x 3 SSD x 7 treatments [3 C sources x 2 C isotopes + 1 control] x 3 replicates).

DNA extraction, fractionation and Illumina paired-end sequencing

Total DNA was extracted from approximately 1 g of soil using a bead-beating and chloroform-isoamylalcohol DNA extraction method as described by Frey et al. (2006) and quantified with the PicoGreen assay (Invitrogen, Carlsbad, CA, USA). DNA enriched in ^{13}C (^{13}C-DNA) was retrieved according to the protocol originally described by Neufeld et al. (2007b) and used by Zumsteg et al. (2013b). Briefly, approximately 5 µg DNA were suspended in 4.8 mL CsCl buffer and adjusted to an optical density of 1.4029 ± 0.0002 with a Refracto 30PX (Mettler-Toledo GmbH, Greifensee, Switzerland), which corresponds

to a volumetric density of 1.723 g mL^{-1} CsCl. The samples were sealed in 1/2x2 PA tubes (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer instructions and ultracentrifugated (VTi-65.1 vertical rotor and optimaTM L-80 XPultracentrifuge, Beckman Coulter) at 177 000 g for 40 h. Fourteen 300 µL DNA fractions per sample were collected drop-wise from the bottom of the tube and their optical density was measured with a Refracto 30PX to ensure formation of a density gradient. DNA of each fraction was precipitated with 1.2 mL polyethylglycerol buffer (30% PEG6000 and 1.5 M NaCl, Sigma-Aldrich, St-Louis, MO, USA) by centrifugation (16 000 g, 30 min), washed with 150 µL 70% ethanol, eluted in 30 µL AE buffer (Qiagen GmbH, Hilden, Germany) and quantified with PicoGreen. DNA fractions extracted from samples amended with ^{12}C-labeled material were used to determine the location of heavy ^{13}C-DNA fractions retrieved from samples supplied with ^{13}C-labeled material (Fig. S1 in the Supplementary Information) as described by Wang et al. (2015). Heavy fractions with buoyant densities between 1.72 and 1.74 g mL^{-1} CsCl representing the heavy ^{13}C-DNA and light fractions with buoyant densities between 1.68 and 1.69 g mL^{-1} CsCl representing the light ^{12}C-DNA were pooled and quantified with PicoGreen.

Amplicon production was conducted according to methods described in previous studies (Hartmann et al., 2015; Rime et al., 2015). Briefly, the fraction pools were diluted to 2 ng µL^{-1} and pretreated with 1 µg BSA mL^{-1} at 95°C for 5 min to inactivate PCR-inhibiting substances. Target-specific PCR were done in triplicates to amplify the hypervariable V3-V4 region of the prokaryotic (archaeal and bacterial) ribosomal small subunit (16S rRNA) and the ITS2 region of the fungal internal transcribed spacer (ITS). Each PCR was done on ^{12}C- and ^{13}C-DNA pools, as well as on DNA from control samples (no C), with a 10 ng DNA template in a final volume of 50 µL. The V3-V4 region of the prokaryotic 16S rRNA gene was amplified with the newly modified primers 341Fh (CCTAYGGGDBGCWSCAG) and 806Rh (GGACTACNVGGGTHTCTAAT) (Frey et al. 2016), while the ITS2 region of the

eukaryotic ribosomal operon was amplified with degenerate versions of the ITS3 (CAHCGATGAAGAACGYRG) and ITS4 (TCCTSCGCTTATTGATATGC) primers recently published by Tedersoo et al. (2014). An in-silico evaluation of the primer pairs revealed a similar coverage for bacteria, archaea, and fungi (around 90%). The 5' ends of the primers were tagged with the CS1 (forward primers) and CS2 (reverse primers) adapters required for multiplexing samples using the Fluidigm Access Array™ System (Fluidigm, South San Francisco, CA, USA). The PCR conditions to amplify the prokaryotic 16S fragments and eukaryotic ITS2 fragments consisted of an initial denaturation at 95°C for 15 min, 35 cycles of denaturation at 94°C for 40 s, an annealing at 58°C for 40 s and an elongation at 72°C for 1 min followed by a final elongation at 72°C for 10 min. Amplicons were sent to the Génome Québec Innovation Center at McGill University (Montreal, Canada) for barcoding using the Fluidigm Access Array™ technology (Fluidigm, South San Francisco, CA, USA) and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA). Raw sequences are available as publicly accessible SRA (ENA: PRJEB10588).

Processing of Illumina paired-end reads

Forward and reverse reads were merged with PEAR v.0.9.5 (Zhang et al., 2014) and poor-quality or unassembled reads were removed based on default parameters. Merged reads were submitted to stringent quality-filtering criteria. Reads containing any mismatches to the primer sequences or ambiguous bases were discarded with the *trim.seqs* function in Mothur v.1.33 (Schloss et al., 2009). Primer sequences of the remaining reads were trimmed at this step. Further quality filtering and clustering into operational taxonomic units (OTUs) was largely performed according to the UPARSE pipeline (Edgar, 2013) implemented in USEARCH v.7.0.1090. Primer-trimmed reads were quality-filtered with the USEARCH *fastq_filter* function with a maximum error threshold (maxee) of 0.25. Singleton

reads were removed with the *sortsize* function to avoid OTU inflation. Sequences were clustered into OTUs at 97% sequence identity using the *cluster_otu* function including an "on-the-fly" chimera detection algorithm. The representative OTU sequences were submitted to an additional chimera-check control against the RDP classifier training datasets for 16S and ITS2 available on-line with the *uchime_ref* function (Edgar et al., 2011). All reads were mapped to the OTU representative sequences with the *usearch_global* function. Taxonomy assignment of prokaryotic and fungal OTUs was conducted in Mothur using a naïve Bayesian classifier (Wang et al., 2007) with a bootstrap support of 60% against the Greengenes (DeSantis et al., 2006; McDonald et al., 2012) and UNITE (version 7, 01.08.2015; Abarenkov et al., 2010) reference databases, respectively. The primers used to amplify the ITS2 region might also target other eukaryotic groups. Therefore, we conducted an additional taxonomic assignment of the ITS2 OTUs against a custom-made reference database based on eukaryotic ITS2 sequences retrieved from the NCBI GenBank database (Benson et al., 2005). Since DNA sequences from *Chlorella*, *Penicillium* and *Festuca* might arise from the added C sources, we identified and removed the sequences affiliated to these organisms from both $16S_{V3-V4}$ and ITS2 sequences before analyses.

CO_2 and $\delta^{13}C$ measurements

In parallel with the SIP approach, 10 g dry-equivalent soil microcosms were set up in triplicates at the same temperature with the same C source:soil ratio previously described. These microcosms were further used to calculate CO_2 emission rates and measure $\delta^{13}C$ signatures of CO_2 emitted after C addition. Because the isotope ratio mass spectrometer (IRMS; Delta V Advantage, Thermo Fischer Scientific) used to detect $\delta^{13}C$ signatures of CO_2 emitted was not equipped for measuring high percentage of ^{13}C atoms, we diluted the added ^{13}C-labeled material with ^{12}C-labeled material (ratio approximately 1:1000) before

homogenizing the C sources with the soils to ensure that the added C sources were used by soil microorganisms and that the emitted CO_2 did not result from the decomposition of the native soil organic matter (priming effect). The microcosms were placed in custom-made gas-tight 1L jars equipped with a septum (Hartmann et al., 2010) 1, 3, 6, 9, 13 and 21 days after C addition. Headspace air was sampled 0, 12 and 24 hours after jar closure with a 20 mL syringe and injected into pre-evacuated 12 mL Exetainer vials (Labco Limited, Lampeter, United Kingdom). The CO_2 concentrations and $\delta^{13}C$ were measured with a gas chromatograph (Trace GC Ultra, Thermo Fischer Scientific, Waltham, MA, USA) coupled with an IRMS. CO_2 emission rates were calculated by fitting linear regressions through the CO_2 concentrations over headspace sampling time. The goodness of the fit (r^2) was calculated for each rate and was very good in general ($r^2 > 0.95$). Based on CO_2 emission rates, we estimated the amount of C emitted throughout the incubation time as a proportion of the amount of added C sources. Delta ^{13}C values were expressed as per mil (‰) in relation to the Vienna-Pee Dee Belemnite gauged reference materials.

Statistical analyses

Differences in soil parameters, in CO_2 emission rates and in $\delta^{13}C$ signatures were assessed by conducting a repeated-measures split-plot ANOVA in R v.3.1.0 (R development Core Team, 2012). To assess differences among individual means of each soil parameter, CO_2 emission rates and $\delta^{13}C$ signatures among incubation times, SSD and C sources, we conducted Tukey Honest Significant Difference (HSD) tests with the function *HSD.test* implemented in the R package *agricolae* (Mendiburu, 2012). Changes in β-diversity were investigated based on Bray-Curtis dissimilarity matrices obtained from square-root transformed relative abundances of OTU tables not previously rarefied, following recommendations outlined by McMurdie and Holmes (2014) and similar to the procedure implemented by Hartmann et al. (2014) and Rime et al. (2016). Permutational

multivariate ANOVA (PERMANOVA) and analysis of similarity (ANOSIM) were conducted in R with the *adonis* and *anosim* functions (number of permutations = 9999) implemented in the *vegan* package (Oksanen et al., 2012) to assess structural differences in microbial communities. Since differences assessed by PERMANOVA may arise due to within-treatment dispersion, we also conducted an analysis of homogeneity of group dispersion (Dispersion) with the *betadisper* function (number of permutations = 9999) in the *vegan* package. Changes in community structures were displayed with principal coordinate analysis (PCO). Differences in community structures of microbial populations present in ^{13}C-DNA fraction, i.e. the populations involved in C utilization, were assessed by pairwise comparisons and p-values were corrected with the Holm method (Holm, 1979). Differences in relative abundances of the dominant prokaryotic and fungal phyla, as well as proteobacterial classes (>1% of the total dataset) were assessed by conducting a repeated-measures split-plot ANOVA. To identify OTUs that were predominant in heavy ^{13}C-DNA fractions, we investigated $\log_2$-fold changes in OTU counts fitted with a negative binomial distribution, as recommended by McMurdie and Holmes (2014). We tested changes in OTU abundances against a null hypothesis with log-likelihood ratio tests, as implemented in the *DESeq2* R package (Love et al., 2014). P-values were corrected for multiple testing with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) and OTUs with adjusted p-values < 0.05 ($P<0.05$) were considered significant. We selected OTUs with abundances that increased in ^{13}C-DNA fractions, i.e. those with positive $\log_2$-fold change values, and mapped them in taxonomic networks generated in Cytoscape v.3.0.2 (Shannon et al., 2003) to explore habitat/treatment-OTU association patterns as previously demonstrated (Hartmann et al., 2015). All graphs except the taxonomic networks were produced with the *graphics* (R development Core Team, 2012) and *ggplot2* R packages (Wickham, 2009).

Results

Characteristics of C sources and SSD used in incubation experiments

The different C sources supplied had similar C content (on average 48%) but differed in nitrogen (N) content (*Chlorella*: 6.5%; *Penicillium*: 1.9%; *Festuca*: 2.0%). The different SSD investigated also differed in physio-chemical parameters. Generally, most of the nutrients analysed (e.g., C, N, and S) were significantly ($P<0.05$, Table 1) lower in supraglacial sediments and barren soils compared to vegetated soils. Soil pH and sand were highest in supraglacial sediments compared to the other SSD.

CO_2 emission and $\delta^{13}C$ signature

CO_2 emission rates and their $\delta^{13}C$ signatures varied with C sources, incubation time and SSD (Fig. 1 and Table 2; Table S1 in the Supplementary Information). The microbial communities in vegetated soils rapidly started to use the added C after one day, while those in supraglacial sediments and barren soils used the supplied C with a delay of one to three days. In supraglacial sediments and barren soils, the highest CO_2 emission rates occurred three days after addition of *Chlorella*. In contrast, CO_2 emission rates in vegetated soils were highest one day after addition of *Chlorella* and *Festuca*, remained constant after three days of incubation and then decreased over time (Fig. 1a). At the end of the incubation period (21 days), CO_2 emission rates remained higher in barren and vegetated soils amended with C ($P<0.05$) while the rates were similar between C amended supraglacial sediments and controls ($P>0.05$). We integrated the CO_2 emission in the experiments over the period of incubation to estimate the amount of C emitted from each treatment and each SSD. We found that much of the C added to the vegetated soils was respired (*Chlorella*: 70.2% of the added C, *Penicillium*: 52.6%, *Festuca*: 48.6%) while less C was emitted from barren soils (*Chlorella*: 54.5%, *Penicillium*: 34.5%, *Festuca*: 37.1%)

and supraglacial sediments (*Chlorella*: 47.3%, *Penicillium*: 22.4%, *Festuca*: 26.6%). However, the fact that all SSD amended with C still showed positive $\delta^{13}C$ values at the end of the incubation period (21 days) indicates that not all material had been consumed (Fig. 1b).

Density fractionation and recovery of microbial communities in ^{12}C-light and ^{13}C-DNA heavy fractions

We recovered 14 DNA fractions distributed along a buoyant density gradient ranging from approximately 1.67 to 1.76 g mL^{-1} CsCl after ultracentrifugation (Fig. S1). Based on CsCl buoyant gradients obtained with DNA extracted from ^{12}C-amended soils, we determined three "light" ^{12}C-DNA fractions (buoyant density: 1.678-1.689 g mL^{-1} CsCl) as well as three "heavy" ^{13}C-DNA fractions (buoyant density: 1.718-1.736 g mL^{-1} CsCl). The densities of heavy fractions were chosen where only small amount of DNA was detected in CsCl gradients obtained from ^{12}C-amended soils and control soils (no C) to ensure that DNA recovered in heavy fractions from samples amended with ^{13}C-labeled material resulted from isotopic enrichment and not from higher GC content (Wang et al., 2015). Using these fractions, we successfully amplified and sequenced prokaryotic 16S$_{V3-V4}$ and eukaryotic ITS2 ribosomal target segments. After quality filtering, we obtained 4 210 852 (22 280 ± 5576 per sample) and 4 070 897 (21 560 ± 12 078) merged 16S$_{V3-V4}$ and ITS2 reads, respectively, which corresponded to 8666 (1002 ± 719) prokaryotic and 3489 (183 ± 70) eukaryotic OTUs. Among the eukaryotic OTUs, we excluded those not classified as fungi (893 OTUs of the total OTU table, 3% of the total sequence dataset) to focus on fungal communities. A detailed description of the prokaryotic and fungal communities is given in SupplementaryResults.doc (Supplementary Information).

Effects of C sources on microbial community structures

Both prokaryotic and fungal communities differed in β-diversity among SSD after addition of C sources. Structural patterns were also distinct between prokaryotic and fungal C utilizers. Prokaryotic and fungal communities structurally differed among C sources, incubation time, SSD, and DNA fractions as revealed by PERMANOVA and ANOSIM (Table 3 and Table S2) and displayed by PCO ordinations (Fig. 2). The PCOs that displayed differences in prokaryotic community structures showed that, at two and six days after C addition, the samples were clearly discriminated between light and heavy DNA fractions and among SSD. Further, the native microbial communities (no C) of each SSD clustered with their respective communities in light ^{12}C-DNA fractions with the exception of the native community of supraglacial sediments. At day 21, the prokaryotic communities still differed among SSD although the difference between communities in light and heavy DNA fractions became smaller. Conversely, the PCOs for fungal communities did not show a clear separation between communities in light and heavy DNA fractions but revealed clear differences among communities in the distinct SSD at all sampling times. The structural differences in prokaryotic communities were partly due to within-treatment dispersion among C sources, SSD and DNA fractions, while differences in fungal community structure were partly due to heterogeneity among C sources and SSD (Table 3). The amount of variability in prokaryotic and fungal communities was higher for SSD (Variability>21%) than for C sources (Variability>6%) and incubation time (Variability<3%) while the variability for DNA fractions were higher for the prokaryotic communities (Variability: 11%) than for the fungal communities (Variability: 3%). The relative abundances of the prokaryotic and fungal taxa in heavy ^{13}C-DNA fractions also differed from those in native communities and in light ^{12}C-DNA fractions and varied among C sources and with time (Fig. S2 and Table S3). Additionally, pairwise comparisons of prokaryotic and fungal populations involved in the utilization of a specific C source showed

that the prokaryotic populations were generally similar in barren and vegetated soils but differed from those in supraglacial sediments while fungal C utilizer populations mainly differed among all SSD (Table S4).

Identification of OTUs present in heavy ^{13}C-DNA fractions

Our OTU-specific analyses were restricted to OTUs found in distinct SSD supplied with different C sources. We did not focus on changes with incubation time since most of the structural variability within prokaryotic and fungal communities occurred among SSD and C sources (Table 3). We investigated significant (adjusted P<0.05) log$_2$-fold changes in OTU counts between light ^{12}C- and heavy ^{13}C-DNA fractions in samples supplied with ^{13}C-material at all sampling times to identify prokaryotic and fungal OTUs that were prominent in heavy ^{13}C-DNA fractions (Fig. S3). Overall, the microbial populations involved in C utilization (based on their presence in the heavy ^{13}C-DNA fraction) were dominated by representatives affiliated to *Actinobacteria*, *Bacteroidetes*, *Alpha-*, *Beta-*, *Gammaproteobacteria* and *Ascomycota* (Figs. 3 and 4).

We identified 144 prokaryotic and 15 fungal OTUs significantly (adjusted P<0.05) enriched in heavy ^{13}C-DNA, representing 44.6% and 9.6% of the total prokaryotic and fungal communities, respectively (Fig. 3; Tables S5 and S6). The majority of the OTUs (82%) affiliated to *Bacteroidetes* were predominant in SSD supplied with *Chlorella* while no clear association with a particular C source was observed in the dominant proteobacterial classes and in the phyla *Actinobacteria* and *Ascomycota* (Fig. 3). For example, most of the dominant betaproteobacterial OTUs in the heavy ^{13}C-DNA fraction were found in more than one SSD independently from the C source supplied. The investigation of prokaryotic OTUs with log$_2$-fold change values greater than three (i.e. representing an eight-fold increase in OTU abundance in the heavy ^{13}C-DNA fraction relative to the light ^{12}C-DNA

fraction) revealed that many OTUs were affiliated to different orders (*Sphingobacteriales*, *Burkholderiales* and *Myxococcales*) or families (*Microbacteriaceae*, *Chitinophagaceae*, *Comamonadaceae*, *Oxalobacteraceae* and *Xanthomonadaceae*; *Methylacidiphilaceae*) (Fig. 4). The prokaryotic OTUs classified at the genus level with $\log_2$-fold change values greater than three consisted of diverse *Actinobacteria* (*Arthrobacter*, *Cellulomonas*, *Cryobacterium* and *Kitasatospora*), *Bacteroidetes* (*Dyadobacter* and *Flectobacillus*), *Proteobacteria* (*Hydrogenophaga*, *Janthinobacterium*, *Massilia*, *Pleomorphomonas*, *Pseudomonas* and *Stenotrophomonas*) and *Verrucomicrobia* (*Opitutus*). Among the fungal communities, particular OTUs affiliated to the ascomycete genera *Fontanospora*, *Lecythophora, Trichoderma and Acremonium* showed a strong increase in OTU abundance in the heavy ^{13}C-DNA fraction relative to the light ^{12}C-DNA fraction (Fig. 4). Conversely, only one predominant basidiomycete OTU (genus Malassezia) in the heavy ^{13}C-DNA fraction responded significantly to any of the C sources (Figs. 3 and 4).

Discussion

Impact of different C sources and SSD on CO_2 emission and microbial community structures

Patterns of CO_2 emission rates in nutrient-poor supraglacial sediments and barren soils differed from those found in vegetated soils after addition of the three different C sources (Figure 1), agreeing with results from Eichorst and Kuske (2012) who reported variable C utilization of ^{13}C-cellulose in various soil types. The high CO_2 emission rates and the highly positive $\delta^{13}C$ signatures recorded after addition of ^{13}C-labeled substrates indicate that the emitted CO_2 mainly originated from the utilization of the supplied organic material (e.g. labile polysaccharides) by fast-growing microorganisms (Kindler et al., 2009) rather than from the decomposition of the native soil organic matter (i.e. priming effect), corroborating the findings reported by Guelland et al. (2013). Despite the clear differences in CO_2

emission rates observed among SSD and C sources supplied, it remains unclear whether these differences were due to changes in microbial communities, differences in edaphic parameters, or both. For example, the differences in CO_2 emission patterns among C sources and SSD may be due to differences in macro- and micronutrient contents in the supplied C sources and the investigated SSD as reported by Göransson et al. (2011).

Microorganisms in vegetated soils utilized and respired the added C sources more rapidly most probably due to lower nutrient limitation and higher initial microbial biomass indicated by the high DNA concentrations extracted from those samples (Table 1). Conversely, in supraglacial sediments and barren soils, microorganisms also responded to the added C sources but with a delay (three days) in order to mobilize nutrients progressively from the supplied material. CO_2 emission rates after addition of *Penicillium* were lower than those measured after addition of *Chlorella* or *Festuca* in all SSD, which might be due to differences in the chemical composition of the C sources (*Penicillium*: chitin (Rizza and Kornfeld, 1969); *Chlorella*: hemicellulose and glycoprotein (Northcote et al., 1958); *Festuca*: lignocellulose (Gunnarsson et al., 2008)) and the higher recalcitrance nature of chitin (Trofymow et al., 1983), the main constitutive compound of the fungal cell wall (Rizza and Kornfeld, 1969), relative to that of cellulose (Baldrian et al., 2010, 2011). Moreover, microorganisms have a lower potential to degrade chitin in bulk soils (low activity of microbial chitinase producers) than those in the rhizosphere forefield soils (Töwe et al., 2010), suggesting that chitin can be less efficiently degraded in bulk soils as used in this incubation experiment. We therefore assume that not only C and nutrient contents but also the chemical structure of the supplied organic material influenced microbial C utilization.

Among microbial C utilizers, we identified specific OTUs predominantly occurring in the heavy ^{13}C-DNA fractions by evaluating differential changes in OTU counts among C sources, SSD and DNA fractions (Love et al., 2014; McMurdie and Holmes, 2014; Pepe-

Ranney et al., 2015). Distributions of abundant prokaryotic phyla in the light and heavy DNA fractions resembled each other 21 days after addition of the ^{13}C-labeled C sources (Figure S2) which suggest that the ^{13}C-assimilating microbial communities are dominated by an opportunistic subset of the total microbial community as pointed out by others (Deslippe et al., 2015; Schmidt et al., 2015). We have previously used longer incubation times (up to 60 days) in SIP experiments (Zumsteg et al. 2013b) and it was not possible to make clear separation between "heavy" and the "light" fractions 22 and 60 days after the addition of the labeled substrate. Shorter incubations times (up to 8 days) limit the movement of label beyond primary carbon consumers via biomass turnover (Zumsteg et al., 2013b; Leung et al, 2016).

In our study, both prokaryotic and fungal communities were significantly influenced by C sources and predominant OTUs in the heavy ^{13}C-DNA fractions were mainly present in more than one SSD and associated with more than one C source (Tables S5 and S6). However, prokaryotic populations involved in the utilization of particular C sources (defined by their presence in ^{13}C-DNA fraction) resembled each other irrespective of SSD while fungal populations in ^{13}C-DNA fractions differed among SSD (Figure 2). We assume that fungal populations able to utilize distinct C sources in soils of the Damma glacier are more influenced by soil environmental parameters than the prokaryotic C utilizers. Brown and Jumpponen (2014) reported that a high proportion of fungal OTUs were less randomly distributed than bacterial OTUs in soil chronosequences along a glacier forefield, suggesting that fungi have more specific habitat requirements in comparison to bacteria. In addition, Zumsteg et al. (2011) used a reciprocal transplant experiment to show that the fungal communities in the barren soils of the Damma glacier forefield were more influenced by changes in soil temperature and moisture than bacterial communities.

Taxa-specific associations with C sources and SSD: do soils in glacial

forefields harbor generalists or specialists?

Drawing conclusions on ecosystem functioning based on phylogenetic information is a tenuous exercise given the lack of knowledge concerning the majority of OTUs found in environmental samples. Nevertheless, here, we aimed to highlight salient patterns in specific OTUs with putative ecological roles that have previously been proposed in the literature in order to provide a deeper understanding of microbial C utilizers in soils. Our data suggest that the addition of different C sources did not exert uniform responses within closely related members (e.g. within a phylum) but led to very specific compositional shifts at lower taxonomic levels (Fig. 3). Although the soil is usually characterized by a tremendous bacterial and fungal diversity (Coleman and Whitman, 2005), only limited subsets of organisms are assumed to contribute to certain processes (e.g. C utilization).

Microbial groups that play an important role in decomposition of complex organic materials such as *Proteobacteria, Actinobacteria and Ascomycota* revealed a heterogeneous response (Figs. 3 and 4), suggesting that different taxa within these phyla are specialists for certain types of high molecular weight substrates. In particular, we found that the prokaryotic OTUs prominent in the heavy fractions of the ^{13}C treatments included copiotrophs (*sensu* Fierer et al., 2007) from diverse taxa of the *Bacteroidetes* and *Proteobacteri*a. Many of these members (e.g. *Massilia, Janthinobacterium, Oxalobacteraceae, Comamonadaceae* and *Burkholderiales*) utilized multiple C sources (Fig. 3) and are known to degrade a wide variety of organic compounds with a key role in cellulose and hemicellulose degradation (Eichorst and Kuske, 2012; Semenov et al., 2012; Štursová et al., 2012; Pinnel et al., 2014; Verastegui et al., 2014; Leung et al., 2016). Interestingly, these previous studies were conducted in forest and agricultural soils, which suggest that similar bacterial taxa are involved in the utilization of various C sources in both developing and mature soils.

Specially, we found OTUs related to the proteobacterial genus *Janthinobacterium*

that were specifically involved in C utilization in supraglacial sediments and barren soils but not in vegetated soils. OTUs belonging to this genus were also involved in degrading organic matter in soils of the McMurdo Dry Valley (Antarctica) (Schwartz et al., 2014) and in weathering rock minerals in barren soils of the Damma glacier forefield (Frey et al., 2010). Given the crucial roles in C utilization and nutrient dynamics in initial nutrient-depleted soils, OTUs related to this genus might be key microorganisms in recently deglaciated soils.

Previous DNA-SIP studies also identified that members of the Gammaproteobacteria were associated with cellulose degradation in soil environments (Bernard et al., 2007; Eichorst and Kuske, 2012). In our study, *Pseudomonas* spp. within the Gammaproteobacteria detected in the heavy fraction of the ^{13}C treatments was the main C utilizer. Some bacterial OTUs from the *Myxococcales* (*Deltaproteobacteria*) also appeared to assimilate the various C sources, which is in agreement with previous studies that members of this order are involved in lignocellulose degradation (Darjany et al., 2014)

In contrast to the association patterns observed between Proteobacteria and multiple C sources, OTUs belonging to the phylum *Bacteroidetes* (e.g. the family *Chitinophagaceae* and the genera *Flectobacillus* and *Dyadobacter*) were specifically associated with C from *Chlorella* in the different SSD. This phylum largely consists of copiotrophs able to use various organic compounds (Fierer et al., 2007). In addition, OTUs related to the genera *Flectobacillus* and *Dyadobacter* are known to dwell in soils and to degrade organic compounds such as starch (Krieg et al., 2010; Lee et al., 2010). Previous studies reported an increase in relative abundance of *Bacteroidetes* related to increasing soil N (Nemergut et al., 2008; Zhang et al., 2013; Koyama et al., 2014). Among the C sources used in our experiments, *Chlorella* had substantially lower C:N ratio compared to the other sources and the additional N supply provided by the *Chlorella* treatment may have favored representatives of *Bacteroidetes*. The presence of OTUs from the

Chitinophagaceae in the heavy ^{13}C-DNA fraction across all time points in this study was interesting because of their potential ability to decompose chitin, a fungal cell wall constituent. *Chitinophaga* spp. has been identified as decomposers of dried rice callus (Li et al., 2011), indicating that their proliferation responds to easily decomposable plant constituents. From these findings we suggest that prokaryotic C utilizers mainly consist of generalist copiotrophs (*Bacteroidetes* and *Proteobacteria*) able to degrade rapidly labile C sources originating from glacial environments.

The addition of the different C sources also favored the development of particular populations related to Actinobacteria. Several OTUs associated with *Kitasatospora*, *Arthrobacter* and *Cellulomonas* being the most abundant genera could be characterized as consumers of the added C sources. *Arthrobacter* has been reported as potential primary utilizer of easily available C (Semenov et al., 2012). Reguera and Leschine (2001) reported that isolates affiliated to the actinobacterial genus *Cellulomonas* can produce both cellulase and chitinase, representing a competitive advantage for acquiring C in natural habitats. Members of this genus were reported to be active in degrading cellulose and cellobiose in soils (Haichar et al., 2007; Cañizares et al., 2011; Schellenberger et al., 2010), which indicates that bacteria affiliated to this genus might be crucial in litter degradation. *Kitasatospora* were highly enriched in the heavy fractions of ^{13}C-labelled sources in developing soils, which has not been documented before. *Kitasatospora* are known to be celluloytic bacteria (Ulrich et al., 2008). This finding provides impetus for further studies of *Kitasatospora* to determine its ability to use easily available or recalcitrant C sources in soils.

Opitutus (Verrucomicrobia) were enriched in the heavy fractions of the ^{13}C treatments, but its role in C assimilation in developing soils is yet unknown. Only one species of this genus has been described, i.e. *Opitutus terrae*, an obligate anaerobe isolated from a rice paddy soil (Chin et al., 2001). *Opitutus* spp. has previously been

described as key C assimilator after the incorporation of fresh potato tissue in mature soils (Semenov et al., 2012). So far, no other study has revealed *Opitutus* spp. as an important C utilizer in developing soils, but they appear to be key organisms in glacial soils as they were abundant in the heavy fractions of the ^{13}C treatments.

Despite the crucial role of fungi in soil decomposition processes (Zifcakova and Baldrian, 2012; Van der Wal et al., 2013), the identity of the main fungal C utilizers has remained elusive in alpine glacial forefields. With the evaluation of differential OTU counts, we found that fungal OTUs able to utilize the different C sources among the SSD investigated consisted of *Ascomycota,* which is in agreement with previous SIP-DNA studies showing that cellulolytic and hemicellulolytic fungal OTUs largely consist of *Ascomycota* (Štursová et al., 2012; Leung et al., 2016). This is the first report of the genera *Fontanospora* and *Lecythophora* involved in the assimilation of C sources in soils. Representatives of the genus *Fontanospora* are abundant in barren soils of the Damma glacier forefield (Rime et al., 2015, 2016), whereas representatives of the genus *Lecythophora* were reported to be active in the degradation of wooden structures in Antarctica (Held et al., 2006). Both genera were highly enriched in the heavy ^{13}C-DNA fractions after the addition with *Festuca* and might therefore represent important fungal decomposers of plant debris in glacial soils. The vegetated soils investigated in our study were originally covered by herbaceous plants (e.g. *Festuca rubra*) and woody shrubs (e.g. *Salix sp.*) (Bernasconi et al., 2011).

This is also the first report of representatives affiliated to the order *Chaetothyriales* to be enriched in ^{13}C-DNA fractions. Little is known on the ecology of these organisms but earlier investigations have shown that members of this order are slow-growing melanized fungi inhabiting rocks in harsh environments (Sterflinger et al., 1999). It is unclear whether these fungal populations are saprophytic or biotrophic (Geiser et al., 2006), but we assume that these fungal decomposers are mainly growing on decaying plant debris or fungal

hyphae.

Interestingly, we found for the first time in SIP studies parasitic fungi (*Acremonium* and *Trichoderma*) to be very important organism in the assimilation of C sources in soils. Due to their lignocellulolytic capabilities (Jimenez et al., 2014), these fungi can actively utilize C from plant residues, such as *Festuca*, which represents an advantage for establishment in vegetated soils recently formed after glacier retreat.

Although the relative abundance of *Basidiomycota* was relatively high in native communities in the different SSD investigated (~20% on average), it decreased drastically in soils supplied with additional C sources (Fig. S2b), indicating that most of the *Basidiomycota* present in distinct soils of the Damma glacier area were not significantly contributing to C utilization. We assume that basidiomycetous fungi are outcompeted by saprotrophs when a source of organic material is supplied to glacial soils, although cellulolytic and hemicellulolytic capabilities are widespread in basidiomycetous yeasts (primarily *Cryptococcus*) (Štursová et al., 2012; Leung et al., 2016).

Here, only one basidiomycetes OTU, affiliated to the genus *Malassezia,* was prominent in ^{13}C-DNA fractions. This is the first report of this basidiomycetous yeast being involved in the assimilation of C substrates in SIP studies although *Malassezia* has been suggested to act as sugar monomer cheaters in the decomposition of lignocellulose (Jimenez et al. 2014). This genus is known to occur in soils associated with nematodes (Adam et al., 2014) or on the skin of warm-blooded animals (Coelho et al., 2013). Interestingly, due to its small genome, this basidiomycete lacks most of the genes encoding carbohydrate-degradation enzymes and mainly depends on external sources of labile C and lipids, which makes it a perfect commensal or parasite (Coelho et al., 2013). We therefore suggest that this yeast might have profited from C-derived breakdown products by other microorganisms, such as ascomycetous saprotrophs and bacterial copiotrophs.

It is important to note that SIP experiments might suffer from drawbacks related to cross-feeding (i.e. the incorporation of ^{13}C into DNA of secondary consumers or microbial commensals) (Neufeld et al., 2007a; Bell et al., 2011; Stursová et al., 2012). Therefore, it cannot be excluded that the detected active microorganisms are able to assimilate C by grazing upon C utilizing metabolic products of other organisms that hydrolyzed ^{13}C-labelled substrates in the soils. For example, some fungi might have incorporated ^{13}C by bacterivory on cellulose degrading bacteria, suggesting that fungi contribute to the biopolymer degrading food webs in soil by top-down control of the bacterial community (Jimenez et al., 2014). Its impact on soil food webs and the distinction between direct degradation of ^{13}C-labelled substrates, degradation of their hydrolysis products, or grazing-mediated incorporation of ^{13}C warrants further studies.

Conclusions

Our study reveals that both prokaryotic and fungal communities were significantly influenced by the different C sources. The addition of different C sources did not exert uniform responses within closely related members (e.g. within a phylum) but led to very specific compositional shifts at lower taxonomic levels indicating that the ^{13}C-assimilating microbial communities are dominated by an opportunistic subset of the total microbial community. Prokaryotic C utilizers mainly consist of generalist copiotrophs able to degrade rapidly labile C sources originating from *Chlorella* and *Festuca*. The degradation of labile sources of C is the main driver of high CO_2 emissions from these soils. The generalist prokaryotic C utilizers identified consist of weathering-active Betaproteobacteria known to be able to solubilize minerals from granite (e.g. *Comamonadaceae*, *Oxalobacteraceae* and *Janthinobacterium*) commonly found in all SSD. Microorganisms with this particular physiological ability might have an advantage in C utilization since they can obtain nutrients directly from minerals. In contrast, despite the crucial role of fungi in soil

decomposition processes, the identity of the main fungal C utilizers has remained elusive in alpine glacial forefields. Fungal OTUs able to utilize the different C sources consisted of *Ascomycota* either saprophytic or parasitic fungi. These fungi can actively utilize C from plant residues, such as *Festuca*, which represents an advantage for establishment in vegetated soils recently formed after glacier retreat. However, it cannot be excluded that the detected active fungi are able to assimilate C by grazing upon C utilizing metabolic products of bacteria that hydrolyzed ^{13}C-labelled substrates in the soils. This study demonstrates that glacial forefields contain microbial communities that are active, versatile and highly adapted to extreme oligotrophic conditions. Many of the active organisms described in this study have an important role in weathering triggering the necessary conditions for soil formation and establishment of higher plants.

Conflict of interest

The authors declare no conflict of interest.

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Table and Figure legends

Table 1. Characteristics of the stages of soil development (surface soils down to 2 cm depth) at the beginning of the incubation. The values are the means ± 1 SE (n=3).

Table 2. Effects of incubation time (Time), stages of soil development (SSD) and C sources (C; *Chlorella*, *Penicillium* or *Festuca*) on CO_2 emission rates (CO_2) and their $\delta^{13}C$ signatures ($\delta^{13}C$).

Table 3. Effects of incubation time (Time), stages of soil development (SSD), C sources (C; *Chlorella*, *Penicillium* or *Festuca*) and DNA fraction (light and heavy) on prokaryotic and fungal β-diversity. For clarity, we only show F-values for the individual factors while F-values for interaction terms between factors are given in Table S2 (Supplementary Information).

Fig. 1. Effects of different C sources (*Chlorella*, *Penicillium* and *Festuca*) on CO_2 emission rates (a) and their $\delta^{13}C$ signatures (b) for different incubation times and in different stages of soil development (SSD) (supraglacial sediments, barren soils and vegetated soils; separated horizontally). Controls (no C) and the different C sources are represented with different colors as described in the figure. Mean CO_2 emission rates and $\delta^{13}C$ signatures ± 1 SE (n=3) are displayed for all incubation times, SSD and C sources except for the vegetated soils that received no C one day after incubation start, where n=2.

Fig. 2. Effects of C sources (*Chlorella*, *Penicillium* and *Festuca*) on prokaryotic (a) and fungal (b) β-diversities at three different incubation times in distinct stages of soil development (SSD) displayed with principal coordinate analysis (PCO). SSD are

represented with different symbols. C sources and native microbial communities (no C) are depicted with different colors. Heavy ^{13}C-DNA fractions are represented with solid symbols while light ^{12}C-DNA fractions are represented with open symbols. The variability of the PCO axes are given in parentheses.

Fig. 3. Taxonomic networks representing the distribution of the detected archaeal, bacterial and fungal OTUs across the different taxonomic branches. Nodes represent OTUs and node sizes represent their relative abundance (square-root). Edges represent the taxonomic path from the root (that is, archaea, bacteria or fungi) to the species level, and OTUs were placed at the level of the lowest possible assignment. OTUs significantly associated with the light ^{12}C-DNA (adjusted $P<0.05$, $\log_2$-fold change<0, see also Fig. S3) are represented by transparent downward arrows, whereas OTUs significantly associated with the heavy ^{13}C-DNA fractions ($\log_2$-fold change>0) are represented by colored symbols (see legend for details). When OTUs prominently found in the heavy ^{13}C-DNA fraction were associated with several SSD or C sources (mixed), they were depicted with the symbol "diamond" (for several SSD) and the color "black" (for several C sources). To highlight predominant OTUs in the heavy ^{13}C-DNA fraction, we colored their taxonomic paths according to their respective phyla or proteobacterial classes. The number of OTUs and their relative abundances in the total datasets are given in parentheses for each highlighted group. Clusters not enriched in ^{13}C (present in the light ^{12}C-DNA fraction) are labeled in roman numbers. I: TM7; II: Acidobacteria; III: Planctomycetes; IV: OD1; V: Chloroflexi; VI: Gemmatimonadetes; VII: Zygomycota.

Fig. 4. Prokaryotic and fungal OTUs predominant in the heavy ^{13}C-DNA fraction for all incubation times (day 2, 6 and 21), stages of soil development (supraglacial sediments, barren soils and vegetated soils) and C sources (*Chlorella*, *Penicillium* and *Festuca*).

Among the prokaryotic OTUs, only those that drastically changed in relative abundances between heavy ^{13}C- and light ^{12}C-DNA fractions (log$_2$-fold change>3) are displayed while all fungal OTUs that changed between these fractions are shown (log$_2$-fold change>0). The taxonomic affiliation at the phylum and the lowest successfully classified level (g__: genus; f__: family; o__: order; c__: class; p__: phylum) are given for each OTU. For clarity, the points represents the mean relative abundance of each OTU while the standard error of each mean value (n=3) are not displayed but are available in Tables S5 and S6 in the Supplementary Information.

Supplementary Information

Supplementary information are available in the on-line version of this article on the journal web-site page.

DNA sequences: raw prokaryotic (16S$_{V3-4}$) and fungal (ITS2) ribosomal sequences provided as separate paired fastq files (forward and reverse reads) and uploaded as publicly available SRA (ENA: PRJEB10588).

SupplementaryResults.doc: Overall description of the prokaryotic and fungal community datasets.

Table S1: Results of the post-hoc Tukey HSD tests conducted on CO_2 emission rates (CO_2) and δ ^{13}C signatures (δ ^{13}C) measured at different incubation times (Time) in distinct stages of soil development (SSD) after addition of C sources. Different letters indicate significant differences among factor levels. The results of the main tests are reported in Table 1 and the mean values are reported in Fig. 1.

Table S2: Interaction terms from tests investigating changes in prokaryotic and fungal β–diversity as a function of incubation time (Time), stages of soil development (SSD), C source (C) and DNA fraction (Fraction). The tests for the main factors are reported in Table 3.

Table S3: Effects of incubation time (Time), stages of soil development (SSD), C source (C) and DNA fraction (Fraction) on the relative abundances of the dominant (>1% total dataset) prokaryotic and fungal taxa (phyla and proteobacteria classes).

Table S4: Pairwise comparisons among prokaryotic and fungal C utilizer populations (heavy ^{13}C-DNA fraction) present in distinct stages of soil development (SSD) at three times of incubation (Time) after addition of C sources. To analyze differences in community structure among C utilizers populations among SSD, we report comparisons among SSD supplied with the same C source.

Table S5: Prokaryotic OTUs predominant in ^{13}C-DNA at three incubation times in distinct stages of soil development (SSD) after addition of C sources. Taxonomic paths, mean relative abundances in both ^{12}C- and ^{13}C-DNA fractions, and associated standard errors of the mean (n=3), log$_2$-fold change values, p-values and adjusted p-values are given for each OTU.

Table S6: Fungal OTUs predominant in ^{13}C-DNA at three incubation times in distinct stages of soil development (SSD) after addition of C sources. Taxonomic paths, mean relative abundances in both ^{12}C- and ^{13}C-DNA fractions and associated standard errors of the mean (n=3), log$_2$-fold change values, p-values and adjusted p-values are given for each OTU.

Fig. S1: Characteristic profiles of the relative abundance of DNA concentrations in the different fractions retrieved along the CsCl buoyant density gradient after ultracentrifugation. Samples amended with ^{12}C-labeled material (a) were used to identify "light" and "heavy" DNA fractions along the density gradient and to confirm that the ultracentrifugation process was not affected by the different C sources added. Fourteen DNA fractions were recovered and were distributed along a buoyant density gradient ranging from approximately 1.67 to 1.76 g mL^{-1} CsCl. The grey areas show fractions of

DNA selected to represent the microbial communities enriched in ^{12}C while the red areas show fractions of DNAs selected to represent the microbial communities enriched in ^{13}C (b).

Fig. S2. Changes in relative abundances of the dominant (>1% of the total dataset) prokaryotic phyla and proteobacterial classes (a) and fungal phyla (b) for different incubation times and distinct stages of soil development (SSD) amended with different C sources. Relative abundance of each taxon is given for both light ^{12}C- and heavy ^{13}C-DNA fractions as well as for native communities (no C). The dominant taxa are represented with different colors and phyla constituing less than 1% of the total dataset are grouped as "Others".

Fig. S3: Relative abundances of prokaryotic (a) and fungal (b) OTUs, represented as dots, in heavy ^{13}C-DNA fractions (y-axis) in comparison to light ^{12}C-DNA fractions (x-axis). Note that the axes have different scales. The values of the $\log_2$-fold change are mapped for each OTU in red or blue according to the continuous scale given in the figure legend. The dashed red line represents a 1:1 ratio of relative abundances based on sequence numbers between heavy ^{13}C-DNA fractions and light ^{12}C-DNA fractions.

Table 1. Characteristics of the stages of soil development at the beginning of the incubation. The values represent means ± SE (n=3).

Parameters[1]	Supraglacial	Barren	Vegetated	F-values ($F_{2,6}$)[†]
Sand (%)	92.0±2.0	55.0±1.0	77.0±1.0	335.6***[‡]
Silt (%)	7.0±2.0	43.0±1.0	19.0±0.0	538.6***
Clay (%)	1.0±0.0	2.0±0.0	4.0±1.0	10.2*
Moisture (%)	24.2±4.4	29.1±3.6	36.2±18.8	0.9[n.s.]
WHC (%)	13.4±4.0	34.1±6.2	58.4±12.1	30.7**
pH_{H2O}	6.7±0.3	5.4±0.1	4.9±0.2	55.0**
TC (%)	0.1±0.1	0.2±0.1	2.1±0.8	20.3**
TN (%)	n.d.	n.d.	0.2±0.1	-
DOC ($\mu g\ g^{-1}$)	64.0±17.7	84.4±26.8	262.2±98.3	9.6*
DON ($\mu g\ g^{-1}$)	3.6±1.6	5.2±2.3	30.9±15.0	10.2*
NO_3^- ($\mu g\ g^{-1}$)	n.d.	n.d.	10.3±1.4	-
NH_4^+ ($\mu g\ g^{-1}$)	2.2±0.9	4.0±2.4	8.9±1.4	15.7*
SO_4^{2-} ($\mu g\ g^{-1}$)	1.6±0.1	2.6±0.2	6.2±2.2	9.9*
PO_4^{3-} ($\mu g\ g^{-1}$)	n.d.	n.d.	8.6±6.7	-
DNA ($ng\ g^{-1}$)	284±61	1454±300	7297±2996	12.6*

[1]Abbreviations :

Supraglacial: supraglacial sediments, Barren: barren soils, Vegetated: vegetated soils, Moisture: gravimetric soil moisture, WHC: water-holding capacity, pH_{H2O}: soil pH measured in water, TC: total carbon, DOC: dissolved organic carbon, TN: total nitrogen, DON: dissolved organic nitrogen, NO_3^-: nitrate, NH_4^+: ammonium, SO_4^{2-}: sulfate, PO_4^{3-}: phosphate, DNA: DNA concentrations.

n.d.: not detected

[†]: F-ratios were conducted as main test of significance where the nominator and denominator degrees of freedom are given in parentheses.

[‡]: Significance thresholds: ***: $p<0.001$, **: $p<0.01$, *:$p<0.05$, n.s.: not significant, -: no test.

Table 2. Effects of incubation time (Time), stages of soil development (SSD) and C sources (C; *Chlorella*, *Penicillium* or *Festuca*) on CO_2 emission rates (CO_2) and their $\delta^{13}C$ signatures ($\delta^{13}C$).

Factors[†]	CO_2	$\delta^{13}C$
	F-values	
Time ($F_{2,72}$)	57.2***[‡]	32.2***
SSD ($F_{2,72}$)	44.5***	1.5[n.s.]
C ($F_{3,72}$)	102.9***	7.7***
Time x Soil ($F_{4,72}$)	1.2[n.s.]	4.0*
Time x C ($F_{6,72}$)	9.0***	5.5**
Soil x C ($F_{6,72}$)	0.5[n.s.]	1.7[n.s.]
Time x Soil x C ($F_{12,72}$)	1.1[n.s.]	3.3**

[†]: Statistical tests of significance were based on F-ratios. The indices indicate the degrees of freedom and error terms.

[‡]: Significance thresholds: ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, n.s.: not-significant.

Table 3. Effects of incubation time (Time), stages of soil development (SSD), C sources (C; *Chlorella*, *Penicillium* or *Festuca*) and DNA fraction (light and heavy) on prokaryotic and fungal β-diversity. For clarity, we only show F-values for the individual factors while F-values for interaction terms between factors are given in Table S2 (Supplementary Information).

Factors[1]	β-diversity			
Prokaryotes	PERMANOVA Pseudo-F	Variability (%)	ANOSIM R[††]	Dispersion F- value
Time (F[†]$_{2,4}$)	4.8***‡	2.2	0.03**	0.5[n.s.]
SSD ($F_{2,12}$)	46.6***	21.7	0.50***	5.6**.
C ($F_{3,54}$)	9.1***	6.4	0.08***	8.1***
Fraction[2] ($F_{1,54}$)	47.9***	11.2	0.19***	9.9**
Fungi				
Time (F[†]$_{2,4}$)	2.7***	1.6	0.01[n.s.]	0.1[n.s.]
SSD ($F_{2,12}$)	48.4***	29.0	0.78***	7.9***
C ($F_{3,54}$)	5.4***	4.8	0.12***	6.6***
Fraction[2] ($F_{1,54}$)	10.5***	3.2	0.05***	1.8[n.s.]

[1]Abbreviations:

PERMANOVA: permutational multivariate ANOVA; Variability: variability explained by each factor; ANOSIM: analysis of similarity; Dispersion: homogeneity of within-treatment variation.

[2]: The factor Fraction represents the differences between communities of light ^{12}C- and heavy ^{13}C-DNA fractions recovered after ultracentrifugation.

[†]: Statistical tests of significance were based on F-ratios. The indices indicate the degrees of freedom and error terms.

[††]: Statistic R values were investigated to assess compositional differences.

[‡]: Significance thresholds: ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, n.s.: not-significant.

16

17

Bacteria
(144 OTUs | 44.6%)

Firmicutes
(1 OTU | 0.1%)

Armatimonadetes
(2 OTUs | 0.2%)

Verrucomicrobia
(2 OTUs | 0.4%)

Gammaproteobacteria
(19 OTUs | 10.6%)

Actinobacteria
(14 OTUs | 4.4%)

Cyanobacteria
(1 OTU | <0.1%)

Betaproteobacteria
(58 OTUs | 20.4%)

Thermi
(3 OTUs | 0.1%)

Alphaproteobacteria
(24 OTUs | 6.0%)

Deltaproteobacteria
(3 OTUs | 0.3%)

Bacteroidetes
(17 OTUs | 1.8%)

Archaea
(0 OTU | 0%)

Fungi
(15 OTUs | 9.6%)

Ascomycota
(14 OTUs | 9.5%)

Basidiomycota
(1 OTU | 0.1%)

OTUs in light ^{12}C-DNA fractions:

OTUs in heavy ^{13}C-DNA fractions:

SSD
△ Supraglacial
○ Barren
□ Vegetated
◇ mixed

C source
● Chlorella
● Penicillium
● Festuca
● mixed

20

21

46

Taxonomy

Prokaryotes	p__Actinobacteria	g__Cellulomonas	OTU_38
	p__Actinobacteria	g__Cryobacterium	OTU_5694
	p__Actinobacteria	f__Microbacteriaceae	OTU_3527
	p__Actinobacteria	f__Microbacteriaceae	OTU_924
	p__Actinobacteria	g__Arthrobacter	OTU_19
	p__Actinobacteria	g__Kitasatospora	OTU_13
	p__Bacteroidetes	f__Chitinophagaceae	OTU_7867
	p__Bacteroidetes	g__Dyadobacter	OTU_626
	p__Bacteroidetes	g__Flectobacillus	OTU_110
	p__Bacteroidetes	o__Sphingobacteriales	OTU_3836
	p__Proteobacteria	g__Pleomorphomonas	OTU_2564
	p__Proteobacteria	g__Sphingomonas	OTU_86
	p__Proteobacteria	g__Hydrogenophaga	OTU_49
	p__Proteobacteria	g__Hydrogenophaga	OTU_757
	p__Proteobacteria	f__Comamonadaceae	OTU_5000
	p__Proteobacteria	f__Comamonadaceae	OTU_6655
	p__Proteobacteria	f__Comamonadaceae	OTU_779
	p__Proteobacteria	g__Janthinobacterium	OTU_420
	p__Proteobacteria	g__Janthinobacterium	OTU_5702
	p__Proteobacteria	g__Massilia	OTU_6469
	p__Proteobacteria	f__Oxalobacteraceae	OTU_4139
	p__Proteobacteria	o__Burkholderiales	OTU_4014
	p__Proteobacteria	o__Burkholderiales	OTU_5741
	p__Proteobacteria	o__Myxococcales	OTU_1135
	p__Proteobacteria	o__Myxococcales	OTU_991
	p__Proteobacteria	g__Pseudomonas	OTU_2948
	p__Proteobacteria	g__Stenotrophomonas	OTU_8615
	p__Proteobacteria	f__Xanthomonadaceae	OTU_8787
	p__Verrucomicrobia	f__Methylacidiphilaceae	OTU_5266
	p__Verrucomicrobia	g__Opitutus	OTU_64
Fungi	p__Ascomycota	g__Fontanospora	OTU_4
	p__Ascomycota	g__Curvularia	OTU_482
	p__Ascomycota	o__Chaetothyriales	OTU_75
	p__Ascomycota	g__Tetracladium	OTU_3421
	p__Ascomycota	g__Lecythophora	OTU_20
	p__Ascomycota	g__Lecythophora	OTU_8
	p__Ascomycota	g__Trichoderma	OTU_108
	p__Ascomycota	g__Acremonium	OTU_289
	p__Ascomycota	g__Neonectria	OTU_81
	p__Ascomycota	f__Nectriaceae	OTU_133
	p__Ascomycota	g__Cephalotrichum	OTU_192
	p__Ascomycota		OTU_447
	p__Ascomycota		OTU_844
	p__Ascomycota		OTU_3119
	p__Basidiomycota	g__Malassezia	OTU_417

Relative abundance (%)

22

47