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

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23 **Abstract**

24 Microbial communities and soil carbon (C) have been shown to vary in response to
25 increasing vegetation cover during soil development after deglaciation. However, little is
26 known about the ability of microorganisms to utilize various C sources in glacier forefield
27 soils. We supplied ecologically relevant ¹³C-labeled C sources (*Chlorella*, *Penicillium* and
28 *Festuca*) to three distinct environments (supraglacial sediments, barren soils and
29 vegetated soils) of the Damma glacier area to monitor ¹³CO₂ production. We identified
30 prokaryotic and fungal populations able to utilize these sources by using DNA-stable
31 isotope probing coupled with Illumina MiSeq sequencing of ribosomal markers. A high
32 initial ¹³CO₂ pulse indicated that ¹³C-labeled microbial and plant material were consumed.
33 The ¹³C-enriched DNA results indicated that betaproteobacterial taxa affiliated to the
34 families *Oxalobacteraceae* and *Comamonadaceae* were important players in C utilization
35 from different sources and present in all environments. In contrast, different fungal taxa
36 played different roles in C degradation depending on the soil environment. Overall, our
37 findings reveal that C utilization is driven by similar prokaryotic populations along a glacier
38 forefield, while the distribution of active fungal populations are more influenced by
39 environmental factors.

40 **Keywords**

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

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49 **Introduction**

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

55 Several studies have focused on soil C dynamics under climate change (Sistla et
56 al., 2013; van Groenigen et al., 2014), but the importance of the different sources of C to C
57 dynamics during soil development in glacial forefields still lacks quantification. Recently
58 deglaciated soils depleted in C receive organic C from autochthonous microbial production
59 (Freeman et al., 2009), from allochthonous sources (wind-deposited pollen and fungal
60 spores as well as plant and faunal debris) (Ohtonen et al., 1999; Hodkinson et al., 2003)
61 and from ancient ice-trapped organic matter released with glacier melting (Bardgett et al.,
62 2007). Microbial necromass also accumulates as a non-negligible C pool in initial soils
63 (Schurig et al., 2013) and can be utilized by other microorganisms (Zumsteg et al., 2013b).
64 After plant establishment, soil C stocks increase substantially (Egli et al., 2010; Kabala and
65 Zapart, 2012; Smittenberg et al., 2012) and C quality changes (Dümig et al., 2011) with the
66 accumulation of organic C from plant litter leading to higher C fluxes (Guelland et al.,
67 2013). Such changes in resource quantity and quality are known to influence microbial
68 communities in soils (Goldfarb et al., 2011). Despite the close relationship between
69 microorganisms and the soil C cycle (Schimel and Schaeffer, 2012), only a few studies
70 have investigated the utilization of different C sources by microbial communities thriving in
71 distinct stages of soil development (SSD) of a glacier forefield. Some pioneering work
72 along this line has been done in the Damma glacier forefield. Esperschütz et al. (2011)
73 incubated ¹³C-labeled plant litter in different SSD in the Damma glacier forefield and

74 observed only slight changes in ^{13}C -enriched phospholipid fatty acid (PLFA) patterns,
75 suggesting that similar microbial communities are actively involved in the C dynamics
76 throughout the forefield. However, the phylogenetic resolution of PLFA is not sufficient to
77 identify the main players in the utilization of C sources (Dumont and Murrell, 2005). By
78 using a DNA-stable isotope probing (SIP) approach, Zumsteg et al. (2013b) observed that
79 algal and fungal cell fragments were crucial C sources in C-depleted barren soils of the
80 Damma glacier forefield. These studies yielded novel information about C assimilation in
81 developing soils, but the methods used were limited in phylogenetic resolution and
82 breadth, and therefore they could not identify bacterial and fungal taxa utilizing C from
83 different organic sources during soil development.

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

99 incorporation of isotopes into microbial DNA used for isopycnic fractionation. Our study
100 specifically addressed the following questions. 1) Does CO₂ released during incubation
101 and its $\delta^{13}\text{C}$ signature vary among the different C sources supplied and in the distinct SSD
102 investigated? 2) What are the main prokaryotic and fungal taxa that utilize the different C
103 sources in pristine glacier forefield environments? 3) Do prokaryotic and fungal C utilizers
104 differ among distinct SSD?

105

106 **Experimental procedure**

107 **Site description**

108 Soil samples (surface soils down to 2 cm depth) from three different stages of soil
109 development (referred to hereafter as SSD: supraglacial sediments, barren soils and
110 vegetated soils) were collected in the Damma glacier area in July during the growing
111 season. The Damma glacier area is located in the Swiss Central Alps and has a typical
112 alpine climate with large seasonal temperature fluctuations (from -2°C to 8°C, on yearly
113 average) as well as a high precipitation regime (2300 mm per year). The Damma glacier
114 forefield has been described extensively in previous studies characterizing the dynamics of
115 this ecosystem (Bernasconi et al., 2011; 2008; Brunner et al., 2011; Göransson et al.,
116 2011; Zumsteg et al., 2012; Frey et al., 2013), which provides a wealthy database of
117 information that can be compared with this study. The different SSD used in this study
118 were selected to represent different soil ecosystems typical of the Damma glacier area.
119 Supraglacial sediments were selected to represent the initial SSD in the Damma glacier
120 area while barren and vegetated soils were investigated because they represent two
121 distinct stages of the soil chronosequence along the Damma glacier forefield. The different
122 SSD differ in vegetation and in microbial community compositions (Rime et al., 2015). Soil
123 texture was determined with the hydrometer technique and gravimetric soil moisture was

124 calculated by measuring differences in weight after oven-drying at 105°C overnight. Dried
125 soils were used to measure water-holding capacity and 2 g of homogenized dried soil were
126 milled to measure total C and N contents with a CN analyzer (Shimadzu, Tokyo, Japan).
127 Dried soil was extracted with milliQ water (1:10 m/v) with an overhead shaker overnight to
128 measure soil pH with a FEP20-FiveEasy pH meter (Mettler-Toledo GmbH, Greifensee,
129 Switzerland), dissolved organic C and N with a TOC-v analyzer (Shimadzu), nitrate, sulfate
130 and phosphate concentrations by ion chromatography with an IC:DX-120 chromatograph
131 (Dionex Corp., Sunnyvale, CA, USA) and ammonium concentration with an FIAS 300
132 (Perkin-Elmer, Waltham, MA, USA), as previously described (Rime et al., 2015).

133

134 **Experimental design**

135 We conducted a stable isotope probing (SIP) microcosm experiment under controlled
136 conditions (temperature: 10°C, no light) for 21 days. The microcosms consisted of
137 approximately 10 g dry soil equivalent previously homogenized through a 2 mm sieve and
138 placed in 100 mL polypropylene pots (Sarstedt AG & Co., Nümbrecht, Germany). Samples
139 were previously equilibrated at 10°C for three days which represents the average
140 temperature in the Damma glacier forefield (Zumsteg et al., 2013a). Samples of each SSD
141 received either no additional C (no C, control treatment) or were supplied with 20 mg of
142 three distinct C sources separately, each of which was labeled with either ¹²C- or ¹³C
143 (*Chlorella* sp. cells, *Penicillium* sp. mycelium, or *Festuca* sp. leaves; referred hereafter
144 simply as *Chlorella*, *Penicillium* and *Festuca*). These sources of C were selected because
145 these organisms had previously been isolated in the Damma glacier area (*Chlorella*:
146 identified by Frey et al. (2010) and experimentally used by Zumsteg et al. (2013b);
147 *Penicillium*: identified by Brunner et al. (2011) and experimentally used by Zumsteg et al.
148 (2013b); *Festuca*: Bernasconi et al. (2011)). The added material was homogenized through

149 a mill with a sieve size of 250 μm prior to mixing with the soils to enable homogenous
150 mixing and to obtain organic fragments with a particle size greater than 250 μm . Such a
151 particle size ensures that most of the cells were still intact and represents realistic
152 fragments found in these soils (Schurig et al., 2013) and utilized by microorganisms after
153 the initial degradation by macro- and microfauna. ^{13}C -labeled *Chlorella* and *Festuca* were
154 produced by IsoLife bv (% ^{13}C atom > 97%, Wageningen, The Netherlands) while ^{13}C -
155 labeled *Penicillium* was produced in-house with ^{13}C -glucose (% ^{13}C atom > 99%,
156 Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) according to the method
157 described by Zumsteg et al. (2013b). Since ^{13}C -labeled *Penicillium* material was only
158 produced with uniformly and highly labeled ^{13}C -glucose, we assume that this C source was
159 highly labeled with ^{13}C (% ^{13}C atom > 90%). The soils incubated were, on the other hand,
160 characterized by negative $\delta^{13}\text{C}$ values (-22.5 ‰). The C and N contents of the C sources
161 were measured with a CN analyzer. C contents of the added C sources were similar (48%)
162 while the N contents were higher in *Chlorella* (6.5%) than in *Penicillium* (1.9%) and
163 *Festuca* (2.0%). During the incubation, the microcosms were watered by spraying distilled
164 water every two days to compensate loss by evaporation and keep the gravimetric soil
165 moisture constant. The microcosms were destructively sampled 2, 6 and 21 days after the
166 additional C was supplied and kept frozen at -80°C until DNA extraction. Overall, we set up
167 189 microcosms (3 incubation times x 3 SSD x 7 treatments [3 C sources x 2 C isotopes +
168 1 control] x 3 replicates).

169

170 **DNA extraction, fractionation and Illumina paired-end sequencing**

171 Total DNA was extracted from approximately 1 g of soil using a bead-beating and
172 chloroform-isoamylalcohol DNA extraction method as described by Frey et al. (2006) and
173 quantified with the PicoGreen assay (Invitrogen, Carlsbad, CA, USA). DNA enriched in ^{13}C

174 (^{13}C -DNA) was retrieved according to the protocol originally described by Neufeld et al.
175 (2007b) and used by Zumsteg et al. (2013b). Briefly, approximately 5 μg DNA were
176 suspended in 4.8 mL CsCl buffer and adjusted to an optical density of 1.4029 ± 0.0002
177 with a Refracto 30PX (Mettler-Toledo GmbH, Greifensee, Switzerland), which corresponds
178 to a volumetric density of 1.723 g mL^{-1} CsCl. The samples were sealed in 1/2x2 PA tubes
179 (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer instructions and
180 ultracentrifugated (VTi-65.1 vertical rotor and optimaTM L-80 XPultracentrifuge, Beckman
181 Coulter) at $177\,000 \text{ g}$ for 40 h. Fourteen 300 μL DNA fractions per sample were collected
182 drop-wise from the bottom of the tube and their optical density was measured with a
183 Refracto 30PX to ensure formation of a density gradient. DNA of each fraction was
184 precipitated with 1.2 mL polyethylglycerol buffer (30% PEG6000 and 1.5 M NaCl, Sigma-
185 Aldrich, St-Louis, MO, USA) by centrifugation ($16\,000 \text{ g}$, 30 min), washed with 150 μL 70%
186 ethanol, eluted in 30 μL AE buffer (Qiagen GmbH, Hilden, Germany) and quantified with
187 PicoGreen. DNA fractions extracted from samples amended with ^{12}C -labeled material were
188 used to determine the location of heavy ^{13}C -DNA fractions retrieved from samples supplied
189 with ^{13}C -labeled material (Fig. S1 in the Supplementary Information) as described by Wang
190 et al. (2015). Heavy fractions with buoyant densities between 1.72 and 1.74 g mL^{-1} CsCl
191 representing the heavy ^{13}C -DNA and light fractions with buoyant densities between 1.68
192 and 1.69 g mL^{-1} CsCl representing the light ^{12}C -DNA were pooled and quantified with
193 PicoGreen.

194 Amplicon production was conducted according to methods described in previous studies
195 (Hartmann et al., 2015; Rime et al., 2015). Briefly, the fraction pools were diluted to 2 ng
196 μL^{-1} and pretreated with $1 \mu\text{g BSA mL}^{-1}$ at 95°C for 5 min to inactivate PCR-inhibiting
197 substances. Target-specific PCR were done in triplicates to amplify the hypervariable V3-
198 V4 region of the prokaryotic (archaeal and bacterial) ribosomal small subunit (16S rRNA)

199 and the ITS2 region of the fungal internal transcribed spacer (ITS). Each PCR was done
200 on ¹²C- and ¹³C-DNA pools, as well as on DNA from control samples (no C), with a 10 ng
201 DNA template in a final volume of 50 µL. The V3-V4 region of the prokaryotic 16S rRNA
202 gene was amplified with the newly modified primers 341Fh (CCTAYGGGDBGCWSCAG)
203 and 806Rh (GGACTACNVGGGTHTCTAAT) (Frey et al. 2016), while the ITS2 region of the
204 eukaryotic ribosomal operon was amplified with degenerate versions of the ITS3
205 (CAHCGATGAAGAACGYRG) and ITS4 (TCCTSCGCTTATTGATATGC) primers recently
206 published by Tedersoo et al. (2014). An in-silico evaluation of the primer pairs revealed a
207 similar coverage for bacteria, archaea, and fungi (around 90%). The 5' ends of the primers
208 were tagged with the CS1 (forward primers) and CS2 (reverse primers) adapters required
209 for multiplexing samples using the Fluidigm Access Array™ System (Fluidigm, South San
210 Francisco, CA, USA). The PCR conditions to amplify the prokaryotic 16S fragments and
211 eukaryotic ITS2 fragments consisted of an initial denaturation at 95°C for 15 min, 35 cycles
212 of denaturation at 94°C for 40 s, an annealing at 58°C for 40 s and an elongation at 72°C
213 for 1 min followed by a final elongation at 72°C for 10 min. Amplicons were sent to the
214 Génome Québec Innovation Center at McGill University (Montreal, Canada) for barcoding
215 using the Fluidigm Access Array™ technology (Fluidigm, South San Francisco, CA, USA)
216 and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego,
217 CA, USA). Raw sequences are available as publicly accessible SRA (ENA:
218 PRJEB10588).

219

220 **Processing of Illumina paired-end reads**

221 Forward and reverse reads were merged with PEAR v.0.9.5 (Zhang et al., 2014) and poor-
222 quality or unassembled reads were removed based on default parameters. Merged reads
223 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.

247

248 **CO₂ and δ¹³C measurements**

249 In parallel with the SIP approach, 10 g dry-equivalent soil microcosms were set up in
250 triplicates at the same temperature with the same C source:soil ratio previously described.
251 These microcosms were further used to calculate CO₂ emission rates and measure $\delta^{13}\text{C}$
252 signatures of CO₂ emitted after C addition. Because the isotope ratio mass spectrometer
253 (IRMS; Delta V Advantage, Thermo Fischer Scientific) used to detect $\delta^{13}\text{C}$ signatures of
254 CO₂ emitted was not equipped for measuring high percentage of ¹³C atoms, we diluted the
255 added ¹³C-labeled material with ¹²C-labeled material (ratio approximately 1:1000) before
256 homogenizing the C sources with the soils to ensure that the added C sources were used
257 by soil microorganisms and that the emitted CO₂ did not result from the decomposition of
258 the native soil organic matter (priming effect). The microcosms were placed in custom-
259 made gas-tight 1L jars equipped with a septum (Hartmann et al., 2010) 1, 3, 6, 9, 13 and
260 21 days after C addition. Headspace air was sampled 0, 12 and 24 hours after jar closure
261 with a 20 mL syringe and injected into pre-evacuated 12 mL Exetainer vials (Labco
262 Limited, Lampeter, United Kingdom). The CO₂ concentrations and $\delta^{13}\text{C}$ were measured
263 with a gas chromatograph (Trace GC Ultra, Thermo Fischer Scientific, Waltham, MA, USA)
264 coupled with an IRMS. CO₂ emission rates were calculated by fitting linear regressions
265 through the CO₂ concentrations over headspace sampling time. The goodness of the fit
266 (r^2) was calculated for each rate and was very good in general ($r^2 > 0.95$). Based on CO₂
267 emission rates, we estimated the amount of C emitted throughout the incubation time as a
268 proportion of the amount of added C sources. Delta ¹³C values were expressed as per mil
269 (‰) in relation to the Vienna-Pee Dee Belemnite gauged reference materials.

270

271 **Statistical analyses**

272 Differences in soil parameters, in CO₂ emission rates and in $\delta^{13}\text{C}$ signatures were
273 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, CO₂ emission rates and $\delta^{13}\text{C}$ 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 ¹³C-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 ¹³C-DNA fractions, we investigated log₂-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.

CO₂ emission and $\delta^{13}\text{C}$ signature

CO₂ emission rates and their $\delta^{13}\text{C}$ 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 CO₂ emission rates occurred three days after addition of *Chlorella*. In contrast, CO₂ emission rates in

324 vegetated soils were highest one day after addition of *Chlorella* and *Festuca*, remained
325 constant after three days of incubation and then decreased over time (Fig. 1a). At the end
326 of the incubation period (21 days), CO₂ emission rates remained higher in barren and
327 vegetated soils amended with C ($P < 0.05$) while the rates were similar between C amended
328 supraglacial sediments and controls ($P > 0.05$). We integrated the CO₂ emission in the
329 experiments over the period of incubation to estimate the amount of C emitted from each
330 treatment and each SSD. We found that much of the C added to the vegetated soils was
331 respired (*Chlorella*: 70.2% of the added C, *Penicillium*: 52.6%, *Festuca*: 48.6%) while less
332 C was emitted from barren soils (*Chlorella*: 54.5%, *Penicillium*: 34.5%, *Festuca*: 37.1%)
333 and supraglacial sediments (*Chlorella*: 47.3%, *Penicillium*: 22.4%, *Festuca*: 26.6%).
334 However, the fact that all SSD amended with C still showed positive $\delta^{13}\text{C}$ values at the end
335 of the incubation period (21 days) indicates that not all material had been consumed (Fig.
336 1b).

337

338 **Density fractionation and recovery of microbial communities in ¹²C-light and ¹³C-** 339 **DNA heavy fractions**

340 We recovered 14 DNA fractions distributed along a buoyant density gradient ranging from
341 approximately 1.67 to 1.76 g mL⁻¹ CsCl after ultracentrifugation (Fig. S1). Based on CsCl
342 buoyant gradients obtained with DNA extracted from ¹²C-amended soils, we determined
343 three “light” ¹²C-DNA fractions (buoyant density: 1.678-1.689 g mL⁻¹ CsCl) as well as three
344 “heavy” ¹³C-DNA fractions (buoyant density: 1.718-1.736 g mL⁻¹ CsCl). The densities of
345 heavy fractions were chosen where only small amount of DNA was detected in CsCl
346 gradients obtained from ¹²C-amended soils and control soils (no C) to ensure that DNA
347 recovered in heavy fractions from samples amended with ¹³C-labeled material resulted
348 from isotopic enrichment and not from higher GC content (Wang et al., 2015). Using these

349 fractions, we successfully amplified and sequenced prokaryotic 16S_{V3-V4} and eukaryotic
350 ITS2 ribosomal target segments. After quality filtering, we obtained 4 210 852 (22 280 ±
351 5576 per sample) and 4 070 897 (21 560 ± 12 078) merged 16S_{V3-V4} and ITS2 reads,
352 respectively, which corresponded to 8666 (1002 ± 719) prokaryotic and 3489 (183 ± 70)
353 eukaryotic OTUs. Among the eukaryotic OTUs, we excluded those not classified as fungi
354 (893 OTUs of the total OTU table, 3% of the total sequence dataset) to focus on fungal
355 communities. A detailed description of the prokaryotic and fungal communities is given in
356 SupplementaryResults.doc (Supplementary Information).

357

358 **Effects of C sources on microbial community structures**

359 Both prokaryotic and fungal communities differed in β-diversity among SSD after addition
360 of C sources. Structural patterns were also distinct between prokaryotic and fungal C
361 utilizers. Prokaryotic and fungal communities structurally differed among C sources,
362 incubation time, SSD, and DNA fractions as revealed by PERMANOVA and ANOSIM
363 (Table 3 and Table S2) and displayed by PCO ordinations (Fig. 2). The PCOs that
364 displayed differences in prokaryotic community structures showed that, at two and six days
365 after C addition, the samples were clearly discriminated between light and heavy DNA
366 fractions and among SSD. Further, the native microbial communities (no C) of each SSD
367 clustered with their respective communities in light ¹²C-DNA fractions with the exception of
368 the native community of supraglacial sediments. At day 21, the prokaryotic communities
369 still differed among SSD although the difference between communities in light and heavy
370 DNA fractions became smaller. Conversely, the PCOs for fungal communities did not show
371 a clear separation between communities in light and heavy DNA fractions but revealed
372 clear differences among communities in the distinct SSD at all sampling times. The
373 structural differences in prokaryotic communities were partly due to within-treatment

374 dispersion among C sources, SSD and DNA fractions, while differences in fungal
375 community structure were partly due to heterogeneity among C sources and SSD (Table
376 3). The amount of variability in prokaryotic and fungal communities was higher for SSD
377 (Variability>21%) than for C sources (Variability>6%) and incubation time (Variability<3%)
378 while the variability for DNA fractions were higher for the prokaryotic communities
379 (Variability: 11%) than for the fungal communities (Variability: 3%). The relative
380 abundances of the prokaryotic and fungal taxa in heavy ¹³C-DNA fractions also differed
381 from those in native communities and in light ¹²C-DNA fractions and varied among C
382 sources and with time (Fig. S2 and Table S3). Additionally, pairwise comparisons of
383 prokaryotic and fungal populations involved in the utilization of a specific C source showed
384 that the prokaryotic populations were generally similar in barren and vegetated soils but
385 differed from those in supraglacial sediments while fungal C utilizer populations mainly
386 differed among all SSD (Table S4).

387

388 **Identification of OTUs present in heavy ¹³C-DNA fractions**

389 Our OTU-specific analyses were restricted to OTUs found in distinct SSD supplied with
390 different C sources. We did not focus on changes with incubation time since most of the
391 structural variability within prokaryotic and fungal communities occurred among SSD and
392 C sources (Table 3). We investigated significant (adjusted P<0.05) log₂-fold changes in
393 OTU counts between light ¹²C- and heavy ¹³C-DNA fractions in samples supplied with ¹³C-
394 material at all sampling times to identify prokaryotic and fungal OTUs that were prominent
395 in heavy ¹³C-DNA fractions (Fig. S3). Overall, the microbial populations involved in C
396 utilization (based on their presence in the heavy ¹³C-DNA fraction) were dominated by
397 representatives affiliated to *Actinobacteria*, *Bacteroidetes*, *Alpha*-, *Beta*-,
398 *Gammaproteobacteria* and *Ascomycota* (Figs. 3 and 4).

399 We identified 144 prokaryotic and 15 fungal OTUs significantly (adjusted $P < 0.05$)
400 enriched in heavy ^{13}C -DNA, representing 44.6% and 9.6% of the total prokaryotic and
401 fungal communities, respectively (Fig. 3; Tables S5 and S6). The majority of the OTUs
402 (82%) affiliated to *Bacteroidetes* were predominant in SSD supplied with *Chlorella* while no
403 clear association with a particular C source was observed in the dominant proteobacterial
404 classes and in the phyla *Actinobacteria* and *Ascomycota* (Fig. 3). For example, most of the
405 dominant betaproteobacterial OTUs in the heavy ^{13}C -DNA fraction were found in more
406 than one SSD independently from the C source supplied. The investigation of prokaryotic
407 OTUs with $\log_2$ -fold change values greater than three (i.e. representing an eight-fold
408 increase in OTU abundance in the heavy ^{13}C -DNA fraction relative to the light ^{12}C -DNA
409 fraction) revealed that many OTUs were affiliated to different orders (*Sphingobacteriales*,
410 *Burkholderiales* and *Myxococcales*) or families (*Microbacteriaceae*, *Chitinophagaceae*,
411 *Comamonadaceae*, *Oxalobacteraceae* and *Xanthomonadaceae*; *Methylococcaceae*)
412 (Fig. 4). The prokaryotic OTUs classified at the genus level with $\log_2$ -fold change values
413 greater than three consisted of diverse *Actinobacteria* (*Arthrobacter*, *Cellulomonas*,
414 *Cryobacterium* and *Kitasatospora*), *Bacteroidetes* (*Dyadobacter* and *Flectobacillus*),
415 *Proteobacteria* (*Hydrogenophaga*, *Janthinobacterium*, *Massilia*, *Pleomorphomonas*,
416 *Pseudomonas* and *Stenotrophomonas*) and *Verrucomicrobia* (*Opitutus*). Among the fungal
417 communities, particular OTUs affiliated to the ascomycete genera *Fontanospora*,
418 *Lecythophora*, *Trichoderma* and *Acremonium* showed a strong increase in OTU
419 abundance in the heavy ^{13}C -DNA fraction relative to the light ^{12}C -DNA fraction (Fig. 4).
420 Conversely, only one predominant basidiomycete OTU (genus *Malassezia*) in the heavy
421 ^{13}C -DNA fraction responded significantly to any of the C sources (Figs. 3 and 4).

422

423 Discussion

424 **Impact of different C sources and SSD on CO₂ emission and microbial community** 425 **structures**

426 Patterns of CO₂ emission rates in nutrient-poor supraglacial sediments and barren soils
427 differed from those found in vegetated soils after addition of the three different C sources
428 (Figure 1), agreeing with results from Eichorst and Kuske (2012) who reported variable C
429 utilization of ¹³C-cellulose in various soil types. The high CO₂ emission rates and the highly
430 positive δ¹³C signatures recorded after addition of ¹³C-labeled substrates indicate that the
431 emitted CO₂ mainly originated from the utilization of the supplied organic material (e.g.
432 labile polysaccharides) by fast-growing microorganisms (Kindler et al., 2009) rather than
433 from the decomposition of the native soil organic matter (i.e. priming effect), corroborating
434 the findings reported by Guelland et al. (2013). Despite the clear differences in CO₂
435 emission rates observed among SSD and C sources supplied, it remains unclear whether
436 these differences were due to changes in microbial communities, differences in edaphic
437 parameters, or both. For example, the differences in CO₂ emission patterns among C
438 sources and SSD may be due to differences in macro- and micronutrient contents in the
439 supplied C sources and the investigated SSD as reported by Göransson et al. (2011).

440 Microorganisms in vegetated soils utilized and respired the added C sources more
441 rapidly most probably due to lower nutrient limitation and higher initial microbial biomass
442 indicated by the high DNA concentrations extracted from those samples (Table 1).
443 Conversely, in supraglacial sediments and barren soils, microorganisms also responded to
444 the added C sources but with a delay (three days) in order to mobilize nutrients
445 progressively from the supplied material. CO₂ emission rates after addition of *Penicillium*
446 were lower than those measured after addition of *Chlorella* or *Festuca* in all SSD, which
447 might be due to differences in the chemical composition of the C sources (*Penicillium*:
448 chitin (Rizza and Kornfeld, 1969); *Chlorella*: hemicellulose and glycoprotein (Northcote et

449 al., 1958); *Festuca*: lignocellulose (Gunnarsson et al., 2008)) and the higher recalcitrance
450 nature of chitin (Trofymow et al., 1983), the main constitutive compound of the fungal cell
451 wall (Rizza and Kornfeld, 1969), relative to that of cellulose (Baldrian et al., 2010, 2011).
452 Moreover, microorganisms have a lower potential to degrade chitin in bulk soils (low
453 activity of microbial chitinase producers) than those in the rhizosphere forefield soils (Töwe
454 et al., 2010), suggesting that chitin can be less efficiently degraded in bulk soils as used in
455 this incubation experiment. We therefore assume that not only C and nutrient contents but
456 also the chemical structure of the supplied organic material influenced microbial C
457 utilization.

458 Among microbial C utilizers, we identified specific OTUs predominantly occurring in
459 the heavy ^{13}C -DNA fractions by evaluating differential changes in OTU counts among C
460 sources, SSD and DNA fractions (Love et al., 2014; McMurdie and Holmes, 2014; Pepe-
461 Ranney et al., 2015). Distributions of abundant prokaryotic phyla in the light and heavy
462 DNA fractions resembled each other 21 days after addition of the ^{13}C -labeled C sources
463 (Figure S2) which suggest that the ^{13}C -assimilating microbial communities are dominated
464 by an opportunistic subset of the total microbial community as pointed out by others
465 (Deslippe et al., 2015; Schmidt et al., 2015). We have previously used longer incubation
466 times (up to 60 days) in SIP experiments (Zumsteg et al. 2013b) and it was not possible to
467 make clear separation between “heavy” and the “light” fractions 22 and 60 days after the
468 addition of the labeled substrate. Shorter incubations times (up to 8 days) limit the
469 movement of label beyond primary carbon consumers via biomass turnover (Zumsteg et
470 al., 2013b; Leung et al, 2016).

471 In our study, both prokaryotic and fungal communities were significantly influenced
472 by C sources and predominant OTUs in the heavy ^{13}C -DNA fractions were mainly present
473 in more than one SSD and associated with more than one C source (Tables S5 and S6).

474 However, prokaryotic populations involved in the utilization of particular C sources (defined
475 by their presence in ^{13}C -DNA fraction) resembled each other irrespective of SSD while
476 fungal populations in ^{13}C -DNA fractions differed among SSD (Figure 2). We assume that
477 fungal populations able to utilize distinct C sources in soils of the Damma glacier are more
478 influenced by soil environmental parameters than the prokaryotic C utilizers. Brown and
479 Jumpponen (2014) reported that a high proportion of fungal OTUs were less randomly
480 distributed than bacterial OTUs in soil chronosequences along a glacier forefield,
481 suggesting that fungi have more specific habitat requirements in comparison to bacteria. In
482 addition, Zumsteg et al. (2011) used a reciprocal transplant experiment to show that the
483 fungal communities in the barren soils of the Damma glacier forefield were more
484 influenced by changes in soil temperature and moisture than bacterial communities.

485

486 **Taxa-specific associations with C sources and SSD: do soils in glacial**
487 **forefields harbor generalists or specialists?**

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

498 Microbial groups that play an important role in decomposition of complex organic

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

512 Specially, we found OTUs related to the proteobacterial genus *Janthinobacterium*
513 that were specifically involved in C utilization in supraglacial sediments and barren soils
514 but not in vegetated soils. OTUs belonging to this genus were also involved in degrading
515 organic matter in soils of the McMurdo Dry Valley (Antarctica) (Schwartz et al., 2014) and
516 in weathering rock minerals in barren soils of the Damma glacier forefield (Frey et al.,
517 2010). Given the crucial roles in C utilization and nutrient dynamics in initial nutrient-
518 depleted soils, OTUs related to this genus might be key microorganisms in recently
519 deglaciated soils.

520 Previous DNA-SIP studies also identified that members of the
521 Gammaproteobacteria were associated with cellulose degradation in soil environments
522 (Bernard et al., 2007; Eichorst and Kuske, 2012). In our study, *Pseudomonas* spp. within
523 the Gammaproteobacteria detected in the heavy fraction of the ^{13}C treatments was the

524 main C utilizer. Some bacterial OTUs from the *Myxococcales* (*Deltaproteobacteria*) also
525 appeared to assimilate the various C sources, which is in agreement with previous studies
526 that members of this order are involved in lignocellulose degradation (Darjany et al., 2014)

527 In contrast to the association patterns observed between Proteobacteria and
528 multiple C sources, OTUs belonging to the phylum *Bacteroidetes* (e.g. the family
529 *Chitinophagaceae* and the genera *Flectobacillus* and *Dyadobacter*) were specifically
530 associated with C from *Chlorella* in the different SSD. This phylum largely consists of
531 copiotrophs able to use various organic compounds (Fierer et al., 2007). In addition, OTUs
532 related to the genera *Flectobacillus* and *Dyadobacter* are known to dwell in soils and to
533 degrade organic compounds such as starch (Krieg et al., 2010; Lee et al., 2010). Previous
534 studies reported an increase in relative abundance of *Bacteroidetes* related to increasing
535 soil N (Nemergut et al., 2008; Zhang et al., 2013; Koyama et al., 2014). Among the C
536 sources used in our experiments, *Chlorella* had substantially lower C:N ratio compared to
537 the other sources and the additional N supply provided by the *Chlorella* treatment may
538 have favored representatives of *Bacteroidetes*. The presence of OTUs from the
539 *Chitinophagaceae* in the heavy ¹³C-DNA fraction across all time points in this study was
540 interesting because of their potential ability to decompose chitin, a fungal cell wall
541 constituent. *Chitinophaga* spp. has been identified as decomposers of dried rice callus (Li
542 et al., 2011), indicating that their proliferation responds to easily decomposable plant
543 constituents. From these findings we suggest that prokaryotic C utilizers mainly consist of
544 generalist copiotrophs (*Bacteroidetes* and *Proteobacteria*) able to degrade rapidly labile C
545 sources originating from glacial environments.

546 The addition of the different C sources also favored the development of particular
547 populations related to Actinobacteria. Several OTUs associated with *Kitasatospora*,
548 *Arthrobacter* and *Cellulomonas* being the most abundant genera could be characterized as

549 consumers of the added C sources. *Arthrobacter* has been reported as potential primary
550 utilizer of easily available C (Semenov et al., 2012). Reguera and Leschine (2001)
551 reported that isolates affiliated to the actinobacterial genus *Cellulomonas* can produce
552 both cellulase and chitinase, representing a competitive advantage for acquiring C in
553 natural habitats. Members of this genus were reported to be active in degrading cellulose
554 and cellobiose in soils (Haichar et al., 2007; Cañizares et al., 2011; Schellenberger et al.,
555 2010), which indicates that bacteria affiliated to this genus might be crucial in litter
556 degradation. *Kitasatospora* were highly enriched in the heavy fractions of ^{13}C -labelled
557 sources in developing soils, which has not been documented before. *Kitasatospora* are
558 known to be cellulolytic bacteria (Ulrich et al., 2008). This finding provides impetus for
559 further studies of *Kitasatospora* to determine its ability to use easily available or recalcitrant
560 C sources in soils.

561 *Opitutus* (Verrucomicrobia) were enriched in the heavy fractions of the ^{13}C
562 treatments, but its role in C assimilation in developing soils is yet unknown. Only one
563 species of this genus has been described, i.e. *Opitutus terrae*, an obligate anaerobe
564 isolated from a rice paddy soil (Chin et al., 2001). *Opitutus* spp. has previously been
565 described as key C assimilator after the incorporation of fresh potato tissue in mature soils
566 (Semenov et al., 2012). So far, no other study has revealed *Opitutus* spp. as an important
567 C utilizer in developing soils, but they appear to be key organisms in glacial soils as they
568 were abundant in the heavy fractions of the ^{13}C treatments.

569 Despite the crucial role of fungi in soil decomposition processes (Zifcakova and
570 Baldrian, 2012; Van der Wal et al., 2013), the identity of the main fungal C utilizers has
571 remained elusive in alpine glacial forefields. With the evaluation of differential OTU counts,
572 we found that fungal OTUs able to utilize the different C sources among the SSD
573 investigated consisted of *Ascomycota*, which is in agreement with previous SIP-DNA

574 studies showing that cellulolytic and hemicellulolytic fungal OTUs largely consist of
575 *Ascomycota* (Štursová et al., 2012; Leung et al., 2016). This is the first report of the genera
576 *Fontanospora* and *Lecythophora* involved in the assimilation of C sources in soils.
577 Representatives of the genus *Fontanospora* are abundant in barren soils of the Damma
578 glacier forefield (Rime et al., 2015, 2016), whereas representatives of the genus
579 *Lecythophora* were reported to be active in the degradation of wooden structures in
580 Antarctica (Held et al., 2006). Both genera were highly enriched in the heavy ^{13}C -DNA
581 fractions after the addition with *Festuca* and might therefore represent important fungal
582 decomposers of plant debris in glacial soils. The vegetated soils investigated in our study
583 were originally covered by herbaceous plants (e.g. *Festuca rubra*) and woody shrubs (e.g.
584 *Salix sp.*) (Bernasconi et al., 2011).

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

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

597 Although the relative abundance of *Basidiomycota* was relatively high in native
598 communities in the different SSD investigated (~20% on average), it decreased drastically

599 in soils supplied with additional C sources (Fig. S2b), indicating that most of the
600 *Basidiomycota* present in distinct soils of the Damma glacier area were not significantly
601 contributing to C utilization. We assume that basidiomycetous fungi are outcompeted by
602 saprotrophs when a source of organic material is supplied to glacial soils, although
603 cellulolytic and hemicellulolytic capabilities are widespread in basidiomycetous yeasts
604 (primarily *Cryptococcus*) (Štursová et al., 2012; Leung et al., 2016).

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

617 It is important to note that SIP experiments might suffer from drawbacks related to
618 cross-feeding (i.e. the incorporation of ^{13}C into DNA of secondary consumers or microbial
619 commensals) (Neufeld et al., 2007a; Bell et al., 2011; Štursová et al., 2012). Therefore, it
620 cannot be excluded that the detected active microorganisms are able to assimilate C by
621 grazing upon C utilizing metabolic products of other organisms that hydrolyzed ^{13}C -
622 labelled substrates in the soils. For example, some fungi might have incorporated ^{13}C by
623 bacterivory on cellulose degrading bacteria, suggesting that fungi contribute to the

624 biopolymer degrading food webs in soil by top-down control of the bacterial community
625 (Jimenez et al., 2014). Its impact on soil food webs and the distinction between direct
626 degradation of ^{13}C -labelled substrates, degradation of their hydrolysis products, or grazing-
627 mediated incorporation of ^{13}C warrants further studies.

628

629 **Conclusions**

630 Our study reveals that both prokaryotic and fungal communities were significantly
631 influenced by the different C sources. The addition of different C sources did not exert
632 uniform responses within closely related members (e.g. within a phylum) but led to very
633 specific compositional shifts at lower taxonomic levels indicating that the ^{13}C -assimilating
634 microbial communities are dominated by an opportunistic subset of the total microbial
635 community. Prokaryotic C utilizers mainly consist of generalist copiotrophs able to degrade
636 rapidly labile C sources originating from *Chlorella* and *Festuca*. The degradation of labile
637 sources of C is the main driver of high CO_2 emissions from these soils. The generalist
638 prokaryotic C utilizers identified consist of weathering-active Betaproteobacteria known to
639 be able to solubilize minerals from granite (e.g. *Comamonadaceae*, *Oxalobacteraceae* and
640 *Janthinobacterium*) commonly found in all SSD. Microorganisms with this particular
641 physiological ability might have an advantage in C utilization since they can obtain
642 nutrients directly from minerals. In contrast, despite the crucial role of fungi in soil
643 decomposition processes, the identity of the main fungal C utilizers has remained elusive
644 in alpine glacial forefields. Fungal OTUs able to utilize the different C sources consisted of
645 *Ascomycota* either saprophytic or parasitic fungi. These fungi can actively utilize C from
646 plant residues, such as *Festuca*, which represents an advantage for establishment in
647 vegetated soils recently formed after glacier retreat. However, it cannot be excluded that
648 the detected active fungi are able to assimilate C by grazing upon C utilizing metabolic

649 products of bacteria that hydrolyzed ^{13}C -labelled substrates in the soils. This study
650 demonstrates that glacial forefields contain microbial communities that are active, versatile
651 and highly adapted to extreme oligotrophic conditions. Many of the active organisms
652 described in this study have an important role in weathering triggering the necessary
653 conditions for soil formation and establishment of higher plants.

654

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955

956 **Table and Figure legends**

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

959

960 **Table 2.** Effects of incubation time (Time), stages of soil development (SSD) and C
961 sources (C; *Chlorella*, *Penicillium* or *Festuca*) on CO₂ emission rates (CO₂) and their $\delta^{13}\text{C}$
962 signatures ($\delta^{13}\text{C}$).

963

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

969

970 **Fig. 1.** Effects of different C sources (*Chlorella*, *Penicillium* and *Festuca*) on CO₂ emission
971 rates (a) and their $\delta^{13}\text{C}$ signatures (b) for different incubation times and in different stages
972 of soil development (SSD) (supraglacial sediments, barren soils and vegetated soils;
973 separated horizontally). Controls (no C) and the different C sources are represented with
974 different colors as described in the figure. Mean CO₂ emission rates and $\delta^{13}\text{C}$ signatures $\pm$
975 1 SE (n=3) are displayed for all incubation times, SSD and C sources except for the
976 vegetated soils that received no C one day after incubation start, where n=2.

977

978 **Fig. 2.** Effects of C sources (*Chlorella*, *Penicillium* and *Festuca*) on prokaryotic (a) and
979 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.

985

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.

1003

Fig. 4. Prokaryotic and fungal OTUs predominant in the heavy ^{13}C -DNA fraction for all

1005 incubation times (day 2, 6 and 21), stages of soil development (supraglacial sediments,
1006 barren soils and vegetated soils) and C sources (*Chlorella*, *Penicillium* and *Festuca*).
1007 Among the prokaryotic OTUs, only those that drastically changed in relative abundances
1008 between heavy ^{13}C - and light ^{12}C -DNA fractions ($\log_2$ -fold change >3) are displayed while
1009 all fungal OTUs that changed between these fractions are shown ($\log_2$ -fold change >0).
1010 The taxonomic affiliation at the phylum and the lowest successfully classified level (g__:
1011 genus; f__: family; o__: order; c__: class; p__: phylum) are given for each OTU. For clarity,
1012 the points represents the mean relative abundance of each OTU while the standard error
1013 of each mean value (n=3) are not displayed but are available in Tables S5 and S6 in the
1014 Supplementary Information.

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

Parameters ¹	Supraglacial	Barren	Vegetated	F-values (F _{2,6}) [†]
Sand (%)	92.0 $\pm$ 2.0	55.0 $\pm$ 1.0	77.0 $\pm$ 1.0	335.6*** [‡]
Silt (%)	7.0 $\pm$ 2.0	43.0 $\pm$ 1.0	19.0 $\pm$ 0.0	538.6***
Clay (%)	1.0 $\pm$ 0.0	2.0 $\pm$ 0.0	4.0 $\pm$ 1.0	10.2*
Moisture (%)	24.2 $\pm$ 4.4	29.1 $\pm$ 3.6	36.2 $\pm$ 18.8	0.9 ^{n.s.}
WHC (%)	13.4 $\pm$ 4.0	34.1 $\pm$ 6.2	58.4 $\pm$ 12.1	30.7**
pH _{H2O}	6.7 $\pm$ 0.3	5.4 $\pm$ 0.1	4.9 $\pm$ 0.2	55.0**
TC (%)	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	2.1 $\pm$ 0.8	20.3**
TN (%)	n.d.	n.d.	0.2 $\pm$ 0.1	-
DOC ($\mu\text{g g}^{-1}$)	64.0 $\pm$ 17.7	84.4 $\pm$ 26.8	262.2 $\pm$ 98.3	9.6*
DON ($\mu\text{g g}^{-1}$)	3.6 $\pm$ 1.6	5.2 $\pm$ 2.3	30.9 $\pm$ 15.0	10.2*
NO ₃ ⁻ ($\mu\text{g g}^{-1}$)	n.d.	n.d.	10.3 $\pm$ 1.4	-
NH ₄ ⁺ ($\mu\text{g g}^{-1}$)	2.2 $\pm$ 0.9	4.0 $\pm$ 2.4	8.9 $\pm$ 1.4	15.7*
SO ₄ ²⁻ ($\mu\text{g g}^{-1}$)	1.6 $\pm$ 0.1	2.6 $\pm$ 0.2	6.2 $\pm$ 2.2	9.9*
PO ₄ ³⁻ ($\mu\text{g g}^{-1}$)	n.d.	n.d.	8.6 $\pm$ 6.7	-
DNA (ng g ⁻¹)	284 $\pm$ 61	1454 $\pm$ 300	7297 $\pm$ 2996	12.6*

¹Abbreviations :

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, NO₃⁻: nitrate, NH₄⁺: ammonium, SO₄²⁻: sulfate, PO₄³⁻: 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 CO₂ emission rates (CO₂) and their $\delta^{13}\text{C}$ signatures ($\delta^{13}\text{C}$).

Factors [†]	CO ₂	$\delta^{13}\text{C}$
	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			
	PERMANOVA	Variability	ANOSIM	Dispersion
	Pseudo-F	(%)	R ^{††}	F- value
<i>Prokaryotes</i>				
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**
<i>Fungi</i>				
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.}

[†]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 ¹²C- and heavy ¹³C-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.

Figure 2 **SSD**

△ Supraglacial ○ Barren □ Vegetated

C source

◆ No C ◆ Chlorella ◆ Penicillium ◆ Festuca

(a)

Day 2

Prokaryotes

Day 6

Day 21

(b)

Fungi

Figure 3

Taxonomy

Prokaryotes

p__Actinobacteria	g__Cellulomonas
p__Actinobacteria	g__Cryobacterium
p__Actinobacteria	f__Microbacteriaceae
p__Actinobacteria	f__Microbacteriaceae
p__Actinobacteria	g__Arthrobacter
p__Actinobacteria	g__Kitasatospora
p__Bacteroidetes	f__Chitinophagaceae
p__Bacteroidetes	g__Dyadobacter
p__Bacteroidetes	g__Flectobacillus
p__Bacteroidetes	o__Sphingobacteriales
p__Proteobacteria	g__Pleomorphomonas
p__Proteobacteria	g__Sphingomonas
p__Proteobacteria	g__Hydrogenophaga
p__Proteobacteria	g__Hydrogenophaga
p__Proteobacteria	f__Comamonadaceae
p__Proteobacteria	f__Comamonadaceae
p__Proteobacteria	f__Comamonadaceae
p__Proteobacteria	g__Janthinobacterium
p__Proteobacteria	g__Janthinobacterium
p__Proteobacteria	g__Massilia
p__Proteobacteria	f__Oxalobacteraceae
p__Proteobacteria	o__Burkholderiales
p__Proteobacteria	o__Burkholderiales
p__Proteobacteria	o__Myxococcales
p__Proteobacteria	o__Myxococcales
p__Proteobacteria	g__Pseudomonas
p__Proteobacteria	g__Stenotrophomonas
p__Proteobacteria	f__Xanthomonadaceae
p__Verrucomicrobia	f__Methylacidiphilaceae
p__Verrucomicrobia	g__Opitutus

Fungi

p__Ascomycota	g__Fontanospora
p__Ascomycota	g__Curvularia
p__Ascomycota	o__Chaetothyriales
p__Ascomycota	g__Tetracladium
p__Ascomycota	g__Lecythophora
p__Ascomycota	g__Lecythophora
p__Ascomycota	g__Trichoderma
p__Ascomycota	g__Acremonium
p__Ascomycota	g__Neonectria
p__Ascomycota	f__Nectriaceae
p__Ascomycota	g__Cephalotrichum
p__Ascomycota	
p__Ascomycota	
p__Ascomycota	
p__Ascomycota	
p__Basidiomycota	g__Malassezia

OTU_38
OTU_5694
OTU_3527
OTU_924
OTU_19
OTU_13
OTU_7867
OTU_626
OTU_110
OTU_3836
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OTU_86
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OTU_5000
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OTU_8
OTU_108
OTU_289
OTU_81
OTU_133
OTU_192
OTU_447
OTU_844
OTU_3119
OTU_417