Soil Biology & Biochemistry 98 (2016) 30–41

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

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

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ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form
17 March 2016
Accepted 22 March 2016
Available online 9 April 2016

Keywords:
Illumina MiSeq sequencing
DNA-stable isotope probing
Glacier forefield
Microbiota

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.

1. 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

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http://dx.doi.org/10.1016/j.soilbio.2016.03.012
0038-0717/

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 δ^{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?

2. Experimental procedure

2.1. 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; 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).

2.2. 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 et 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).

2.3. 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