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Microbial food web dynamics along a soil chronosequence of a glacier forefield

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Abstract

Microbial food webs are critical for efficient nutrient turnover providing the basis for functional and stable ecosystems. However, the successional development of such microbial food webs and their role in "young" ecosystems is unclear. Due to a continuous glacier retreat since the middle of the 19th century, glacier forefields have expanded offering an excellent opportunity to study food web development at differently developed soils. In the present study, litter degradation and the corresponding C fluxes into microbial communities were investigated along the forefield of the Damma glacier (Switzerland). ^{13}C-enriched litter of the pioneering plant *Leucanthemopsis alpina* (L.) Heywood was incorporated into the soil at sites that have been free from ice for approximately 10, 60, 100 and more than 700 years. The structure and function of microbial communities were identified by ^{13}C analysis of phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL). Results showed increasing microbial diversity and biomass, and enhanced proliferation of bacterial groups as ecosystem development progressed. Initially, litter decomposition proceeded faster at the more developed sites, but at the end of the experiment loss of litter mass was similar at all sites, once the more easily-degradable litter fraction was processed. As a result incorporation of ^{13}C into microbial biomass was more evident during the first weeks of litter decomposition. ^{13}C enrichments of both PLEL and PUFA biomarkers following litter incorporation were observed at all sites, suggesting similar microbial foodwebs at all stages of soil development. Nonetheless, the contribution of bacteria and actinomycetes to litter turnover became more pronounced as soil age increased in detriment of archaea, fungi and protozoa, more prominent in recently deglaciated terrain.

1 Introduction

Glaciers are retreating in many mountainous areas of the world due to global warming (Oerlemans, 2005) leaving behind new terrestrial habitats that are colonized by

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organisms (Bardgett et al., 2007). This is usually a gradual process that results in a chronosequence. The latter can be defined as a set of soils that share a common community of organisms, climate, parent material and slope, but differ in regard with the length of time that materials have been exposed to weathering and soil formation. As glaciers recede, parent materials become exposed and physical and biogeochemical weathering convert primary minerals to secondary minerals releasing plant nutrient elements in soluble forms. Initially, the newly exposed surface typically has a high proportion of rocks, lacks vegetation, is sandy and contains few nutrients (Schütte et al., 2009). As substrate colonization by plants and microorganisms occurs, organic material starts to accumulate that can be further transformed; as a result complex food webs develop and stratification into horizons of increasing physical and chemical complexity becomes more prominent (Chapin et al., 1994; Bardgett et al., 2007). These features make chronosequences ideal to study the initial and successional stages of soil and ecosystem development as well as the role of plant-microbe interactions at different time points of soil evolution.

Various studies in alpine regions have recently provided novel insights into microbial community dynamics and their importance in nutrient cycling in these environments. For instance, extensive colonization of granite surfaces could play a crucial role in initial soil formation and mineral dissolution (Frey et al., 2010), but also wind-driven transport of organic material (insects, grasses, seeds) has to be considered (Jumpponen et al., 1999). Although the first phase of soil development is dominated by geological and chemical processes, also biology plays a major role. For example autotrophic fixation of C is also known to be an important process during the early stages of soil formation providing, in turn, nutrient sources for heterotrophic microorganisms (Walker and del Moral, 2003). Diazotrophic communities have been shown to be highly diverse following glacier retreat and could have a major role in N cycling in these ecosystems (Duc et al., 2009). Heterotrophic communities may also be highly abundant at initial soil stages (Tscherko et al., 2003), for instance, in response to ancient C released during glacier retreat. Microbial communities in glacier forefields have also been shown to

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respond to changes in ecosystem development (e.g. C and N content) and vegetation (Tscherko et al., 2005; Miniaci et al., 2007).

Pioneer plants can strongly alter parent materials and stimulate microbe-plant interactions through root morphology, rhizodeposition and litter production (Bardgett et al., 1999; Bardgett and Walker, 2004). The plant residues are transformed by microorganisms into humus and other new organic substances enhancing the capacity of the soil to hold water and nutrients. This positive feedback accelerates the development of more complex plant communities and microbial food web structures (Edwards et al., 2006). Rhizodeposits of pioneering plants such as *Leucanthemopsis alpina* (L.) Heywood (*L. alpina*) have been shown to cause significant shifts in the microbial community structure of rhizosphere soil compared to bulk soil at glacier forefields (Edwards et al., 2006). Due to their simple molecular structure root exudates are readily assimilable by single microbes (Baudoin et al., 2003). By contrast, the transformation of litter requires different specialized microorganisms acting as functional units to degrade substances like lignin or cellulose (Dilly et al., 2004; Fioretto et al., 2005). Although microbial interactions during litter degradation might be critical for ecosystem development in such scenarios (Bardgett and Walker, 2004), little is known about microbial dynamics of this process and the main players involved.

To improve our understanding of litter degradation and the role of microbial communities during the initial stages of soil and ecosystem formation, a field incubation experiment using ^{13}C labelled plant litter was carried out in a chronosequence in the Damma Glacier forefield (Switzerland). Plant litter of *L. alpina* was incubated into soil at different locations in the forefield of the Damma Glacier, because *L. alpina* was previously described as a pioneering plant in this area (Edwards et al., 2006; Miniaci et al., 2007). Microbes involved in litter degradation were identified by following the ^{13}C label into phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL) (Gattinger et al., 2003; White et al., 1979; Zelles, 1999). We hypothesized a different and more complex microbial community structure as soil development progresses. In addition, we expected litter degradation rates to increase accordingly along the chronosequence

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as a result of better developed microbial foodwebs. To test this, litter degradation and incorporation of ^{13}C-labelled litter products by microbial communities were studied at four different locations (10, 60, 100 and 700 years old) in bulk and litter amended soil.

2 Material and methods

2.1 Production of labelled plant litter

L. alpina was sowed in plastic pans filled with prick out substrate in a greenhouse approach. Seeds were watered daily and incubated for 8–10 days at +20/+15 °C (day/night) and 75–85% relative humidity until germination. Subsequently, plants were transferred into plastic pots (4 plants per pot) of 12 cm in diameter, filled with a mixture of potting soil, expanded clay and silica sand (1:1:2, v/v/v). The pots were placed in a transparent EFTE-plastic tent (ethylene-tetrafluorethylene, film thickness 80 µm, Koch Membranen GmbH, 83253 Germany; volume ~7000 L) and subjected to a photoperiodic day length of 14 h (additional light by sodium vapour discharge lamps, SONT Agro 400, Philips, NL). Temperature was kept around +25/+18 °C (day/night) and relative humidity between 75–85%. Irrigation was performed daily via irrigation tubes (500 mL/24 h/12 pots). Twice a week 500 mL of Hoagland based fertilizer was added after irrigation (Hoagland, 1920). The ^{13}C labelling was performed according to Esperschütz et al. (2009a). In brief, CO_2 concentration in the tent atmosphere was reduced by plant photosynthesis during the daytime. When the CO_2 concentration in the tent dropped below 350 µmol mol^{1}, enriched $^{13}CO_2$ (δ^{13}C = +170‰ vs. Vienna-Pee Dee Belemnite, Air Liquide, Düsseldorf, Germany) was added to the tent atmosphere, until CO_2 concentration reached 400 µmol mol^{1}. Using this experimental setup, an enriched ^{13}C atmosphere of +90‰ to +110‰ V-PDB was established inside the tent. At night time the tent atmosphere was pumped through vials containing soda lime (sodium and calcium hydroxide) using a membrane pump (N 0135.3 AN.18, KNF Neuberger, Freiburg, Germany) with a flow of 200 L min^{-1} to reduce the CO_2 produced by the plant

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dark respiration. The CO_2 depleted air was pumped into the closed tent again. The system capacity ensured a stable CO_2-concentration of 350 µmol mol^{-1} over night. The CO_2 concentration of the inner and outer tent atmosphere (day/night) was measured continuously with a photo acoustic CO_2-controller (7MB1300, Siemens, Germany, calibration at 400 to 600 µmol mol^{-1} ± 2%). The labelled plants were harvested before flowering after a total growth time of 10 weeks. Leaves and stems were oven-dried (60 °C), homogenized and shredded (0.5–2.0 cm). The obtained labelled plant litter of *L. alpina* (δ^{13}C = 88.4 ± 1.6‰ vs. VPDB) was used in the following experiment.

2.2 Experimental setup

The experiment was carried out in the forefield of the Damma glacier, around 2100 m a.s.l. It is located in the canton Uri in Switzerland (N 46° 38.177′ E 008° 27.677′) facing north/east. The climate of the area is characterized by a short vegetation period (mid of June to end of September) and approximately 2400 mm of rainfall per year. It has a total length of 2.35 km and a total area of 5.09 km^2 (measured in 1973). Since the beginning of measurements in 1921, the Damma glacier has retreated at an average rate of 11 m per year. A detailed site description and site investigation has been done by the BigLink project (http://www.cces.ethz.ch/projects/clench/BigLink). Based on this systematic measurements as well as the Swiss Glacier monitoring network (http://glaciology.ethz.ch/messnetz/ glaciers/damma.html), three locations in the forefield of known exposure time since glacier retreat were chosen: (a) the first location near the glacier tongue (N 46° 38.177′ E 008° 27.677′) with an exposure time of approximately 10 years (T1), (b) a second location (T2) below the 1992 moraine free of ice for nearly 60 years (N 46° 38.177′ E 008° 27.677′), and (c) a third location near the 1928 moraine (N 46° 38.177′ E 008° 27.677′) estimated to be ice free for around 100 years (T3). Outside the forefield, a fourth location (T4) (N 46° 38.177′ E008° 27.677′) free from ice for at least 700 years, served as reference site (Egli et al., 2001). According to Hämmerli et al. (2007), the bedrock material is Aargranite and surface conditions

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vary between fine fluvial sands and coarse sandy areas. The soil in the forefield can be classified as poorly developed regosols, with 76.4 to 83.7% of sand, 10.8 to 15.7% of silt and 5.5 to 7.9% of clay (Lazzaro et al., 2009; Töwe et al., 2010). Soil pH ranged from 4.3 to 5.6; the lowest value was detected at the reference site T4. Increasing concentrations of total C and total N from 0.11% to 6.50% and from 0.004% to 0.442%, respectively, were observed as ecosystem development progressed. The soil $\delta^{13}C$ value was on average −27‰ vs. V-PDB at all locations (Table 1).

Following snow melt in the beginning of June 2008, tubes made of aluminium (20 cm in diameter and 14 cm high) were installed 10 cm deep into the soil and replicated 5 times at every location (T1–T4). Every replicate was composed of three independent tubes: two for soil analysis and one for litterbag study. In the tubes for soil analysis, plant litter (5 g oven-dried) was applied directly below the vegetation cover (2–5 cm depth). For the litter bag experiment, two litter bags (mesh-size 40 µm) with 2.5 g of oven-dried plant material per bag were placed at a similar depth as with the direct litter application. Every tube was protected with steel grids (mesh size 5 mm) against disturbances.

Eight and 12 weeks after litter application one litter bag and one of the tubes containing directly applicated litter was harvested per location and replicate. Sampled litter bags were immediately placed on ice for transportation and kept frozen until further analysis. The tubes were completely removed from the soil, sealed and transported to the lab for further analysis. Control soil was sampled next to the tubes at similar soil depths at the beginning and at the end of the experiments. The soil was immediately sieved at the forefield and stored on ice for transportation.

2.3 Soil and litter bag analysis

Litter degradation rates were calculated based on the loss of litter material in the litter bags during incubation after drying at 60 °C. Soil samples from the tubes were taken from 2–5 cm depth and remaining litter material was carefully removed. An aliquot of the soil samples was dried at 60 °C whereas the rest was stored at −20 °C for

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phospholipid analysis. The dried soil and plant litter material were ball-milled and subsequently analysed for total C, ^{13}C and N content using an elemental analyzer (Eurovector, Milan, Italy) coupled with an isotope ratio mass spectrometer (MAT 253, Thermo Electron, Bremen, Germany). Soil pH was analysed in 0.01 M $CaCl_2$ extracts (DIN ISO 10390).

2.4 Phospholipid analyses

Phospholipid fatty acid (PLFA) and phospholipid ether lipid (PLEL) analyses were performed based on Zelles et al. (1995) and Gattinger et al. (2003). An aliquot of 50 g soil (dry weight) was extracted with 250 mL of methanol, 125 mL of chloroform and 50 mL of phosphate buffer (0.05 M, pH 7). After 2 h of horizontal shaking, 125 mL of water and 125 mL of chloroform were added to promote phase separation. After 24 h the water phase was removed and discarded. The total lipid extract was separated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI 2 g/12 mL; Bond Elut, Analytical Chem International, CA, USA). An aliquot of the phospholipids equivalent to 25 g (dry weight) was used for PLEL extraction as described by Gattinger et al. (2003). The remaining 25 g of the phospholipid extract was further separated into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (see Zelles et al., 1995 for details) to facilitate the identification of fatty acids as well as to obtain a good baseline separation of peaks for isotopic calculations.

Prior to measurements, an internal standard (nonadecanoic acid methyl ester respectively myristic acid methyl ester) was added to calculate absolute amounts of fatty acids. PLFA were analyzed as fatty acid methyl esters (FAME) on a gas chromatograph/mass spectrometry system (5973MSD GC/MS Agilent Technologies, Palo Alto, USA). FAMEs were separated on a polar column (BPX70, SGE GmbH, Griesheim, Germany), 60 ×0.25 mm × 0.25 µm, coated with 70% of cyanopropyl polysilphenylene-siloxane (see Esperschütz et al., 2009b for details). PLEL were measured using an non-polar column (HP 5 capillary column, 50 m length, 0.2 mm internal diameter; coated with a cross-linked 5% phenylmethyl rubber phase with a film thickness

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of 0.3 µm; see Gattinger et al., 2003 for details). The mass spectra of the individual FAME were identified by comparison with established fatty acid libraries (Solvit, CH 6500 – Luzern, Switzerland) using MSD Chemstation (Version D.02.00.237).

The actual δ^{13}C ratio of the individual FAME was corrected for the one C atom that was added during derivatisation (Abrajano et al., 1994). The "isoprenoid-20" PLEL (phytane, i20) was used for (ubiquitous) archaeal abundance (Gattinger et al., 2003). For illustration and data analysis, lipids were summarized in groups according to their chemical character (see Fig. 2 for details). PLFA analysis of *L. alpina* plant litter was performed at the beginning of the experiment as described above. PLFA a16:0, i18:0, 18:1ω11, 18:2ω6,9 and 18:3 were detected in significant amounts. Since PLFA 18:3 was not detected in any soil sample, removal of litter residues prior to extraction was successful and hence all PLFA were taken into account for statistical analysis and interpretation of data.

2.5 Calculations

Stable isotope results were expressed in δ^{13}C or atom percent (AP) according to Eqs. (1) and (2):

$$\delta^{13}\mathrm{C} = [(R_{\mathrm{sample}}/R_{\mathrm{V\text{-}PDB}}) - 1] \cdot 1000 \quad (1)$$

$$^{13}\mathrm{C}_{\mathrm{AP}} = (100 \cdot R_{\mathrm{V\text{-}PDB}} \cdot (\delta^{13}\mathrm{C}/1000 + 1)/(1 + R_{\mathrm{V\text{-}PDB}} \cdot (\delta^{13}\mathrm{C}/1000 + 1)) \quad (2)$$

R_{Sample} and $R_{\mathrm{V\text{-}PDB}}$ represent the ^{13}C to ^{12}C ratios of sample and international standard Vienna-Pee Dee Belemnite (VPDB = 0.0111802), respectively. The relative amount of litter-incorporated ^{13}C (%$^{13}\mathrm{C}_{\mathrm{LITTER}}$) into the total lipid fraction was calculated according to Eq. (3),

$$\%^{13}\mathrm{C}_{\mathrm{LITTER}} = C_{Tx} \cdot (^{13}\mathrm{C}_{Tx} - ^{13}\mathrm{C}_{T0}) \cdot 100/^{13}\mathrm{C}_{\mathrm{added}} \quad (3)$$

where the concentration of the individual C-fraction [ng g^{-1} DW] at timepoint *Tx* was multiplied by its ^{13}C enrichment in atom percent excess (difference between ^{13}C at

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timepoint *Tx* and ^{13}C enrichment at control) and expressed relatively to the amount of added ^{13}C [ng g^{1} DW]. The relative ^{13}C-distribution within total measured phospholipids ($\%^{13}C_{PLDIST}$) was calculated as follows:

$$\%^{13}C_{PLDIST} = \%^{13}C_{LITTER}\ i \cdot 100 / \sum \%^{13}C_{LITTER}\ i \quad (4)$$

where $\%^{13}C_{LITTER}\ i$ represents the relative amount of added ^{13}C in an individual phospholipid *i*, as calculated after Eq. (3). Shannon Diversity (H_k) and Evenness (E_k) were calculated according to Eqs. (5) and (6):

$$H_k = -\sum_{i=1}^{k} p_i \ln p_i \quad (5)$$

$$E_k = \frac{H_k}{\ln k} \quad (6)$$

where *k* represents the total number of fatty acids and p_i the relative abundance of each species (Hill, 1973).

2.6 Statistical analysis

Univariate analysis of variance was carried out using SPSS 11.0, with location (T1–T4) and harvesting treatment (control, 8 weeks, 12 weeks) as independent variables. Two-way analysis of variance (ANOVA) was performed to establish significant interactions between location and treatment. Significant differences for specific variables were identified using Duncan's post-hoc test at $p < 0.05$ following one-way ANOVA. Principal component analysis (PCA) was performed on the variance-covariance matrix utilizing the statistical software *R*. Data for PCA were calculated as means ($n = 5$) of each location (T1–T4) and time point (control, 8 weeks, 12 weeks). Data illustration was performed with Adobe Illustrator CS3 and S-PLUS 8.1.

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3 Results

3.1 Microbial plant litter degradation

After 8 weeks significant plant litter degradation ($p < 0.05$) was observed at all sites T1–T4 (Table 2). Nearly 55% of the applied plant litter was lost at T1, whereas approximately 65% of the litter material was degraded at the other sites. After 12 weeks no further degradation was observed at T2, T3 and T4. At T1 an extra 10% mass loss was detected ($p < 0.05$), resulting in a similar amount of degraded residues at all investigated sites at the end of the experiment. Contamination of the total litter mass loss with soil material was excluded, since the total mass loss correlated with the mass loss of C (linear regression, $R^2 = 0.959$, data not shown). Compared to C the amounts of N degraded during the incubation period were relatively low. As a result, lower C/N ratios were found at the end of the experiment ($p < 0.05$). The label in the plant litter ranged between +81‰ and +89‰ $\delta^{13}C$ (vs V-PDB) and did not significantly change during the incubation period, indicating that the litter material used in the present experimental setup was homogeneously labelled.

The total soil phospholipid (PL) content (nmol g^{-1} DW) increased as ecosystem development progressed, whereas significantly higher values ($p < 0.05$) were detected at the reference site T4 compared to all other sites ($p < 0.05$; Fig. 1a). Due to litter application, the PL content increased irrespective of the site location T1–T4. In general, no differences in PL content were observed between 8 and 12 weeks, except at the reference site T4. In order to compare the amount of ^{13}C incorporated into total PL across different locations in the forefield, the percentage of added ^{13}C that was incorporated into total PL ($\%^{13}C_{LITTER}$) was calculated relative to the total amount of ^{13}C added. Similar to the total PL content, an increasing incorporation of ^{13}C derived from plant litter into total PL was detected at T4 compared to the other sites (Fig. 1b). Between 8 and 12 weeks a slight decrease in ^{13}C values in total PL was observed at T2 and T3, whilst a 50% reduction was recorded at T4 (Fig. 1b).

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3.2 Soil microbial community composition

Shannon diversity and evenness indices were calculated according to Eqs. (5) and (6) respectively on the basis of total PL concentrations and the number of fatty acids detected in the samples (Table 3). In general, diversity increased concomitantly from T1 to T4; however, a very low diversity was detected in the control treatment at T1. A similar trend was observed for Shannon evenness.

Figure 2 shows the ordination of the samples using PCA analysis of the various phospholipid classes. Principal components accounted for 49.5% (PC1) and 23.6% (PC2) of the total variance, respectively. The first principal component (PC1) shows some discrimination between samples from T1 and T2/T3/T4 sites. The PL biomarkers that correlated best with this axis were etherlipids (PLEL) and poly unsaturated fatty acids (PUFA) as well as 10Me, iso, ant and cy PLFA. Etherlipids and PUFA were more abundant in the samples derived from T1 and T2, whilst the opposite was observed for the other groups. The second principal component (PC2) separated samples based on the presence or absence of added litter. The variables that contributed the most to the variance explained by this axis were long-chained and saturated fatty acids (nor>20), as well as saturated branched fatty acids, where the position of branching was unknown (unb); these were less abundant in litter treatments. On the other hand, $-\omega 7$, $-\omega 9$ and $-\omega 11$ mono unsaturated PLFA were more abundant in the litter treatments. While PCA was able to differentiate between control and litter-amended samples, there was no clear separation of samples from T1 T2 and T3 within the litter-amended treatments. However, there was a good separation of both control and litter-amended samples from T4 with regard to the other developmental stages.

3.3 ^{13}C distribution within PL groups

Figure 3 shows the natural ^{13}C abundance (no litter application) of PL groups in soil at the different sites. For most groups, δ^{13}C values ranged around −25‰ at T2, T3 and T4, and showed no statistical differences at $p < 0.05$ between these sites. T1

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showed higher variability of δ^{13}C natural abundance with average values ranging between −1‰ and −20‰ (vs. V-PDB). In general, δ^{13}C natural abundance in PL groups significantly differed between soil samples from T1 and the other sites, but similar values were observed for the PUFA and PLEL groups.

Distribution of ^{13}C derived from plant litter (PL_{DIST}) among the individual groups was calculated according to Eq. (4). Results showed similar incorporation of plant litter ^{13}C among individual PL groups at all sites (Fig. 4). In all cases, the highest plant litter ^{13}C incorporation was reported for PUFA (50%), PLEL (20–30%) and nor< 20 saturated fatty acids (10–15%).

Litter-derived ^{13}C incorporation patterns were also assessed by means of PCA analysis (Fig. 5). Principal components accounted for 72.5% (PC1) and 12.2% (PC2) of the total variance, respectively. Results showed a clear separation between T1 and the other sites along PC1 for both harvesting time points. This component is very similar to that of the total soil microbial community profile (Fig. 2); for T1 higher incorporation of ^{13}C into the PUFA and PLEL groups and lower incorporation at all other groups compared to the other sites was observed. At the reference site T4, a large shift occurred between the first and the second harvesting time point on PC2, which was mainly due to the lack of nor> 20 and −ω5 PLFAs; hence ^{13}C incorporation into these groups after 12 weeks of litter incubation was negligible.

4 Discussion

4.1 Plant litter degradation and microbial biomass

In the present study, all experimental sites were located in a relatively small geographical region and therefore similar climatic and weathering conditions can be assumed. The litter decomposition rate during the first 8 weeks of incubation was high at all sites, and even higher at sites that had been free from ice for at least 60 years (Table 2). This might be explained by the increase in microbial biomass as succession proceeds

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(Fig. 1; Ohtonen et al., 1999). Nonetheless, after 12 weeks more than 50% of the applied plant litter was degraded at all locations along the glacier forefield in agreement with previous studies; for instance, Bradford et al. (2002) described a litter mass loss of around 40% after 5 weeks, although results may vary depending on the type of litter and the mesh size of the bag. It is also possible that the loss of litter mass was not only a result of microbial degradation. Particularly in wet environments, leaching of dissolved organic matter from litter tends to be significant (Chapin et al., 2002). Recently, Gülland and coworkers (unpublished data) found substantial leaching of litter in the Damma glacier forefield. However, the incorporation of litter had a stimulating effect on soil microbial biomass at all investigated sites suggesting that microbial activity was responsible for the observed loss of litter (Dornbush, 2007); after 8 weeks of litter incubation, microbial biomass had doubled at all sites (Fig. 1a). The incorporation of a new C and energy source into the soil, resulted in the stimulation of zymogenous organisms otherwise present in a dormant state in the soil, regardless of the time free from ice. The freshly-added litter material probably contained considerable amounts of relatively easily degradable substances (Heal et al., 1997). As these compounds are consumed, slow-growing cellulose- and lignin-decomposers start to take over and soon opportunistic organisms, that mainly degrade sugars and amino acids, begin to die of starvation (Tate, 1987; Dilly et al., 2003). This was reflected by a decrease in soil microbial biomass at all sites after 8 weeks, except T1 (Fig. 1). Results from ^{13}C incorporation into microbial biomass not only support the above-mentioned trend but also underpin that such turnover becomes faster as soil development progresses (Fig. 1b).

4.2 Soil microbial community profile

Microbial diversity and evenness were significantly lower at T1 compared to the other sites (Table 3). As soil development progresses and the parent material becomes more accessible for plant colonization, complexity increases, nutrient content improves and new potential niches and microniches become available (Loreau, 2001). This in turn, results in enhanced microbial diversity (Hättenschwiler et al., 2005). The harsh alpine

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conditions, however, may limit diversity in these environments as suggested by similar Shannon indices in T2 and T3. Higher evenness usually reflects higher functionality and stability within a system (Legrende and Legrende, 1998). This becomes more apparent when diversity values between control and litter amended treatments are compared. At T1, diversity substantially increased in the litter amended treatments, whereas no changes were observed at the older sites (Table 3). The fact that few changes in evenness were observed following litter incorporation may suggest an increasing stability of the microbial food web irrespective of nutrient sources, as succession proceeds.

The initial microbial community structure as evidenced by PLFA analysis of controls (no litter incorporation) indicated marked differences between the T1 and T2–T3 sites, and T4 (Fig. 2). At T1, the microbial community structure was dominated by PLEL and PUFA; these decreased as soil age increased. According to the literature, these markers indicate a higher proportion of archaea, fungi, microeukaryotes, protozoa and cyanobacteria (Gattinger et al., 2003; Zelles et al., 1999; Zaady et al., 2010; Potts et al., 1987; Villanueva et al., 2004). These organisms form microbial mats in initial ecosystem structures (Villanueva et al., 2004; Stal, 1995) to revert on different skills of C and N fixation systems (Nisbet and Fowler, 1999), which also allow the use of C-CO_2 or old C sources. In contrast to data from Töwe et al. (2010), where higher abundance of archaeal ammonia oxidizers was observed as ecosystem development progressed, results from this study indicated a high proportion of total archaea at T1, based on the. ubiquitious archaeal lipid phytane (Gattinger et al., 2003). In comparison with T1, a more bacterial-dominated microbial population was detected at the control sites T2, T3 and T4. Not only actinomycetes (10Me) and Gram-positives (iso, ant), but also some Gram-negative populations (cy, $-\omega 5$, $-\omega 8$) were higher at the older sites. According to Kramer and Gleixner (2008), both Gram-positive and Gram-negative bacteria prefer plant derived C as a C-source. Moreover, vegetation and plant cover were generally higher at these sites which might have provided better growth conditions for bacteria.

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The microbial community structure detected in control treatments differed from that in the litter treatments at all stages of ecosystem development (Fig. 2). After litter application, an increase of mainly Gram-negative bacteria was observed ($-\omega 7$, $-\omega 9$, $-\omega 11$) that benefited from the fresh and easily available C sources. This is in accordance with other studies postulating Gram-negatives as an important group of bacteria involved in the litter decomposition process (Elfstrand et al., 2008; Kramer and Gleixner, 2008). Despite a significant decrease in microbial biomass between 8 and 12 weeks of incubation, differences between both harvesting time points in PLFA profiles of soils amended with litter were not as pronounced as those found between litter-amended and control samples. This suggests that shifts in the dominant microbial populations from more opportunistic groups to more specialized groups capable to process the more recalcitrant litter components was slow and thus not clearly detectable within the four weeks interval. In addition, differences in the microbial community structure between litter-amended treatments from T1, T2 and T3 were not as prominent as those between T4 and the other sites (Fig. 2). The influence of run-off transport of materials from the glacier end to sites T2 and T3 compared to the reference site T4 (not influenced by run-off from the glacier end), and the lack of major variations in soil chemistry between T2 and T3 may explain the lack of greater differences between the two and also between T2–T3 and T1.

4.3 ^{13}C distribution within PL groups

There was a high natural ^{13}C abundance of individual PL groups in T1 with δ^{13}C values up to +0‰ (vs. V-PDB). We do not attribute these high δ^{13}C values to the direct use of ancient C after glacier retreat as suggested by Bardgett et al. (2007), as the δ^{13}C values of natural soil organic matter were not higher at T1 compared to those found at the other sites. The high natural ^{13}C abundance at T1 might be an indication of cyanobacterial fixation of C (Walker and del Moral, 2003). Due to a smaller isotopic fractionation by the 3-hydroxypropionate pathway compared to the Calvin cycle, ^{13}C enriched signatures may also occur due to C fixation by cyanobacteria (van der

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Meer et al., 2007). By predation and release of the fixed C into the soil matrix and subsequent uptake by other microbes, enriched ^{13}C signatures were possibly transferred into a variety of "non-cyanobacterial" PL groups. For instance high $\delta^{13}C$ values in PLFA biomarkers indicative for Gram-positive and Gram-negative bacteria were also detected. In addition, as ecosystem development progresses, the influence of organic sources from plants increases. Higher plants fixed C through the Calvin cycle resulting in higher influence of $\delta^{13}C$ signatures within the range −20 to −25‰ $\delta^{13}C$ (vs. V-PDB) in biolipids at T2–T3 and T4.

At all sites, PUFA and PLEL groups showed highest litter-derived ^{13}C signatures among the microbial community structure, suggesting that mainly fungi, microeukaryotes, protozoa and archaea were highly active during litter decomposition (Fig. 5). According to Emerenciano et al. (1986), high contents of sesquiterpene lactones are known in the family of *Asteraceae* and up to 7 different compounds have been detected in the *Leucanthemopsis* genus. As such compounds are difficult to degrade (Picman, 1987), fungi might have played an important role as the main litter decomposers in our study. For instance, fungi secrete enzymes that break down the cuticule of dead leaves (Chapin et al., 2002). Schmidt and Lipson (2004) suggest that a high proportion of fungi may dominate the microbial community structure at low temperatures (−5 to 3 °C), decomposing litter and immobilizing N. Frey et al. (2003) postulated a translocation of litter-derived C belowground by decomposer fungi, subsequently stabilized as soil organic matter. However, PUFA may occur not only in fungi, but also among cyanobacteria (Zaady et al., 2010) and γ-proteobacteria (Nichols and McMeekin, 2002), which have been detected in the Damma forefield as well (Duc et al., 2009). Therefore, it might be possible that at the young site, the ^{13}C enrichment in PUFA observed in the control soil (no litter application) originates from cyanobacterial ^{13}C fixation, whereas ^{13}C enrichment in PUFA after litter application indicates decomposition processes by fungi or γ-proteobacteria.

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High amounts of ^{13}C derived from litter were also detected in PLFA nor< 20; however, these lipids are ubiquitously found in all organisms and therefore provide no clear bioindication. Surprisingly, we found ^{13}C enrichment in the PLEL group, characteristic of archaea. To the best of our knowledge this is the first litter decomposition study that includes PLEL as archaeal biomarker (at least in alpine ecosystems), suggesting that archaea might play an important role during initial litter decomposition in alpine ecosystems. However, it remains unclear whether the enrichment observed is due to the direct participation of archaea in litter degradation or to the recycling of ^{13}C from other groups.

It should be noted that results from general PL analyses (Sect. 4.2) showed a stimulation of individual PL groups following litter application which may not necessarily be related to the utilization of litter derived ^{13}C. This might be explained by an increase in soil organic carbon mineralization due to the input of fresh organic carbon residues, the so called "priming effect" (Kuzyakov, 2010). Additionally, during the time between litter application and the first harvest (8 weeks), microbes may have migrated to other sites through deposition of particulate matter, air currents, precipitation or snowmelt (Schütte et al., 2009). The importance of such disturbances and mechanisms was not estimated in the present study.

Similar litter-derived ^{13}C incorporation patterns for all PLFA groups were observed regardless of site age (Fig. 4a and b). These results suggest that initial food web structures also form the basis of later stages of soil development in this kind of environments. This might be related to the demanding specialization required to survive and proliferate in environments characterized by very low temperatures, short vegetation periods and low availability of nutrients. Microbial populations thus have to react rapidly to organic inputs, which might be restricted to the short vegetation period, and also to compete with plants for the scarce nutrients. After 12 weeks a significant shift was observed at all sites; incorporation of ^{13}C into PLEL signatures decreased, whilst an increase was observed in the incorporation of ^{13}C into PUFA markers (Fig. 4a and b). This might be related to (i) lower activity of archaea once that litter rich in carbohydrates

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and sugars has been consumed, as was previously shown for bacteria (Bardgett and Walker, 2004), and (ii) higher contribution of fungi as the litter becomes more recalcitrant for degradation.

5 Conclusions

The main points emerging from this study are:

- Soil development increases microbial biomass, diversity and evenness in glacier foreland resulting in faster turnover rates in response to organic source pulses.
- Soil development strongly influences the structure of the microbial community in glacier forefields with increasing importance of bacteria as soil development progresses.
- Adaptation to the harsh environmental conditions prevailing in alpine ecosystems acts as a strong selective force resulting in similar microbial food web patterns at different stages of soil development.
- Cyanobacteria may play a more important role in C-cycling in alpine ecosystems than previously thought.
- Both Gram-negative bacteria and archaea are significantly involved in C-turnover in response to the incorporation of litter residues in alpine forefields.

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References

Abrajano, J. T. A., Murphy, D. E., Fang, J., Comet, P., and Brooks, J. M.: $^{13}C/^{12}C$ ratios in individual fatty acids of marine mytilids with and without bacterial symbionts, Org. Geochem., 21, 611–617, 1994.

Bai, Q., Gattinger, A., and Zelles, L.: Characterisation of microbial consortia in paddy rice soil by phosholipid analysis, Microb. Ecol., 39, 273–281, 2000.

Bardgett, R. D. and Walker, L. R.: Impact of coloniser plant species on the development of decomposer microbial communities following deglaciation, Soil Biol. Biochem., 36, 555–559, 2004.

Bardgett, R. D., Mawdsley, J. L., Edwards, S., Hobbs, P. J., Rodwell, J. S., and Davies, W. J.: Plant species and nitrogen effects on soil biological properties of temperate upland grasslands, Funct. Ecol., 13, 650–660, 1999.

Bardgett, R. D., Richter, A., Bol, R., Garnett, M. H., Baeumler, R., Xu, X., Lopez-Capel, E., Manning, D. A. C., Hobbs, P. J., Hartley, I. R., and Wanek, W.: Heterotrophic microbial communities use ancient carbon following glacial retreat, Biology Lett., 3, 487–490, 2007.

Baudoin, E., Benizri, E., and Guckert, A.: Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere, Soil Biol. Biochem., 35, 1183–1192, 2003.

Boschker, H. T. S., Nold, S. C., Wellsbury, P., Bos, D., Graaf, W. D., Pel, R., Parkes, R. J., and Cappenberg, T. E.: Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers, Nature, 392, 801–805, 1998.

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Bradford, M. A., Tordoff, G. M., Eggers, T. J., and Newingtont, H. J. E.: Microbiota, fauna, and mesh size interactions in litter decomposition, Oikos, 99, 317–323, 2002.
Butler, J. L., Williams, M. A., Bottomley, P. J., and Myrold, D. D.: Microbial community dynamics associated with rhizosphere carbon flow, Appl. Environ. Microb., 69, 6793–6800, 2003.
Chapin, F. S., Walker, L. R., Fastie, C. L., and Sharman, L. C.: Mechanisms of Primary Succession Following Deglaciation at Glacier Bay, Alaska, Ecol. Monogr., 64, 149–175, 1994.
Chapin, F. S., Matson, P. A., and Mooney, H. A.: Principles of Terrestrial Ecosystem Ecology, Springer, New York, 2002.
Dilly, O., Bloem, J., Vos, A., and Munch, J. C.: Bacterial Diversity in Agricultural Soils during Litter Decomposition, Appl. Environ. Microb., 70, 468–474, 2004.
Dornbush, M. E.: Grasses, litter, and their interaction affect microbial biomass and soil enzyme activity, Soil Biol. Biochem., 39, 2241–2249, 2007.
Duc, L., Neuenschwander, S., Rehrauer, H., Wagner, U., Sobek, J., Schlapbach, R., and Zeyer, J.: Development and experimental validation of a *nifH* oligonucleotide microarray to study diazotrophic communities in a glacier forefield, Environ. Microbiol., 11, 2179–2189, 2009.
Edwards, I., Bürgmann, H., Miniaci, C., and Zeyer, J.: Variation in Microbial Community Composition and Culturability in the Rhizosphere of *Leucanthemopsis alpina* (L.) Heywood and Adjacent Bare Soil Along an Alpine Chronosequence, Microb. Ecol., 52, 679–692, 2006.
Egli, M., Fitze, P., and Mirabella, A.: Weathering and evolution of soils formed on granitic, glacial deposits: results from chronosequences of Swiss alpine environment, Catena, 45, 19–47, 2001.
Elfstrand, S., Lagerlöf, J., Hedlund, K., and Mårtensson, A.: Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling, Soil Biol. Biochem., 40, 2530–2539, 2008.
Emerenciano de P. V., Auxiliadora, M., Kaplan, C., Gottlieb, O. R., Bonfanti de M. M. R., Ferreira, Z. S., and Comegno, L. M. A.: Evolution of sesquiterpene lactones in *asteraceae*, Biochem. Syst. Ecol., 14, 585–589, 1986.
Esperschütz, J., Buegger, F., Winkler, J. B., Munch, J. C., Schloter, M., and Gattinger, A.: Microbial response to exudates in the rhizosphere of young beech trees (*Fagus sylvatica* L.) after dormancy, Soil Biol. Biochem., 41, 1976–1985, 2009a.
Esperschütz, J., Pritsch, K., Gattinger, A., Welzl, G., Haesler, F., Buegger, F., Winkler, J., Munch, J., and Schloter, M.: Influence of chronic ozone stress on carbon translocation pattern into rhizosphere microbial communities of beech trees (*Fagus sylvatica* L.) during a

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growing season, Plant Soil, 323, 85–95, 2009b.
Fioretto, A., Di Nardo, C., Papa, S., and Fuggi, A.: Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem, Soil Biol. Biochem., 37, 1083–1091, 2005.
Frey, S. D., Six, J., and Elliott, E. T.: Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface, Soil Biol. Biochem, 35, 1001–1004, 2003.
Frey, B., Rieder, S. R., Brunner, I., Plötze, M., Koetzsch, S., Lapanje, A., Brandl, H., and Furrer, G.: Weathering-associated bacteria from the Damma Glacier forefield: Physiological capabilities and impact on granite dissolution, Appl. Environ. Microb., 76, 4788–4796, 2010.
Frostegard, A. and Bååth, E.: The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fert. Soils, 22, 59–65, 1996.
Gattinger, A., Ruser, R., Schloter, M., and Munch, J. C.: Microbial community structure varies in different soil zones of a potato field, J. Plant Nutr. Soil Sci., 165, 421–428, 2002.
Gattinger, A., Günthner, A., Schloter, M., and Munch, J. C.: Characterization of *Archaea* in soils by polar lipid analysis, Acta Biotechnol., 23, 21–28, 2003.
Hämmerli, A., Waldhuber, S., Miniaci, C., Zeyer, J. and Bunge, M.: Local expansion and selection of soil bacteria in a glacier forefield, Eur. J. Soil Sci, 58, 1437–1445, 2007.
Hättenschwiler, S., Tiunov, A., V., and Scheu, S.: Biodiversity and litter decomposition in terrestrial ecosystems, Annu. Rev. Ecol. Evol. Syst., 36, 191–218, 2005.
Heal, O. W., Anderson, J. M., and Swift, M. J.: Plant litter quality and decomposition: an historical overview, edited by: Cadisch, G. and Giller, K. E., in: Driven by nature. Plant litter quality and decomposition, CAB International, Wallingford, United Kingdom, 1997.
Hill, M. O.: Diversity and Evenness: A unifying notation and its consequences, Ecology, 54, 427–432, 1973.
Jumpponen, A., Vare, H., Matteson, K. G., Ohtonen, R., and Trappe, J. M.: Characterization of "safe sites" for pioneers in primary succession on recently deglaciated terrain, J. Ecol., 87, 98–105, 1999.
Kramer, C. and Gleixner, G.: Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation, Soil Biol. Biochem., 40, 425–433, 2008.
Kuzyakov, Y.: Priming effects: Interactions between living and dead organic matter, Soil Bio. Biochem., 42, 1363–1371, 2010.
Lazzaro, A., Abegg, C., and Zeyer, J.: Bacterial community structure of glacier forefields on

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siliceous and calcareous bedrock, Eur. J. Soil Sci., 60, 860–870, 2009.
Leake, J. R., Ostle, N. J., Rangel-Castro, J. I., and Johnson, D.: Carbon fluxes from plants through soil organisms determined by field $^{13}CO_2$ pulse-labelling in an upland grassland, Appl. Soil Ecol., 33, 152–175, 2006.
Legrende, P.: Legrende L Numerical ecology, Elsevier, Amstersdam, The Netherlands, 1998.
Loreau, M.: Microbial diversity, producer-decomposer interactions and ecosystem processes: a theoretical model, Proc. R. Soc. London Ser. B, 268, 303–309, 2001.
Lu, Y., Murase, J., Watanabe, A., Sugimoto, A., and Kimura, M.: Linking microbial community dynamics to rhizosphere carbon flow in a wetland rice soil, FEMS Microbiol. Ecol., 48, 179–186, 2004.
Miniaci, C., Bunge, M., Duc, L., Edwards, I., Bürgmann, H., and Zeyer, J.: Effects of pioneering plants on microbial structures and functions in a glacier forefield, Biol. Fert. Soils, 44, 289–297, 2007.
Nichols, D. S. and McMeekin, T. A.: Biomarker techniques to screen for bacteria that produce polyunsaturated fatty acids, J. Microbiol. Meth., 48, 161–170, 2002.
Nilsson, L., Bååth, E., Falkengren-Grerup, U., and Wallander, H.: Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient, Oecologia, 153, 375–384, 2007.
Nisbet, E. G. and Fowler, C. M. R.: Archaen metabolic evolution of microbial mats, P. R. Soc. Lond. B. Bio., 266, 2375–2382, 1999.
Oerlemans, J.: Extracting a climate signal from 169 glacier records, Science, 308, 675–677, 2005.
Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., and Trappe, J.: Ecosystem properties and microbial community changes in primary succession on a glacier forefront, Oecologia, 119, 239–246, 1999.
Ostle, N., Briones, M. J. I., Ineson, P., Cole, L., Staddon, P., and Sleep, D.: Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna, Soil Biol. Biochem., 39, 768–777, 2007.
Picman, A. K.: Persistence and disappearance of the sesquiterpene lactone, isoalantolactone, in soils, Biochem. Syst. Ecol., 15, 361–363, 1987.
Pombo, S. A., Pelz, O., Schroth, M. H., and Zeyer, J.: Field-scale ^{13}C-labeling of phospholipid fatty acids (PLFA) and dissolved inorganic carbon: tracing acetate assimilation and mineralization in a petroleum hydrocarbon-contaminated aquifer, FEMS Microbiol. Ecol., 41,

BGD
8, 1275–1308, 2011

259–267, 2002.
Potts, M., Olie, J. J., Nickels, J. S., Parsons, J., and White, D. C.: Variation in phospholipid ester-linked fatty acids and carotenoids of desiccated nostoc commune (cyanobacteria) from different geographic locations., Appl. Environ. Microb., 53, 4–9, 1987.
Ratledge, C. and Wilkinson, S. G.: Microbial lipids, Academic Press, London, 1989.
Reichardt, W., Mascarina, G., Padre, B., and Doll, J.: Microbial communities of continuously cropped, irrigated rice fields, Appl. Environ. Microb., 63, 233–238, 1997.
Schmidt, S. K. and Lipson, D. A.: Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils Plant Soil, 259, 1–7, 2004.
Schuette, U. M. E., Abdo, Z., Bent, S. J., Williams, C. J., Schneider, G. M., Solheim, B., and Forney, L. J.: Bacterial succession in a glacier foreland of the High Arctic, ISME J., 3, 1258–1268, 2009.
Stal, L. J.: Tansley Review No. 84, Physiological ecology of cyanobacteria in microbial mats and other communities, New Phytol., 131, 1–32, 1995.
Tate, R III.: Soil organic matter, biological and ecological effects, John Wiley & Sons Inc, USA, 291 pp., 1987.
Töwe, S., Albert, A., Kleineidam, K., Brankatschk, R., Dümig, A., Welzl, G., Munch, J., Zeyer, J., and Schloter, M.: Abundance of microbes involved in nitrogen transformation in the rhizosphere of *Leucanthemopsis alpina* (L.) Heywood grown in soils from different sites of the Damma glacier forefield, Microb. Ecol., 60, 762–770, 2010.
Tscherko, D., Rustemeier, J., Richter, A., Wanek, W., and Kandeler, E.: Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps, Eur. J. Soil Sci., 54, 685–696, 2003.
Tscherko, D., Hammesfahr, U., Zeltner, G., Kandeler, E., and Böcker, R.: Plant succession and rhizosphere microbial communities in a recently deglaciated alpine terrain, Basic Appl. Ecol., 6, 367–383, 2005.
Van Der Meer, M. T. J., Schouten, S., Damsté, J. S. S., and Ward, D. M.: Impact of carbon metabolism on ^{13}C signatures of cyanobacteria and green non-sulfur-like bacteria inhabiting a microbial mat from an alkaline siliceous hot spring in Yellowstone National Park (USA), Environ. Microbiol., 9, 482–491, 2007.
Villanueva, L., Navarrete, A., Urmeneta, J., White, D. C., and Guerrero, R.: Combined phospholipid biomarker-16S rRNA gene denaturing gradient gel electrophoresis analysis of bacterial diversity and physiological status in an intertidal microbial mat, Appl. Environ. Microb., 70,

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8, 1275–1308, 2011

6920–6926, 2004.
Walker, L. R. and del Moral, R.: Primary succession and ecosystem rehabilitation, Cambridge, UK, Cambridge University Press, 2003.
White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbie, R. J.: Determination of the sedimentary microbial biomass by extractible lipid phosphate, Oecologia, 40, 51–62, 1979.
Zaady, E., Ben-David, E. A., Sher, Y., Tzirkin, R., and Nejidat, A.: Inferring biological soil crust successional stage using combined PLFA, DGGE, physical and biophysiological analyses, Soil Biol. Biochem., 42, 842–849, 2010.
Zelles, L., Bai, Q. Y., Rackwitz, R., Chadwick, D., and Beese, F.: Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structure in soils, Biol. Fert. Soils, 19, 115–123, 1995.
Zelles, L.: Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review, Biol. Fert. Soils, 29, 111–129, 1999.

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Table 1. Total C and N contents, pH-values and $\delta^{13}C$ natural abundance in soil (means of $n = 5 \pm$ standard deviation) at different stages of ecosystem development (T1–T4).

ecosystem development	pH ($CaCl_2$)	C_{total} [%]	N_{total} [%]	^{13}C [‰ V-PDB]
10 years (T1)	5.6 (0.1)	0.11 (0.09)	0.004 (0.002)	−26.96 (2.39)
60 years (T2)	4.8 (0.1)	0.78 (0.55)	0.048 (0.034)	−26.50 (0.47)
100 years (T3)	5.1 (0.6)	0.62 (0.35)	0.040 (0.028)	−27.51 (0.56)
> 700 years (T4)	4.3 (0.2)	6.50 (3.96)	0.442 (0.252)	−26.72 (0.28)

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Table 2. Plant litter mass loss and selected parameters during the litter incubation period along the Damma forefield (means of $n = 5 \pm$ standard deviation).

		8 weeks				12 weeks			
	initial plant litter	T1	T2	T3	T4	T1	T2	T3	T4
plant litter [g per litterbag DW]	2.50	1.12 (0.11)	0.95 (0.11)	0.91 (0.10)	0.87 (0.12)	0.88 (0.08)	0.84 (0.16)	0.83 (0.31)	0.93 (0.36)
remaining plant litter [%]	100.00	44.68 (4.22)	38.03 (4.52)	36.27 (3.94)	34.90 (4.95)	35.14 (3.10)	33.50 (6.24)	33.21 (12.41)	37.40 (14.40)
C_{total} [g per litterbag DW]	0.96 (0.06)	0.59 (0.15)	0.42 (0.04)	0.42 (0.05)	0.40 (0.04)	0.45 (0.13)	0.38 (0.06)	0.37 (0.14)	0.42 (0.17)
$^{13}C_{total}$ [‰ δ^{13}C V-PDB]	88.35 (1.61)	84.00 (4.42)	82.33 (4.41)	83.47 (4.41)	85.53 (3.09)	86.50 (2.14)	80.19 (7.36)	81.04 (3.15)	81.45 (5.31)
N_{total} [g litterbag DW]	0.04 (0.01)	0.04 (0.00)	0.03 (0.01)	0.03 (0.00)	0.03 (0.00)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
C/N	22.70 (1.22)	13.32 (0.75)	13.31 (0.57)	13.57 (1.11)	13.35 (0.57)	12.98 (0.90)	12.85 (0.88)	13.86 (0.47)	13.40 (1.18)

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Table 3. Microbial diversity and evenness (Shannon indices) at T1–T4 after 8 weeks and 12 weeks of litter application, as well as without litter (control). Values are illustrated as means ($n = 5 \pm$ standard deviation).

	control				8 weeks				12 weeks			
	10	60	100	> 700 y	10	60	100	> 700 y	10	60	100	> 700 y
Shannon diversity	1.600 (0.154)^{a}	2.733 (0.111)^{b}	2.869 (0.082)^{b}	2.869 (0.033)^{b}	2.257 (0.108)^{a}	2.562 (0.108)^{b}	2.478 (0.147)^{b}	2.823 (0.050)^{c}	2.337 (0.0377)^{a}	2.512 (0.053)^{b}	2.698 (0.139)^{c}	21 717 (0.060)^{c}
Shannon evenness	0.667 (0.084)^{a}	0.839 (0.034)^{b}	0.861 (0.025)^{b}	0.850 (0.011)^{b}	0.685 (0.033)^{a}	0.777 (0.032)^{b}	0.752 (0.045)^{b}	0.830 (0.015)^{c}	0.687 (0.011)^{a}	0.762 (0.016)^{b}	0.819 (0.042)^{c}	0.834 (0.018)^{c}

Letters (a,b,c) indicates significant differences $p < 0.05$ within treatments (control, 8 weeks, 12 weeks).

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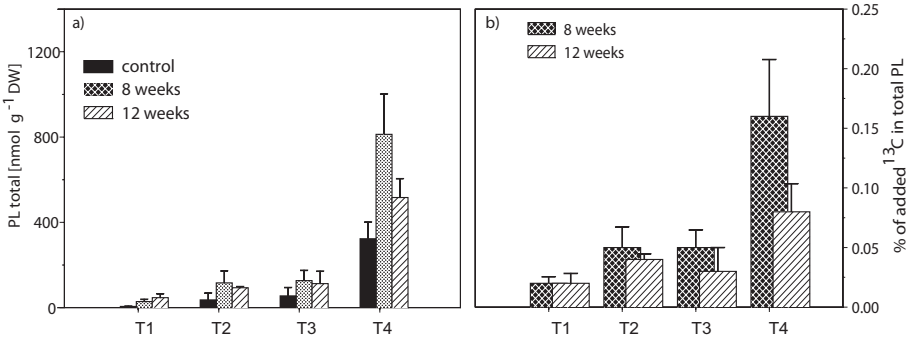

Fig. 1. Total phospholipid (PL) content **(a)** in soil (nmol g^{-1} DW) and the percent of added ^{13}C incorporated into total PL **(b)** at different stages of ecosystem development T1–T4 and individual harvesting time points (means + standard deviation, $n = 5$).

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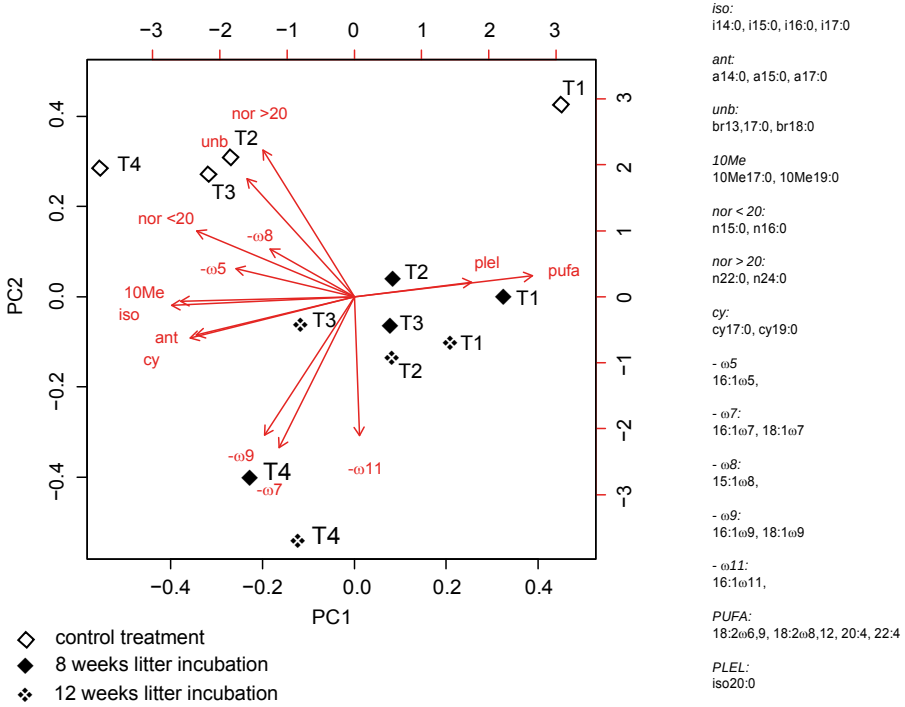

Fig. 2. Illustration of the first (PC1) and the second (PC2) principal components of the principal component analysis (PCA) of all PL groups on mol% basis (means of $n = 5$).

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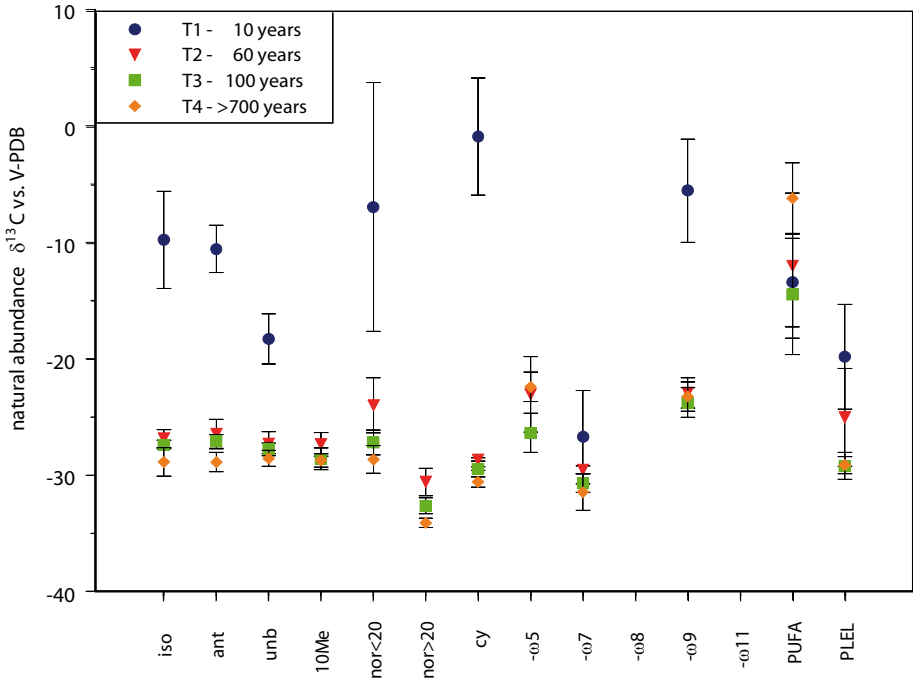

Fig. 3. Natural abundance δ^{13}C values [‰ vs. V-PDB] in individual PL groups in soil without litter application (means ± standard deviation; $n = 5$). PL groups were formed as described in Figs. 2 and 5.

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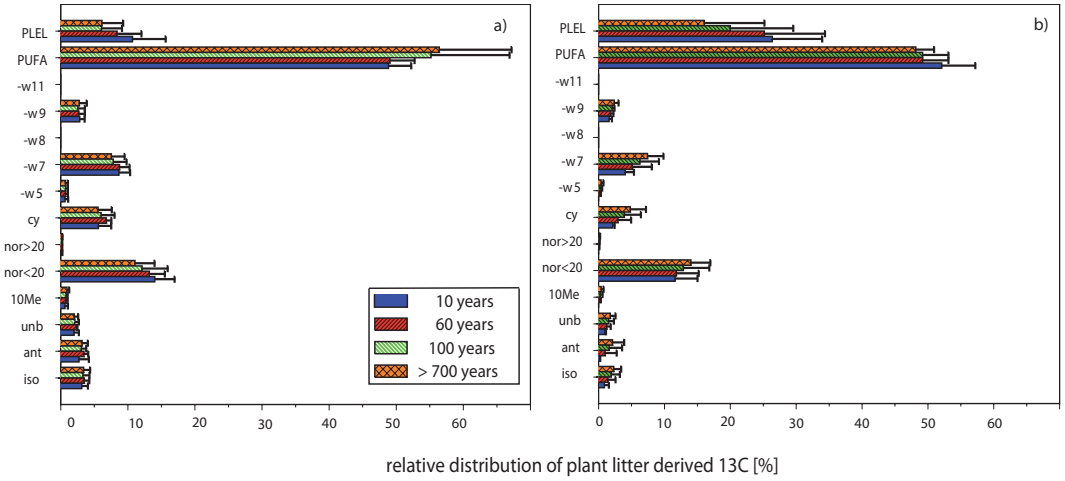

Fig. 4. Distribution of ^{13}C derived from litter within PL groups in % relative to total litter derived ^{13}C into total PL according to Fig. 1 (means + standard deviation; $n = 5$) after 8 weeks **(a)** and 12 weeks **(b)** of litter incubation. PL groups were formed as described in Figs. 2 and 5.

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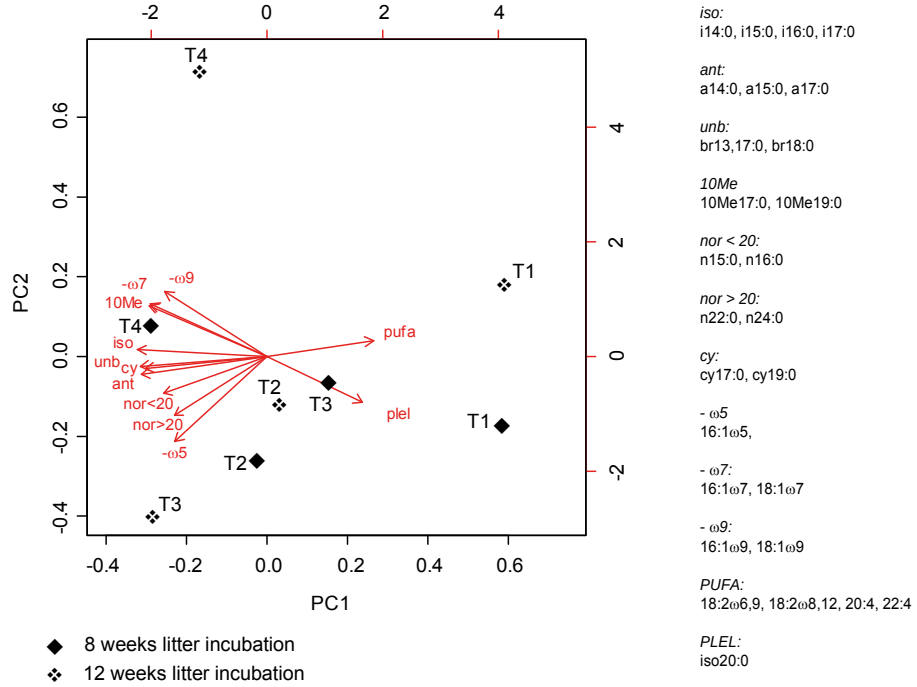

Fig. 5. First (PC1) and second (PC2) principal components of the principal component analysis (PCA) illustrating the distribution of ^{13}C derived from litter within PL groups (in % relative to total ^{13}C derived from litter into total PL according to Fig. 1; means of $n = 5$).

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