Microbial diversity in the forefield of the receding Damma glacier

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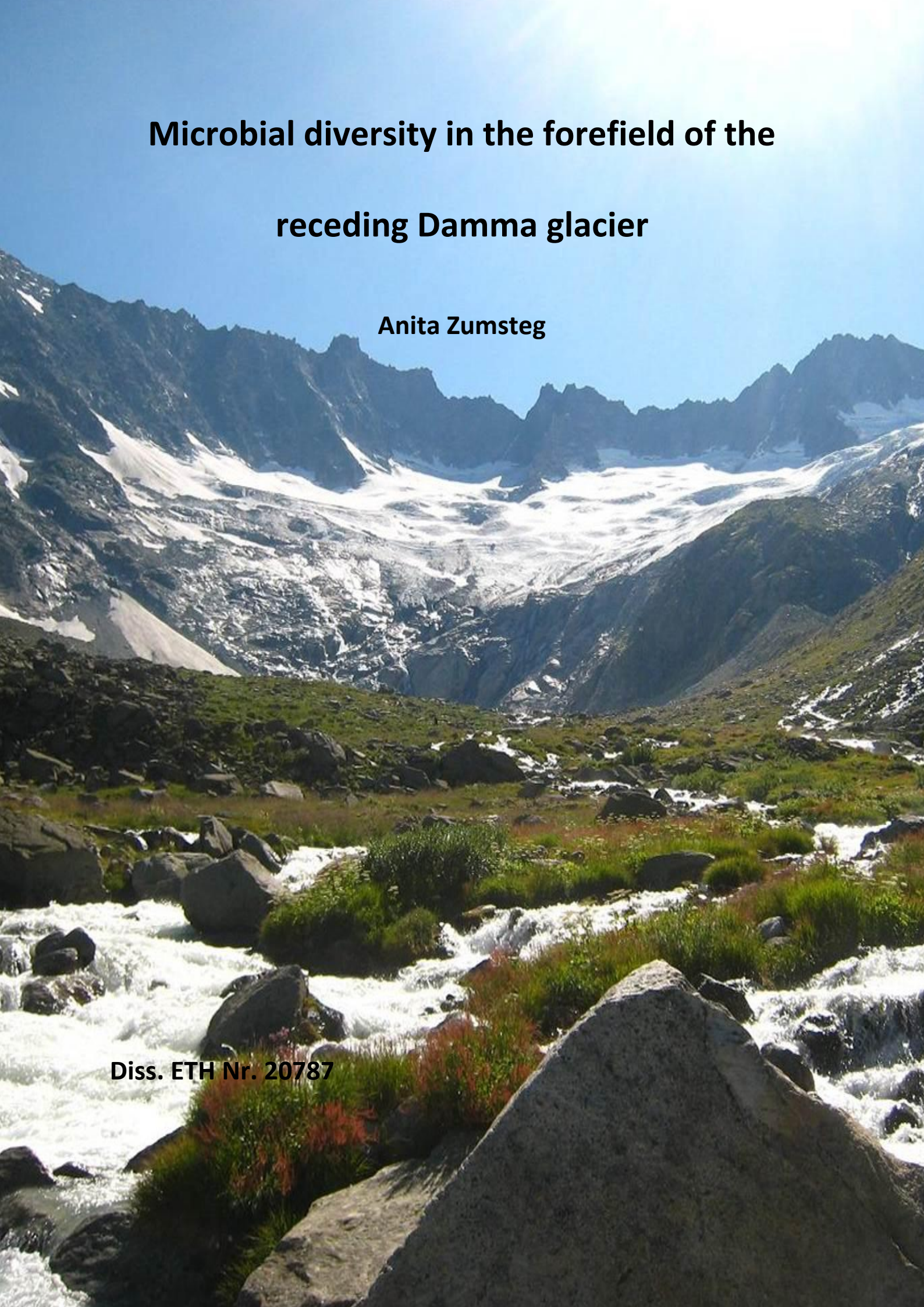

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"Science may set limits to knowledge, but should not set limits to imagination."

Bertrand Russell

CONTENTS

i. SUMMARY

Global temperatures have been increasing steadily since the industrial revolution in the 19th century, and more precisely by 0.2°C per decade during the last 30 years. This leads to a rapid retreat and mass loss of glaciers around the globe. Such a glacier retreat exposes bare soil composed of sand, stones and gravel. Successively after deglaciation, microbes and plants are colonizing this so-called glacier forefield and gradually change it from bare to vegetated soil. Such glacier forefield chronosequences are ideal investigation sites for soil formation, microbial and plant succession as well as microbial adaptation to a changing climate and changes in carbon sources.

During my thesis I investigated the microbial succession, adaptation and carbon usage along the soil age chronosequence of the Damma glacier forefield, located in the Swiss Central Alps. The aim was to elucidate key soil and environmental factors influencing the microbial communities. In a first study, the microbial succession of bacteria, archaea and fungi along the chronosequence, reaching from recently deglaciated bare soil to > 100 year old vegetated soil, was investigated using molecular approaches such as terminal restriction fragment length polymorphism (T-RFLP) and sequence analysis. In a second study, I performed a soil transfer experiment in bare soil along a cross-section near the glacier terminus, in order to investigate the influence of microclimatic parameters such as soil moisture and temperature on microbial communities and their adaptational capabilities to a changing climate. Using molecular approaches and microbial activity measurements, I could show that differences in microclimate and, hence, also in microbial community composition were mainly due to expositional differences between the sites. In a third study, I investigated the ability of bacteria to decompose either fungal (*Penicillium* sp.) or algal (*Chlorella* sp.) biomass in recently deglaciated bare soil where carbon and nitrogen contents are low. In combination with activity measurements and T-RFLP profiling, the stable isotope probing technique was used to elucidate the main bacterial phylotypes incorporating carbon from the supplied biomass.

In the first study, I could show that the Shannon diversity Index (*H*) was highest for bacteria (between 2.3 and 3.4), but there was no clear pattern along the chronosequence. The same was true for the fungal diversity, which was generally lower throughout the forefield (*H* ranged from 0.3 to 1.2). Interestingly, archaean diversity (H) decreased from 2 to 0.2 with increasing soil age. Overall, my findings demonstrate that bacteria, archaea and fungi all exhibit successional patterns along the Damma glacier forefield chronosequence. The autotrophic *Cyanobacteria* and the versatile *Proteobacteria* were abundant in the bare soil and a higher number of sequences from heterotrophic bacteria were found in the older vegetated soil.

There was a distinct shift in archaea community composition, from a dominance of *Euryarchaeota* in the bare soil, to a dominance of *Crenarchaeota* in the older vegetated soil. This could reflect a change in function of the different archaeal groups along the chronosequence, since *Euryarchaeota* are more involved in carbon cycling and *Crenarchaeota* more in nitrogen cycling. Similarly, the fungal community changed from *Ascomycota* to *Basidiomycota* along the chronosequence. This reflects the fact that *Ascomycota* are often-free living, whereas *Basidiomycota* often form close associations with plants. We found that soil parameters such as pH (decreasing from 4.8 to 3.8 with increasing soil age), base saturation (decreasing from 70% to 50%), carbon and nitrogen content (increasing with increasing soil age from 0.07% to 2% and from 0.01% to 0.13% respectively) and other factors such as plant cover (increasing from zero to 100% plant cover) were the main drivers for the community changes I observed along the chronosequence of the Damma glacier forefield.

Environmental parameters that could induce microbial community shifts, which I specifically examined, were soil moisture and soil temperature, which are mainly influenced by the exposition to the sun. In the second study, I investigated the reaction of soil microbial communities to changing temperature and soil moisture regimes by performing a soil transfer experiment, and monitoring the communities for 16 months. This was done in non-vegetated soil in order to minimize confounding effects of soil parameters like organic matter. There I observed a significant ($P < 0.05$) change and an overall increase in microbial activities when soil was transferred from a warmer and drier site to a

colder and moister site, e.g. when the soil temperature decreased and the soil moisture content became more favorable. Therefore soil temperature and soil moisture appeared to be the main limiting factor for microbial activities, as high soil temperatures can lead to the death of certain species and as soil moisture is influencing the nutrient availability. Furthermore, the bacterial and fungal community structures appeared to be significantly ($P < 0.05$) influenced by soil temperature and the soil moisture content in general, reflecting the changes over a longer time span, whereas the microbial activities were reflecting the situation at a certain time point. The bacterial phylogenetic groups that reacted most to changes in the microclimate were *Acidobacteria*, *Actinobacteria*, and *alpha-* and *beta-Proteobacteria.* In all bacterial phylogenetic classes we found species that were resistant to transfer (increasing abundance), whereas others were sensitive to transfer (decreasing abundance). In the fungal community, *Ascomycota* of the phylogenetic class *Pezizomycotina* reacted resistant to the transfer, whereas the mitosporic *Ascomycota* reacted sensitively.

Microbial community composition and activity can also be influenced by the availability of a distinct carbon source. During my third study, I investigated the reaction of the bacterial soil community to the addition of either algal or fungal biomass, both being abundant colonizers of recently deglaciated, carbon depleted soil, representing possible carbon sources for microorganisms. I observed a more efficient utilization of algal biomass than fungal biomass, since CO_2 respiration was 2.5 times higher after the addition of algal biomass compared to the addition of fungal biomass. This could be due to the easier degradability of the algal cell wall material (mainly composed of cellulose, whereas the fungal cell wall is mainly composed of chitin) and the higher nitrogen content. However, only 20% of the added carbon was respired as CO_2, and the rest presumably remained in the soil. Interestingly, the community structure also differed between the two treatments, indicating a preference of certain phylogenetic groups for one carbon source. For instance *Proteobacteria* were the main decomposers of algal biomass, whereas the *Bacteroidetes,* particularly the *Flavobacteria* sp., were the main decomposers of fungal biomass. This corroborated the findings that *Flavobacteria* sp. are indeed adapted to cold environments and thrive in an excess of degradable biomass.

My work shows that even non-vegetated sandy and stony soil harbors a vast diversity of microorganisms, which are able to adapt and survive in harsh environments. As in any ecosystem every organism is linked to other organisms in one way or the other, ensuring the function of the whole ecosystem. This fact is even more important in a glacier forefield, where the organisms strongly rely on the availability of nutrient and carbon sources, forming a complex network of interactions also together with mineral weathering, plant colonization and other factors, eventually leading to soil formation.

ii. ZUSAMMENFASSUNG

Aufgrund der steigenden globalen Durchschnittstemperaturen seit der Industriellen Revolution im 19ten Jahrhundert, und genauer um 0.2°C pro Dekade in den letzten 30 Jahren, verlieren die Gletscher weltweit mehr und mehr an Masse. Dieser Gletscherrückzug setzt neue Böden frei, die aus Sand, Steinen und Kies bestehen. Mit der Zeit besiedeln Mikroben und Pflanzen diesen neuen Lebensraum und der steinige und sandige Untergrund verändert sich dadurch langsam zu einem dicht mit Pflanzen bewachsenen, Humus bedeckten Boden. Solche Bodenaltersabfolgen in Gletschervorfeldern, genannt Chronosequenzen, sind ideale Standorte zur Untersuchung der Bodenbildung und der mikrobiellen und pflanzlichen Sukzession, sowie der mikrobiellen Anpassung an sich verändernde Umweltbedingungen wie zum Beispiel Veränderungen im Klima oder der Kohlenstoffquellen. Während meiner Dissertation habe ich die mikrobielle Sukzession, die Anpassung an sich verändernde mikroklimatische Bedingungen und die Nutzung verschiedener Kohlenstoff Quellen entlang der Damma Gletscher Chronosequenz in den Schweizer Zentralalpen untersucht. Ziel war es, wichtige Boden- und Umwelt-Faktoren, welche die mikrobiellen Lebensgemeinschaften beeinflussen, zu eruieren. In einer ersten Studie wurde die mikrobielle Sukzession von Bakterien, Archaea und Pilzen entlang der Chronosequenz, beginnend mit vor kurzem entgletschertem, steinigem Untergrund bis zu entwickelten, mit Pflanzen bewachsenen, mehr als 100 Jahre alten, Böden untersucht. Die verwendeten molekularen Ansätze waren unter anderem Terminaler-Restriktions-Polymorphismus (T-RFLP) und Sequenzanalysen. Um den Einfluss von mikroklimatischen Parametern wie Bodenfeuchte und Temperatur auf die mikrobiellen Gemeinschaften und ihre Anpassungsfähigkeit in einem sich wandelnden Klima zu untersuchen, führte ich in einer zweiten Studie ein Boden-Transfer Experiment mit vor kurzem entgletschertem Boden durch. Dieser sandige, nicht mit Pflanzen überwachsene Boden wurde entlang eines Temperatur- und Bodenfeuchte-Gradienten, in der Nähe des Gletschers, von der sonnenbeschienen Seite an die schattige Seite und umgekehrt, transferiert. Mit molekularen Methoden sowie Aktivitätsmessungen konnte ich zeigen,

dass das Mikroklima einen grossen Einfluss auf die mikrobiellen Gemeinschaften hat. In meiner dritten Studie untersuchte ich die Fähigkeit von heterotrophen Bakterien, Pilzfragmente (*Penicillium* sp.) als auch Algenfragmente (*Chlorella* sp.) aufzunehmen und zu veratmen. Dies wurde ebenfalls in kürzlich entgletschertem Boden untersucht, da dort der Kohlenstoff und Stickstoff Gehalt niedrig ist. Zur Analyse welche bakteriellen Phylotypen die zugefügten Zellfragmente metaboliseren können, wurde in Verbindung mit Aktivitätsmessungen und RFLP-Profiling, die sogenannte Stable-Isotope-Probing-Technik verwendet.

Mit der ersten Studie konnte ich zeigen, dass Bakterien, Archaea und Pilze eine Sukzession entlang der Chronosequenz zeigen. Die Diversität war am Grössten bei den Bakterien (Shannon Diversitätsindex *H* zwischen 2.3 und 3.4), zeigte jedoch keinen Trend mit steigendem Bodenalter. Auch die Diversität der Pilze folgte keinem Trend, war aber im Allgemeinen tiefer (*H* zwischen 0.3 und 1.3). Die Diversität (*H*) der Archaea hingegen sank markant mit steigendem Bodenalter von $H = 2$ auf 0.2. Neben der Diversität veränderte sich auch die Zusammensetzung der Archaea, Bakterien und Pilzen entlang der Chronosequenz. Die autotrophen *Cyanobakterien* und die vielseitigen *Proteobakterien* waren im kürzlich entgletscherten Boden reichlich vorhanden und es wurde eine höhere Anzahl an heterotrophen Organismen in den älteren Böden gefunden. Interessanterweise beobachtete ich in der Gruppe der Archaea eine Dominanz der *Euryarchaeota* in jungen Böden, während ältere Böden hauptsächlich von den *Crenarchaeota* besiedelt wurden. Dies könnte auch ein Wechsel in der Funktion bedeuten, die die entsprechenden Archaea im Vorfeld ausüben, da *Euryarchaeota* bekanntlich Funktionen im Kohlenstoffkreislauf erfüllen, während die *Crenarchaeota* wichtige Funktionen im Stickstoffkreislauf ausführen. Und auch die Pilze zeigten einen ähnlichen Wechsel entlang der Chronosequenz, wo die meist freilebenden *Ascomyceten* vorzugsweise die jungen Böden besiedelten und die oft mit Pflanzen in Verbindung stehenden *Basidiomyceten* hauptsächlich die älteren Böden. Ich konnte zeigen dass die Bodenparameter pH (sank von pH 4.8 auf 3.8 mit steigendem Bodenalter), Basensättigung (sank von 70% auf 50%), Kohlenstoff- und Stickstoffgehalt (stieg von 0.07% auf 2% für Kohlenstoff und von 0.01% auf 0.13% für Stickstoff) und

andere Parameter wie die Pflanzendecke (stieg von keinen Pflanzen bis zu einer Pflanzendecke von 100%) die wichtigsten bestimmenden Faktoren für diese Sukzession der mikrobiellen Gruppen waren.

Potenzielle Umweltparameter die Einfluss auf die mikrobielle Gemeinschaft haben können, sind die Bodenfeuchte und die Bodentemperatur, welche vor allem durch die Exposition zur Sonne beeinflusst werden. Nachdem ich für meine zweite Studie Boden entlang eines Temperatur und Bodenfeuchte Gradienten transferiert hatte, und die mikrobiellen Gemeinschaften über 16 Monaten untersuchte, beobachtete ich eine signifikante ($P < 0.05$) Zunahme der mikrobiellen Aktivitäten, wenn der Boden aus einem wärmeren und trockeneren Ort zu einem kälteren und feuchteren Standort übertragen wurde, das heisst, wenn die Temperatur und der Feuchtigkeitsgehalt des Bodens günstiger für die Mikroben wurden. Deshalb konnte ich schliessen, dass die Temperatur und die Bodenfeuchte sehr wichtige limitierende Faktoren für die mikrobielle Aktivität sind, da eine zu hohe Temperatur zum Tod einiger Spezies führen kann und eine erhöhte Bodenfeuchte zu einer besseren Nährstoffverfügbarkeit führt. Die Struktur der Populationen von Bakterien und Pilzen auf der anderen Seite, schien stark von der Temperatur und der Bodenfeuchte im Allgemeinen beeinflusst zu werden ($P > 0.05$). Die bakteriellen Gruppen die am meisten auf Veränderungen im Mikroklima reagierten waren die *Acidobakterien*, *Actinobakterien*, *alpha-* und *beta-Proteobakterien*. In all diesen Gruppen gab es Spezies die sensitiv (Abnahme der Abundanz) oder resistent (Zunahme der Abundanz) auf den Bodentransfer reagierten. Bei den Pilzen reagierten die *Pezizomyceten* resistent auf den Bodentransfer, während die mitosporischen *Ascomyceten* sensitiv reagierten.

Neben den oben genannten Boden- und Umweltfaktoren untersuchte ich in meiner dritten Studie zusätzlich den Einfluss von zwei verschiedenen möglichen Kohlenstoffquellen auf die bakterielle Gemeinschaft im Boden. Die zugegebenen Zellfragmente wurden wie erwartet von den Bakterien und Pilzen genutzt und veratmet, sowie von einigen Bakterien in die Zelle eingebaut. Die Zugabe der Biomasse erhöhte allgemein die bakterielle Aktivität. Ich konnte interessanterweise beobachteten, dass die Biomasse der Algen effizienter genutzt wurde als die pilzliche Biomasse, da hier die CO_2

Respiration um das 2.5 fache tiefer war. Dies könnte daran liegen das die Algen erstens mehr Stickstoff enthalten und zweitens deren Zellwand (die aus Zellulose besteht) einfacher abzubauen ist als die pilzliche Zellwand (aus Chitin bestehend). Aber nur 20% des zugefügten Kohlenstoffs wurde als CO_2 veratmet, es ist daher anzunehmen dass der restliche Anteil im Boden verblieben ist. Ausserdem war die bakterielle Zusammensetzung verschieden, je nachdem ob Pilzfragmente oder Algenfragmente zugegeben wurden, was darauf hindeutet das einige Spezies eine Präferenz für eine der beiden Kohlenstoffquellen zeigten. So waren die *Proteobakterien* die Hauptkonsumenten der zugegebenen Algenbiomasse, und die *Bacteroideten*, vor allem die *Flavobacteria* sp., die Hauptkonsumenten der pilzlichen Biomasse. Die *Flavobacteria* sp. sind bekannt für ihre gute Anpassung an kalte Gebiete, und dass sie sich bei einem Überschuss an Kohlenstoff sehr stark vermehren können, was bei meiner Studie, vor allem bei der Zugabe von Pilzbiomasse, ebenfalls gezeigt werden konnte.

Meine Arbeit zeigt, dass sogar der sandige und steinige Boden ohne Pflanzenbewuchs eine grosse mikrobielle Diversität vorweist. Wie in jedem Ökosystem sind die Organismen auch hier alle miteinander verbunden, was das Funktionieren des Systems gewährleistet. Dies ist speziell wichtig in einem Gletschervorfeld, wo die Organismen stark abhängig von Nährstoffressourcen, und als Konsequenz, voneinander sind. Diese Verbundenheit zwischen den Organismen, zusammen mit der Gesteinsverwitterung, der Kolonisation von Pflanzen und anderen Faktoren, führt mit der Zeit zu Bodenbildung.

1. General Introduction

1.1 Glacier Forefields

1.1.1 Formation of Glacier Forefields

The total area covered by ice worldwide is estimated to be 785 ± 100 * 10^3 km^2 (Dyurgerov & Meier, 2005). These massive ice masses consisting of glaciers, ice shelves, ice sheets and ice caps are losing mass rapidly around the globe (Oerlemans, 1994; UNEP, 2011). The most probable cause is a rise in global annual mean temperatures since the beginning of the 19^{th} century and more precisely, of around 0.2°C per decade during the last 30 years (Hansen et al., 2006; Hansen et al., 2010).

The bare soil that is exposed after glacial retreat on land surfaces consists mainly of patches of stones, sand and gravel (Bernasconi et al., 2011) which are heterogeneously distributed. This is due to the dynamical processes of glacier advancing, which induces abrasion and deposition of large rocks, and melting, which involves large amounts of melt water which redistributes glacier deposits (Dumig et al., 2011). In newly deglaciated terrain water can easily intrude into patches of sand and gravel, but also into cracks in stones which are opened by erosion and freeze-thaw cycles, where it acts as a mechanical and chemical weathering agent (Mavris et al., 2010). Chemical weathering is indeed most active in recently deglaciated terrain, where compact rocks are therefore fragmented and water dissolutes the minerals formerly captured. Additionally, the newly deglaciated terrain is quickly colonized by microbes, plants, insects and nematodes (Coulson et al., 2003; Tscherko et al., 2005; Yergeau et al., 2007 a). Especially bacteria and fungi are known to be active in biological weathering (Borin et al., 2010; Frey et al., 2010; Brunner et al., 2011; Lapanje et al., 2011), dissolving minerals from the rock surfaces. Erosion, mechanical, chemical and biological weathering are the initial stages of soil formation, resulting in the dissolution of $CaCO_3$, a decrease in pH, leaching of calcium and magnesium cations, transformations of mineral forms of iron, organic matter accumulation and the formation of aggregate soil structures (Mavris et al., 2010; Bernasconi et al., 2011; Kabala & Zapart, 2012). As nutrient availability is improving with increasing time since

deglaciation, due to the mineral dissolution and weathering, colonizing plants are now able to colonize the deglaciated soil (Ohtonen et al., 1999). Plants enhance biological weathering already started by microbes, as root exudates weather stone surfaces and plant roots enhance crack formation in rocks (Hall & Walton, 1992; Bashan et al., 2002). The slow growing lichens (a symbiotic relationship between green algae or *Cyanobacteria* with fungi) colonize mainly rock surfaces and are also well known to cause biological weathering of rock material. This colonization of plants and lichens and their weathering activities are beneficial for further vegetation succession, as the nutrient content of the soil is increased and the soil surface stabilized (Breen & Levesque, 2008; de los Rios et al., 2011). Years of biological, chemical and mechanical weathering results eventually in the accumulation of organic carbon, an increase in the clay and silt content and the decrease of the skeletal content of the soil (Smittenberg et al., 2012). This ultimately leads to a densely vegetated soil with distinct soil layers (Dumig et al., 2011) (Figure 1).

The succession of soils after glacial retreat, differing in age, texture, biological and chemical properties is called a soil age chronosequence within a glacier forefield (Figure 1). As the glaciers have periodically decreased since 1850 after the last major glacier advance related to the little ice age (Bernasconi et al., 2011), a glacier forefield can represent a succession of soils from a few decades up to 160 years of soil age, depending on the location of the glacier and its rate of retreat. Generally glaciers are located either at high altitudes or in the cold regions of the world, thus manly in the Andes, the Alps, the Rocky Mountains, the Himalayas and around the two Earth Poles.

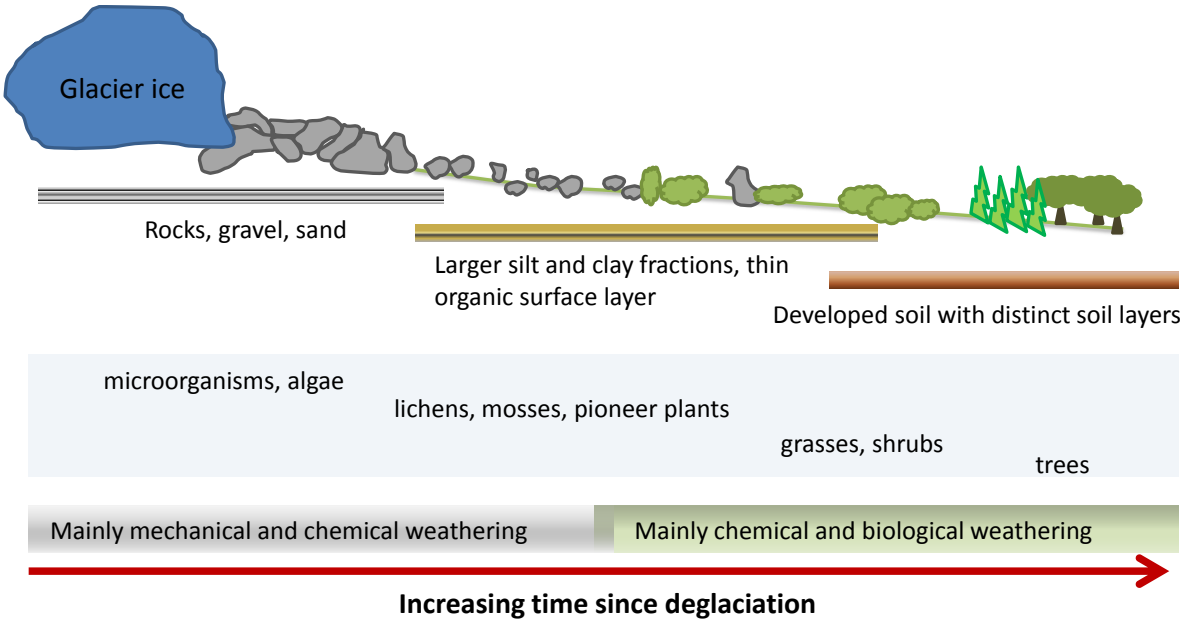


Figure 1: A glacier forefield is characterized by a chronosequence of soils which are differing in texture, biological colonization and weathering type.

1.1.2 Polar Glacier Forefields

The Antarctic continent is covered to a large part by ice (Figure 2). Near the coast, glaciers protrude out of the thick continental ice layer onto ice free regions. The Antarctic Peninsula is especially characterized by around 400 largely independent mountain glaciers draining into ice shelves or into marine tidewater glaciers, but some also terminating on land. The Antarctic Peninsula has experienced a drastic warming in the last 50 years of about 2 – 5 °C per century. The glaciers and glacier forefields there have a great similarity to sub-polar glacial systems (such as coastal Greenland, Svalbard, Patagonia and Alaska), as they are more sensitive to atmospheric warming than the less sensitive cold ice sheets covering the rest of the Antarctic continent (Vaughan, 2006). Research on glacier forefields on the Antarctic Peninsula included studies on soil properties (Strauss et al., 2009; Ramos et al., 2012), studies on airborne microbial diversity (Hughes et al., 2004) and soil microbiology (Yergeau et al., 2007 a; Yergeau et al., 2007 b; Yergeau et al., 2009) amongst others. Other main biological research on soil on the Antarctic continent has been conducted around the Ross Ice Shelf and Victoria Land (Barrett et al., 2006; Niederberger et al., 2008), in the McMurdo dry

valleys (Shravage et al., 2007) and the neighboring glacial runoff called Blood Falls (Mikucki & Priscu, 2007) (amongst others).

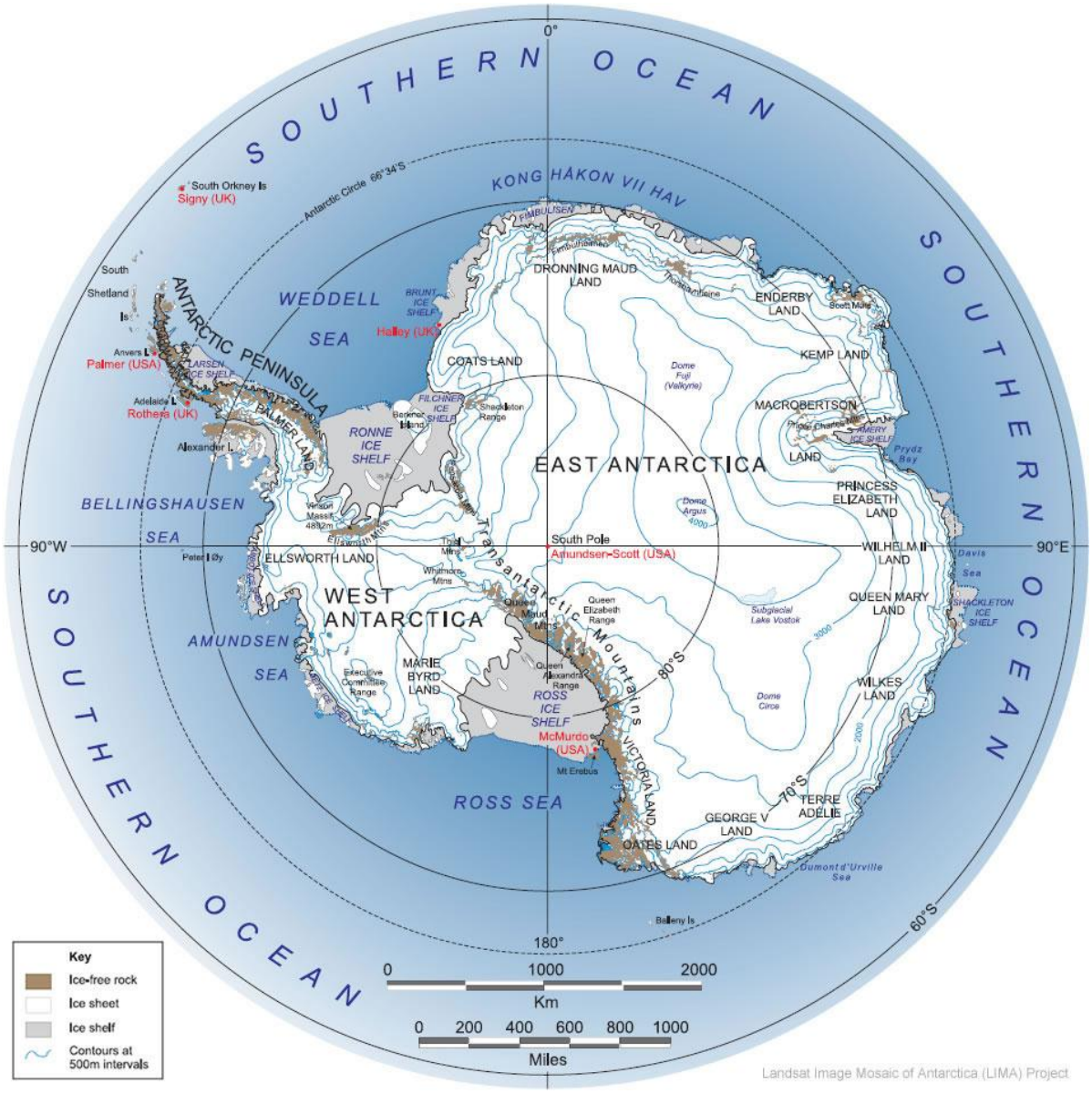


Figure 2: The Antarctic continent (from the Landsat Image Mosaic of Antarctica (LIMA)). On the Antarctic Peninsula (west coast) and in the McMurdo area (south), there are the ice-free zones were most scientific research has been performed.

Compared to Antarctica (without the Antarctic Peninsula), the Arctic experienced a larger increase in temperatures over the past 50 years, and the glaciers are therefore retreating faster (Turner et al., 2007). Arctic glacier forefields are mainly located in Greenland, Svalbard (Norway), North Canada and Russia and are mostly classified as permafrost soils (Figure 3). The vast majority of research performed in microbial ecology in the Arctic has been conducted on the Islands of Svalbard. The research ranges from rock weathering (Borin et al., 2010), the succession of mycorrhizal fungi (Fujiyoshi et al., 2011) and bacteria (Schutte et al., 2010) along glacier forefields to research on cryoconite holes (Sawstrom et al., 2002; Stibal et al., 2008) and on Aeolian fallout on the Islands (Hawes, 2008) just to name a few. Substantial research on glacier forefields is also being conducted in high arctic regions of Canada, investigating the microbial life beneath glaciers (Skidmore et al., 2000), biological soil crusts (Breen & Levesque, 2008) and the effect of low temperatures on biodegradation (Eriksson et al., 2001), whereas in the subarctic Tundra in Sweden the effect on warming on carbon turnover was investigated (Rinnan et al., 2007).

Figure 3: The Northern Polar region (from Schuur et al. (2008)). The soils are mostly classified as Permafrost soils. The sea around the north-Pole is covered by a thick ice sheet. Most scientific research is performed on the islands of Svalbard (Norway) and in the Arctic regions of Canada.

1.1.3 Temperate Glacier Forefields

Temperate glacier forefields are mainly located in mountainous ecosystems like in the Alps, the Andes, the Rocky Mountains and in the Himalayas. These mountain ecosystems have much in common with the polar ecosystems with respect to slow soil formation, short growing season and considerable changes in vegetation cover due to climatic changes over the last decades. However there are a number of differences between these ecosystems: firstly, high altitudes of the temperate zone generally receive more precipitation, temperatures are warmer (annual mean of 2.2 °C in the Alps compared with -11.6 °C in Greenland (Jones et al., 2000), and the snow cover is thicker. Consequently, these soils are better insulated and soil frost is less deep. Secondly, natural disturbances such as soil erosion, rock fall, and avalanches play a more important role in ecosystem functioning (Hagedorn et al., 2010). All these factors lead to a faster ecosystem development after glacier retreat than at higher latitudes. The temperate glaciers, especially in the Alps, are furthermore very well monitored, thus the exact mass and length losses are known as well as high definition meteorological data during the year.

In the European Alps, the observed increase in air temperature was more than twice the increase in global mean temperature over the last 50 years (Cannone et al., 2008). It can be estimated that glaciers in the European Alps lost about half their total volume between 1850 and 1975, another 25% of the remaining amount between 1975 and 2000, and an additional 10% – 15% in the first 5 years of this century (Haeberli et al., 2007). In the last ten years back to 2002, the glaciers lost more volume and surface area than in the 35 years before from 1967 to 2002 (Paul et al., 2004; Cannone et al., 2008). Thus the small glaciers in the Alps might disappear during the next decades. Other temperate glacier areas like the Rocky Mountains, the Andes or the Himalayas are not studied that intensely, but it can be expected that the rates of glacier retreat are of similar extent.

Research activities in temperate glacier forefields range from successional studies in the European Alps (Sigler & Zeyer, 2002 b; Nicol et al., 2005; Tscherko et al., 2005; Lazzaro et al., 2012) to investigations in broader areas in the Rocky Mountains (Jumpponen, 2003; Ley et al., 2004; Lipson &

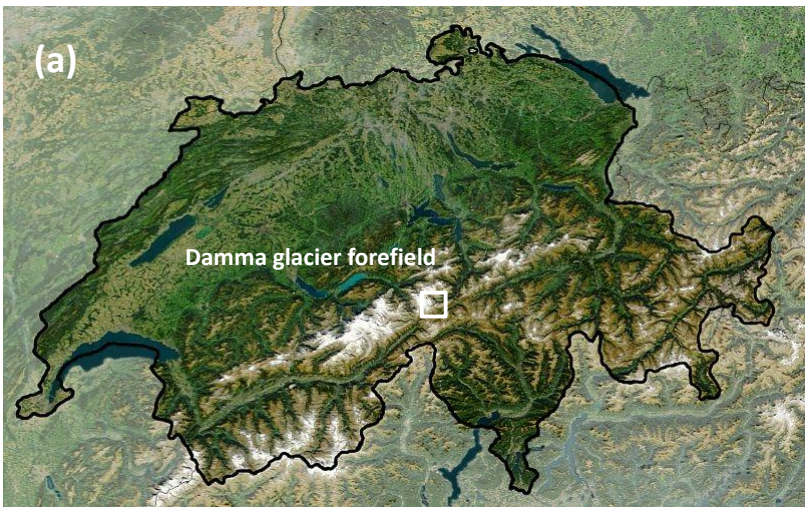
Schmidt, 2004), over to the South American Andes (Schmidt et al., 2008; Garibotti et al., 2011) and increasingly, the Himalayas (Srinivas et al., 2011). This thesis was conducted on the Damma glacier forefield located in the Swiss Central Alps and is introduced in the next sub-chapter.

1.1.4 The Damma Glacier Forefield

The study site for this thesis was the Damma glacier forefield, near Göscheneralp in the Canton of Uri, Switzerland (Figure 4). It is located in the central Aar Massive; at 46°38' N 8°27' E, at an altitude between 1950 m and 2050 m a.s.l.. The Damma glacier forefield has been subjected to extensive interdisciplinary studies through the CESS project BigLink (**Bi**osphere-**g**eosphere interactions: **Link**ing climate change, weathering, soil formation and ecosystem evolution) of the ETH domain (Bernasconi et al., 2011). The mean annual precipitation is about 2400 mm and the mean annual temperature is around 1.8°C. The Damma glacier has retreated at an average rate of 10 m y^{-1} since 1921, with two short advancing periods between 1920 – 1928 and 1970 – 1992, resulting in two terminal moraines within the soil age chronosequence. As a result, the glacier forefield is divided into three soil age classes. In the young section the soil is between 2 to 18 years old, in the intermediate section the soil is between 57 and 79 years old and in the old section the soil is between 108 and 140 years old (Bernasconi et al., 2011) (Figure 4). In 2003, a large block of stagnant ice detached from the main glacier and remained in the valley. This block of ice will be called glacier terminus or dead ice body from now on.

Studies performed on the Damma glacier forefield included investigations on biology, geology and soil development. The biological research included the influence of pioneering plants on microbial communities (Miniaci et al., 2007 a), the diversity of bacteria and fungi along the soil chronosequence (Sigler & Zeyer, 2002 b; Duc et al., 2009; Lazzaro et al., 2012; Welc et al., 2012) and their weathering potential in the recently deglaciated terrain (Frey et al., 2010; Brunner et al., 2011). The other studies investigated the stream water chemistry (Hindshaw et al., 2011) and the isotopic

fractionation and carbon dynamics along the forefield (Kiczka et al., 2011; Smittenberg et al., 2012), amongst others. Most of the studies performed on the Damma glacier forefield focused on the soil age chronosequence, but especially the recently deglaciated terrain without vegetation is an interesting study site for microbial primary colonization, microbial adaptation and on microbial survival strategies in this harsh environment. This however, has not been thoroughly studied yet. Additionally, bare soil provides the possibility to investigate the reaction of microbial communities to a changing climate, as the heterogenic nature of recently deglaciated terrain (large stones, sandy patches and melt water), creates different microclimates in sites close to each other. Furthermore, a small scale investigation of the succession of bacteria, archaea and fungi along the soil age chronosequence is needed to be able to correlate microbial colonization with physico-chemical soil parameters.



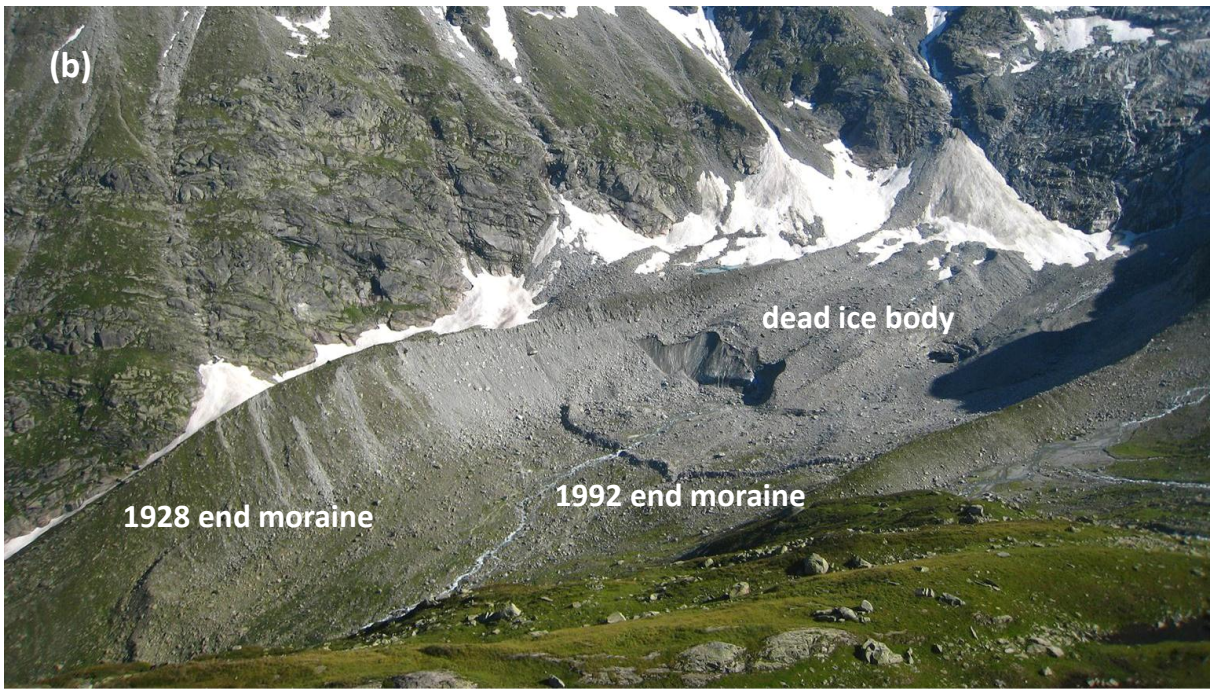


Figure 4: (a) Map of Switzerland with the location of the Damma glacier (from www.psteiner.net). (b) Photograph of the Damma glacier forefield taken in September 2010. The two end moraines of 1992 and 1928 can be seen in the glacier forefield, as well as the stream and the glacier terminus (dead ice body).

Through the ETH project BigLink and its recent nomination as a Critical Zone Observatory (CZO) (Banwart et al., 2011), the Damma glacier forefield has become one of the most intensively studied glacier forefields in the European Alps. The only other glacier studied in a similar extent in the Swiss Alps is the Morteratsch glacier which attracted scientific interest as early as 1912 (Buchanan, 1912). The two glacier forefields are well comparable, as they lie on similar altitudes (1900 to 2400 m.a.s.l.), on granitic bedrock, have similar length and both have a north-to-south orientation, which makes

them susceptible for the same climatic variability over the past century. The only main difference lies in the fact that the Morteratsch glacier retreated faster (Smittenberg et al., 2012) and furthermore did not readvance between 1920 and 1928 and between 1970 and 1992, which makes the Damma glacier forefield an exceptional study site.

The main characteristics of the forefield were found to be similar. In both forefields the pH decreases (from approx. 5.5 to 4.5) with soil age while the clay content increases as do the organic nitrogen and organic carbon contents (0.004% to 0.4% organic N and 0.1% to 4% organic C) (Egli et al., 2006; Bernasconi et al., 2011). However, while the Morteratsch glacier forefield has been intensively studied with regards to mineral weathering (Mavris et al., 2010), organic matter and phosphorous transformation (Egli et al., 2012) and plant succession (Burga et al., 2010), the focus of research on the Damma glacier forefield has been more on the microbial succession (Sigler et al., 2002 a), microbial function (Frey et al., 2010; Brunner et al., 2011; Lapanje et al., 2011), carbon evolution (Smittenberg et al., 2012; Gülland et al., 2012 b) and stream water chemistry (Hindshaw et al., 2011). The extensive studies performed on these two glacier forefields could be combined to give a good overview on initial soil development, primary biological colonization and mineral and elemental transformations along soil age chronosequences in the Central Alps.

1.2 Soil Physico-Chemical Gradients in a Glacier Forefield

1.2.1 Nutrient Availability

During primary microbial and plant succession, mineral nutrients are increasingly incorporated into complex organic matter and nutrient availability changes (Allison et al., 2007). Most notably, the availability of mineral phosphorus (P) declines due to weathering and drainage loss (Chapin et al., 1994; Crews et al., 1995) while carbon (C), nitrogen (N) and sulfur (S) accumulate with time in organic matter (Chapin et al., 1994; Crews et al., 2001; Smittenberg et al., 2012). As a result, the abundance of C, N and S relative to P increases dramatically during succession (Figure 5).

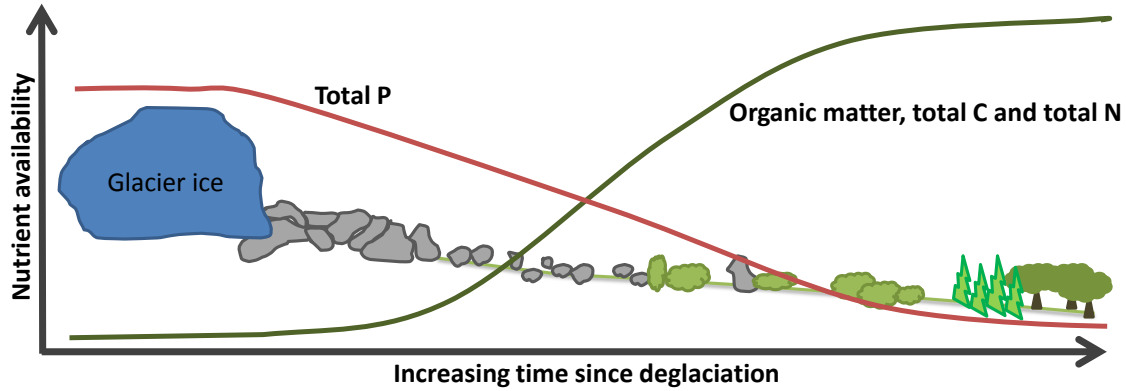


Figure 5: Nutrient contents and availability of the soil change along the forefield chronosequence. Total P decrease with soil age, whereas organic matter and total C and N increases.

Organic matter builds up along the chronosequence. This correlates with higher numbers of microorganisms, plants and animals. After the easily degradable organic matter is decomposed, the remaining soil organic matter becomes older and more recalcitrant and therefore more resistant to decomposition. Eventually this results in a lower availability of organic carbon for microbes with increasing soil age (Goransson et al., 2011) and a higher availability in younger soils (Sigler & Zeyer, 2004). Consequently, the extent of C limitation for microbial growth increases with soil age. But it was found that the N limitation decreased (Goransson et al., 2011).

Not only does nutrient availability change along the chronosequence, it also changes seasonally. During winter, the input of fresh nutrients is almost zero due to the snow cover (Nemergut et al., 2005). Organic nutrients that accumulated on the snow are flushed onto the soil when snow melts in spring. Those nutrients, especially the easily degradable material, are then decomposed during summer. After the easily degradable material has been decomposed, nutrient composition changes from labile to more recalcitrant during autumn and winter (Lazzaro et al., 2012).

1.2.2 Soil Properties

With increasing soil age along the chronosequence (transect), the soil properties change gradually in a glacier forefield. Overall the soil develops from a bare stone and sand environment to developed soils with distinct soil horizons and a dense vegetation cover (Bernasconi et al., 2011). This change in soil characteristics has a large influence on the colonizing microbial communities.

The newly deglaciated terrain is a heterogeneous landscape characterized by large stones, melt-water rivers and batches with smaller stones and sand. Therefore sites close to each other can have very different soil properties. The moist and sandy batches are colonized by microorganisms that are forming a soil surface community, which eventually lead to the development of crusted surfaces (Breen & Levesque, 2008). There the soil has a significantly higher volumetric water content, higher organic carbon content, a greater silt and clay fraction and the soil particles are more stabilized compared to non-crusted soils (Breen & Levesque, 2008). Phototrophic organisms like *Cyanobacteria* furthermore raise the nutrient content of the soil as their photosynthetic activities provide significant input of C and N into the upper soil layers (Freeman et al., 2009). This soil stabilization and the higher water and nutrient content enhances plant establishment (Yoshitake et al., 2010). Plant roots in turn acidify the soil through root exudates, thus further decreasing the pH along the chronosequence (Bernasconi & BigLink, 2008). With time larger rocks are colonized first by lichens, later also by mosses and eventually plants. Chemical, biological and mechanical weathering of larger stones

decreases the skeletal content of the soil over time. Generally the soil texture changes from a high sand content towards a higher clay and silt content (Smittenberg et al., 2012). The dense root system in vegetated soil enhances the water holding capacity and increases the nutrient content further through root exudates and plant litter decomposition by soil microorganisms (Miniaci et al., 2007 a; Esperschütz et al., 2011 b). Overall, in newly deglaciated terrain without vegetation the environmental conditions in the soil vary widely within a close range, whereas older sites that are covered by dense vegetation show less variation.

1.2.3 Microclimatic Gradients

In polar regions most of the soils are classified as permafrost soils, which are frozen for most of the year (McKay et al., 1998). Therefore, temperatures only rise above 0°C in the uppermost soil layers during the summer months (Eriksson et al., 2001; Ramos et al., 2012), with maximums of around 10°C in the Arctic. In the Antarctic, soil temperatures only rarely rise above 0°.

In temperate glacier forefields, soil temperatures are stable and close to the melting point during winter when there is a snow cover. However, temperatures are significantly lower without a snow cover during autumn and spring (Freppaz et al., 2008). Then the soil can undergo freeze-thaw cycles, this consequently enhances stone fractionation and selects for soil microorganisms resistant to these temperature fluctuations. In summer the soil temperature can reach up to 30°C (personal measurement) and solar UV radiation is usually high at high altitudes due to the decreasing amounts of air molecules, aerosols, and clouds in the atmosphere (Schmucki & Philipona, 2002). The light intensity on temperate glacier forefields might therefore be twice as high as at sea level.

Depending on the thermal conductivity and heat capacity of the surface material, soil temperature can vary highly between day and night in recently deglaciated areas (French, 1970). Shading of nearby mountains, large stones or topographical heterogeneity can furthermore create differing microclimates at sites close to each other (Thorn et al., 2008). This is especially the case on glacier

forefields, which are usually located in valleys formed by the formerly larger glacier; there, the shading effect of mountains is very pronounced. Therefore, along a cross-section in front of the glacier terminus, the south-facing site receives more sun during a day than the north-facing site, which is more shaded by mountains. Vegetation cover dampens the effect of high radiation and exposition at later successional stages. Soil temperatures therefore fluctuate less and are generally lower in vegetated than in unvegetated soil (Smith, 1975).

Generally, soil moisture fluctuates seasonally. It is high in winter (under snow cover temperatures are slightly above 0°C, thus the water is liquid at the soil surface), during the snowmelt in spring and after rain events and low in summer after the snow has melted and the soil dries out (Bales et al., 2011). Along the chronosequence, soil moisture is elevated right at the glacier terminus, due to the proximity to melt-water from the ice (Strauss et al., 2009). The soil moisture then decreases in the upper soil layers in areas without vegetation, as the water drains away between stones. Soil moisture again increases in older soil, as it is able to retain more water when the organic layer and the clay fraction is increased (Strauss et al., 2009). Additionally, in recently deglaciated terrain exposition plays a major role, as long sunshine hours enhance evaporation and therefore lead to moister soil at sites shaded by mountains or stones. At later successional stages the vegetation cover shades the soil, thus there is less evaporation and the exposition does not play such a large role in soil moisture. In soil situated close to the glacier stream, soil moisture is mostly high but fluctuates seasonally depending on melt water.

The influences of changing microclimates on microbial communities have only rarely been studied, and the expositional differences between sites investigated have been neglected so far in earlier studies on the Damma glacier forefield. To study the reactions of microbial communities to a changing microclimate and to elucidate the key environmental parameters influencing microbial communities, soil transfer can be performed. There one can create differing microclimates in the same soil environment, therefore ensuring that the change in microclimate is the main factor to

inflict a change in the microbial activities and community structures and not a change in soil properties (Bottomley et al., 2006; Boyle et al., 2006; Lazzaro et al., 2011).

1.3 Microbial Communities along Glacier Forefields

1.3.1 Input of Microbes into Recently Deglaciated Terrain

Shortly after glacier retreat the newly exposed terrain is colonized by microbes despite the generally nutrient-poor environment. As microbes have been shown to survive beneath glaciers (Skidmore et al., 2000) and in glacier ice (Priscu et al., 1999), it is supposed that they colonize the new terrain through the glacier melt-water (Hodson et al., 2008). Additionally microbes can survive in cryoconite holes on top of the glacier ice (Sawstrom et al., 2002; Cameron et al., 2012). From there the microorganisms are then deposited on the newly deglaciated terrain with glacier melt. Later more input takes place through atmospheric deposition of particles containing microorganisms (Burrows et al., 2009) and through rain and snow (Sattler et al., 2001). Furthermore, microorganisms can also be introduced through human activities (Lee, J. E. & Chown, 2009; Olech, 2010). In any case it depends on the environmental factors at the specific site whether an introduced microorganism is able to grow and survive.

1.3.2 Microbial Succession along Glacier Forefields

On newly exposed soil that is devoid of life, a microbial primary succession takes place before plants and animals arrive. The first microbial colonizers ameliorate the local conditions and facilitate colonization for other microorganisms. It was proposed that heterotrophic organisms are the first colonizers on recently deglaciated terrain, before autotroph succession begins (Hodkinson et al., 2002). However, it is now supposed, that primary colonizers like *Cyanobacteria*, green algae, lichenized and free living fungi, mosses, and heterotrophic bacteria all colonize the soil surface simultaneously, often forming biological soil crusts (Breen & Levesque, 2008; de los Rios et al., 2011). Generally it was found that the early stages of microbial succession are dominated by bacteria, while during later stages fungi are dominant, mainly because of their association to plants as mycorrhizal species.

There has been a large interest in studying microbial succession along glacier forefields in recent years. Multiple studies addressed the succession of archaea, bacteria, fungi, algae and lichens along glacier chronosequences (Jumpponen, 2003; Sigler & Zeyer, 2004; Nicol et al., 2005; Garibotti et al., 2011) (amongst others). However, most phylogenetic groups have been investigated separately; therefore there is still a lack of knowledge around the simultaneous colonization and succession of bacteria, archaea and fungi along a glacier forefield chronosequence and also around which soil physico-chemical parameters are driving the microbial succession.

Bacterial succession starts with opportunistic heterotrophs (like *Actinobacteria*, *alpha-* and *beta-Proteobacteria*) colonizing the bare soil near the glacier terminus (Nemergut et al., 2007; Esperschütz et al., 2011 a). Some were primarily found in the young soil, but not in the old soil like the *Comamonadaceae* clade of *beta-Proteobacteria* (Nemergut et al., 2007). Additionally, photosynthetic organisms like *Cyanobacteria* (but also algae) also primarily colonize the young soil, but their numbers decrease with soil age, probably due to shading effects of the increasingly dominant vegetation cover. In the older soil ages, mainly *Acidobacteria*, *Bacteroidetes*, *delta-Proteobacteria*, *Firmicutes* and *Verrucomicrobia* have been found (Nemergut et al., 2007). Many of them are involved in litter decomposition or are associated with plants. There is conflicting information about bacterial phylotype diversity over time following glacier retreat. Some studies found that it increased (Nemergut et al., 2007; Schutte et al., 2010) while others found the opposite (Sigler et al., 2002 a; Sigler & Zeyer, 2002 b; Fierer et al., 2010). Therefore, it seems that the diversity along chronosequences is not coherent between differing glacier forefields. One reason for this incoherence might be the presence of pioneer plants that can increase or reduce the diversity of certain bacterial phylogenetic groups. For instance it was shown that bacterial cell numbers decreased with distance from pioneering plants in a glacier forefield (Miniaci et al., 2007 a). However, this was the opposite for diazotrophic bacteria, as a significantly higher phylotype richness in bulk soil was found compared to rhizosphere soil (Duc et al., 2009). As a glacier forefield offers a

wide range of environmental conditions within itself, it is very difficult to find consistent results across several forefields.

Primary colonization of fungi in recently deglaciated terrain has only been rarely studied, the main focus lying on the mycorrhiza forming *Basidiomycota* and their colonization of plants along the forefield (Alfredsen & Hoiland, 2001). Pioneer soil often lacks inocula of mycorrhizal fungi (Trowbridge & Jumpponen, 2004) and therefore the plants in recently deglaciated soils are not yet colonized by mycorrhiza (Fujiyoshi et al., 2011). Later in succession, plants that form associations of mycorrhizal fungi dominate the vegetation, therefore increasing the diversity and numbers of fungi forming mycorrhizal relationships (Fujiyoshi et al., 2011; Oehl et al., 2011). Furthermore, fungi are associated to algae or *Cyanobacteria* in a symbiosis known as lichens. Lichens are early colonizers of rock surfaces and bare soil, such as recently deglaciated areas. They can contribute to the nitrogen economy in areas where this nutrient is limiting (Breen & Levesque, 2008).

So far archaea have been mostly neglected in microbial studies in glacier forefields. Only one study showed that within the *Crenarchaeota*, Group 1.1b *Crenarchaeota* populations dominate throughout the successional gradient, while Group 1.1c *Crenarchaeota* are present in mature soil only (Nicol et al., 2005). The other main archaeal group, the *Euryarchaeota,* were not studied at all so far. Overall archaeal diversity shows a decrease in diversity and abundance along the soil chronosequence (Esperschütz et al., 2011 a). As archaea are known to survive in extreme conditions around the world, it would be crucial to study their colonization pattern along a glacier forefield chronosequence and link it to changing environmental and soil parameters.

1.3.3 Assessing Phylogenetic Diversity

Evidence is growing that soil harbors most of the planets biodiversity, the reason being that even at small scales one finds a large diversity. In the beginning of microbial research, attempts were made to culture microbes from environmental samples, to be able to classify the microbes according to

their morphological and metabolic properties. However, as only a small portion of microorganisms sampled in the environment can be cultivated, methods for the cultivation-independent phylogenetic identification had to be developed (Rappe & Giovannoni, 2003). As a consequence, the number of for instance bacterial phylogenetic divisions identified has increased from roughly 12 related groups in 1987 (Woese, 1987) based on cultivated organisms to over 40 groups in 1996, mainly through cultivation independent approaches. In that study 12 phylum- or division-level bacterial lineages with no pure culture isolates were identified, which are referred to as "candidate divisions (Hugenholtz et al., 1998). Also the number of such "candidate divisions" increased to more than 36 today, representing groups where only environmental sequences are available with often no information on their function in an ecosystem (Glockner et al., 2010). This illustrates the increase in phylogenetic groups known to researchers today through culture independent techniques.

Generally, a description of a community structure should include the number of species (diversity), the number of cells of each individual species (evenness) and the role of each species in the environment where it was found (Hugenholtz et al., 1998). Therefore, it is obvious that to assess the community structure in a complex ecosystem, thousands of sequences have to be analyzed, which is a long and laborious undertaking. Nevertheless I constructed clone libraries from 16S rRNA or 18S rRNA PCR amplifications to assess phylogenetic diversity, combined with terminal restriction length polymorphism (T-RFLP).

T-RFLP is frequently used as a community fingerprinting method, from which the Shannon Diversity Index (H) can be calculated through the formula $H = [\text{Sum } p_i * (\ln p_i)^2 - (\text{Sum } p_i * \ln p_i)^2]$ where p_i is the intensity of the T-RF (Sigler & Zeyer, 2002 b). It can be coupled with clone library analyses by digesting the clones and therefore assigning specific T-RFs to a phylogenetic species. If the species found has been cultured and investigated before, one can connect the T-RF to a function in the ecosystem. Other diversity indexes are the phylotype richness (S) which is simply the total number of distinct T-RFs in a profile. A widely used diversity index is the Simpsons index (D), which is calculated as $D = 1 - \text{Sum } (p_i)^2$ (Simpson, 1949; Dunbar et al., 2000). Overall one can state that community

structure analysis with T-RFLP is a widely used method, which gives an estimation about the diversity in a sample and, through multivariate statistical methods, one can determine the similarity of communities from different samples. Clone libraries can then be used to assess community composition, which might give an indication of the function a specific species plays in the environment.

Other possible profiling methods are for instance Shotgun sequencing of protein encoding genes (metagenomics), which provides a quantitative phylogenetic assessment of microbial communities (von Mering et al., 2007), or to evaluate the functionality of the microorganisms in an ecosystem (metaproteomics), where the proteins of microbial communities are extracted and the fingerprinting takes place on the proteins instead of the DNA (Maron et al., 2007).

1.4 The Carbon Cycle in Newly Deglaciated Terrain

1.4.1 The Carbon Cycle

The carbon cycle is the biogeochemical cycle by which carbon is exchanged among the biosphere, pedosphere, geosphere, hydrosphere, and atmosphere of Earth. As almost half of the dry mass of life on earth and also half of the organic matter accumulating in soil is composed of carbon, the carbon cycle has a central role in biochemical processes in almost all ecosystems (Chapin et al., 2006).

The cycle depends on autotrophic organisms that produce their own organic compounds, using CO_2 from air or water through photosynthesis (these organisms are called carbon fixers). Photosynthesis follows the reaction $6CO_2 + 6H_2O$ + Light Energy (Sun) → $C_6H_{12}O_6 + 6O_2$. Carbon is then transferred within the biosphere as heterotrophs like bacteria and fungi feed on autotrophic organisms (or parts of them) and decompose dead organic matter (detritus). The degradation and subsequent respiration reaction does need oxygen and is as follows $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$. The carbon therefore leaves the biosphere again through respiration of CO_2. This CO_2 can then be used by autotrophs, and the carbon cycle starts again (Figure 6).

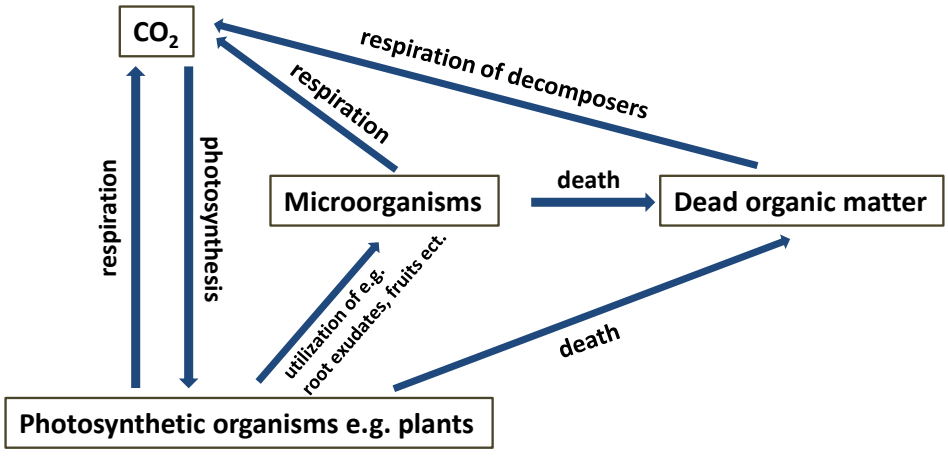


Figure 6: The carbon cycle in soil. Photoautotrophic organisms like algae, *Cyanobacteria* and plants produce organic compounds, using CO_2 from the atmosphere. Organic carbon is then transferred within the biosphere as heterotrophs like for instance bacteria and fungi feed on autotrophic organisms (or parts of them) and decompose dead organic matter like roots and plant litter. Through this degradation and respiration CO_2 is transported to the atmosphere, where it can then be used by autotrophs again.

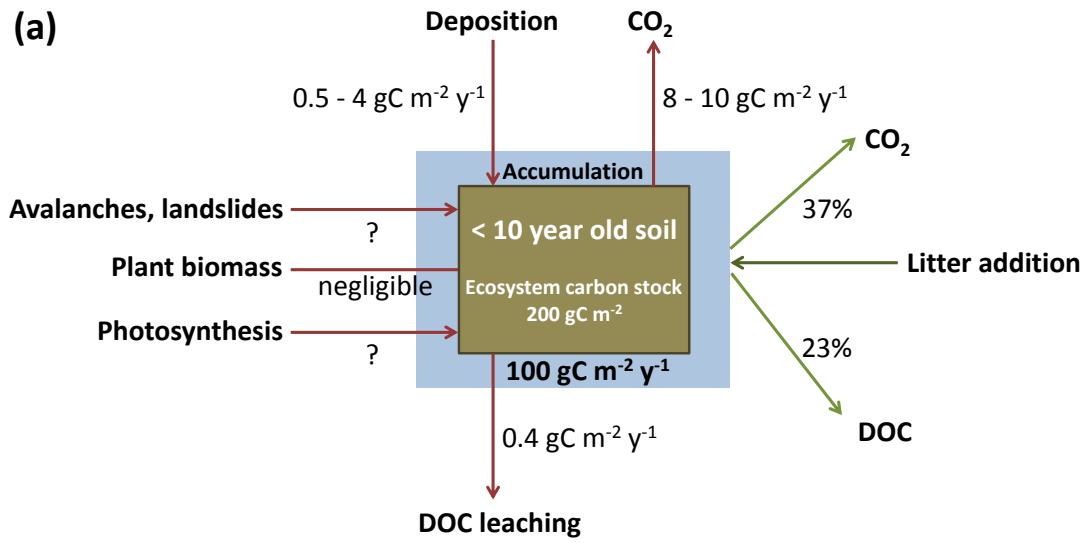
1.4.2 Carbon Pools

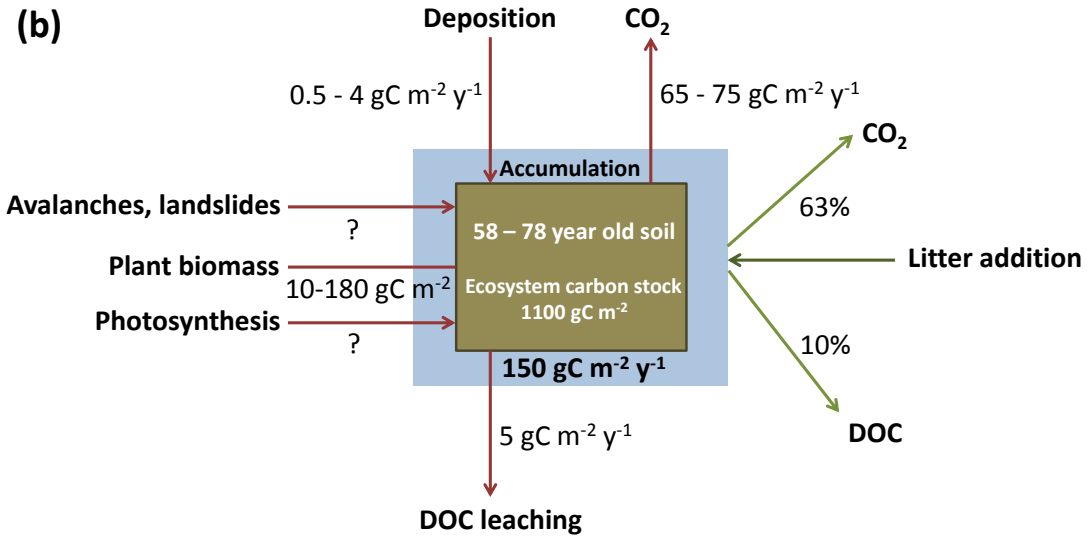
Generally C stocks in glacier forefield soil is composed of ancient, deposited, autotrophically fixed C or of senescent biomass. These C stocks are eventually decomposed by heterotrophic organisms and incorporated into their biomass or partly respired as CO_2.

Surprisingly, organic carbon is already present in the fine granitic sediments right after glacial retreat, in the Damma glacier forefield for instance 0.1 mg C g^{-1} soil was measured right at the glacier terminus, the same amount as in an Austrian glacier forefield (Bardgett et al., 2007; Bernasconi et al., 2011). A probable source is organic matter that was previously beneath the glacier, containing remnants of preglacial soils and geological carbon (Bardgett et al., 2007). For most recently deglaciated soil, radiocarbon-based age estimates indicated ancient C to be the dominant source of soil-respired CO_2 (Gülland et al., 2012 b). Additionally the carbon originates from the surface of the glacier where it is derived from fossil fuel burning (black carbon) or from modern autotrophic sources (Bardgett et al., 2007). As soon as new soil is exposed to wind and weather, further organic carbon is deposited through the atmosphere (Jurado et al., 2008) and through wind from more fertile grounds (Hawes, 2008). It is difficult to find comparable numbers of the amount of carbon deposited with wind, rain and snow on glacier forefields. On the Damma glacier the deposition of carbon with snow was estimated to 0.4 to 1.2 gC m^{-2} y^{-1} (Brankatschk et al., 2011). At an Alpine site in Austria an annual deposition of 3.4 gC m^{-2} y^{-1} was measured (Pogodina, 2003) and in the Colorado Rocky Mountains the Aeolian deposition was around 0.5 gC m^{-2} y^{-1} (Figure 7), there it was furthermore proposed that this is the dominant input of C into high altitude regions (Ley et al., 2004). Freeman et al. (2009) showed that a significant amount of carbon is brought already into a bare soil ecosystem through photosynthetic activities (up to 23.7 gC m^{-2} y^{-1}), despite short summers at the earth poles and the snow cover on temperate glacier forefields, which limits the time for autotrophic acquisition of C (Ley et al., 2004). Therefore, photoautrotrophic C can be a noteworthy input of carbon into the soil ecosystem, even though this primary production of carbon was considered to be low compared to allochthonous carbon input (Stibal et al., 2008). Additional input of carbon into glacier forefield soil can happen through avalanches and landslides, which deposit soil from the side moraines and the

nearby mountains onto the forefield, but this has not been quantified so far. Microbial communities could also decompose microbial derived cell fragments (from dead microorganisms) and use this decaying matter as a carbon source. But this possibility has not been investigated so far in glacier forefields. Overall, the ecosystem carbon stocks of soil less than 10 years of age without vegetation, has been estimated to 200 gC m^{-2} (Smittenberg et al., 2012) (Figure 7).

In later successional stages, the input of carbon to soil through autotrophic activities is much higher due to the growth of plants. Therefore, the contribution of new plant-fixed C to soil CO_2 respiration is as much as 90%. Additionally heterotrophically respired C from accumulated "old" soil organic matter become increasingly important in later successional stages (Gülland et al., 2012 b). It is however, difficult to estimate the net input rates of carbon into soil from plants, as for instance net primary productivity depends on plant species and communities (Torn et al., 2009). The vegetation type furthermore affects tissue chemistry and seasonality of inputs and plant species differ in the proportion of photosynthate partitioned to roots, shoots, or woody structures which are later decomposed at differing rates (Torn et al., 2009). We do however know, that despite the relatively low net primary production, cold ecosystems have relatively high ecosystem carbon contents as the degradation rates are low, reaching 1100 gC m^{-2} at soil ages around 60 – 80 years and up to 3100 gC m^{-2} at soil ages over 110 years (Smittenberg et al., 2012) (Figure 7). Most ecosystem carbon in cold regions is present as organic debris and soil organic carbon (Smittenberg et al., 2012) and the content increases with soil age along a glacier forefield (Conen et al., 2007). One cannot say if this carbon is available to microorganism, as the complex nature of soil organic matter (consisting of organic debris that is partially or fully decomposed to individual molecules, all with different chemical and physical properties, distributed between litter, pores, aggregates and on mineral surfaces with different physico-chemical stabilization mechanisms) expresses varying stabilities against breakdown (Smittenberg et al., 2012). Generally, the C accumulation rates increase from 100 gC m^{-2} y^{-1} in soil ages < 10 years to 150 gC m^{-2} y^{-1} in soil ages of around 80 years and decrease again to 60 gC m^{-2} y^{-1} in older soil ages (Gülland et al., 2012 b) (Figure 7).


(a)
Deposition
CO2
0.5 - 4 gC m-2 y-1
8 - 10 gC m-2 y-1
Accumulation
Avalanches, landslides
?
< 10 year old soil
Plant biomass
negligible
Ecosystem carbon stock
200 gC m-2
Photosynthesis
?
100 gC m-2 y-1
CO2
37%
Litter addition
23%
DOC
0.4 gC m-2 y-1
DOC leaching


(b)
Deposition
CO2
0.5 - 4 gC m-2 y-1
65 - 75 gC m-2 y-1
CO2
Accumulation
63%
Avalanches, landslides
?
58 – 78 year old soil
Litter addition
Plant biomass
10-180 gC m-2
Ecosystem carbon stock
1100 gC m-2
Photosynthesis
?
10%
150 gC m-2 y-1
DOC
5 gC m-2 y-1
DOC leaching

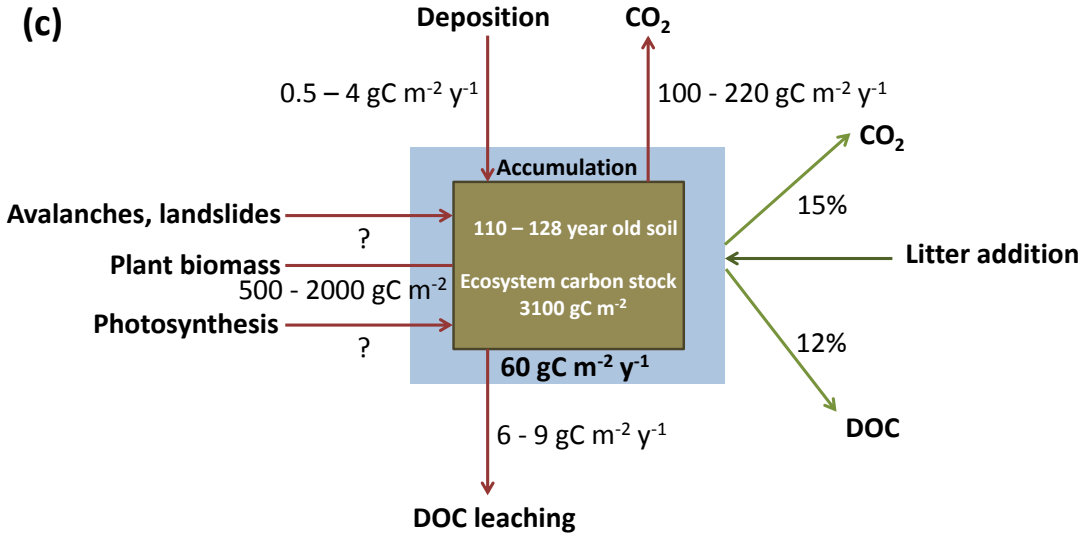

(c)
Deposition
CO2
0.5 – 4 gC m-2 y-1
100 - 220 gC m-2 y-1
CO2
Accumulation
15%
Avalanches, landslides
?
110 – 128 year old soil
Litter addition
Plant biomass
500 - 2000 gC m-2
Ecosystem carbon stock
3100 gC m-2
Photosynthesis
?
12%
60 gC m-2 y-1
DOC
6 - 9 gC m-2 y-1
DOC leaching

Figure 7: (p. 45) Carbon deposition, pools and fluxes in differing soil ages in the Damma glacier forefield, numbers taken from Pogodina (2003), Brankatschk et al. (2011), Smittenberg et al. (2012) and Gülland et al. (2012 a; 2012 b). (a) Recently deglaciated bare soil, where plant biomass is negligible and the input of C through photosynthesis has not been determined. Here a large input of carbon could happen through avalanches and landslides. (b) Intermediate soil ages from 58 to 78 years and (c) older soil ages from 110 to 128 years of age. For those later successional stages the input of carbon through photosynthesis and the senescence of plants is high, but difficult to estimate. The arrows in green represent the CO_2 efflux and DOC leaching data performed at the three soil ages in the Damma glacier forefield (Gülland et al., 2012 a).

1.4.3 Carbon Fluxes

Respiration of CO_2 is the largest efflux of C from a soil ecosystems to the atmosphere, another efflux mechanism is leaching (Torn et al., 2009). Both are decomposition fluxes which are a function of the soil C stock and its decay rate. In the Damma glacier forefield, sources of soil CO_2 effluxes through decomposition change along the chronosequence, due to different degradation times for different carbon compounds. Generally, root exudates, microbial cell contents, and some fresh litter compounds decompose in time scales of hours to months (rarely years). In contrast, organic compounds that are more resistant to decay in general, by association with soil minerals or aggregate structures, are decaying in the range of decades and centuries (Torn et al., 2009). In a natural ecosystems CO_2 fixation and carbon decomposition are influenced by many factors like soil moisture, temperature, substrate quality and the season (Hobbie et al., 2000). One can say that in soil about 50% of the decomposable biomass is respired as CO_2 and 50% is either incorporated into biomass or becomes part of the soil organic matter (Kindler et al., 2006; Miltner et al., 2009). Overall, C losses from soil by soil respiration and dissolved organic carbon leaching increases from 9 to 70 and further to 168 gC m^{-2} y^{-1} along the Damma glacier soil age chronosequence (Figure 7). (Dissolved organic carbon is a leaching product from plants, litter, and humus.)

CO_2 respiration is low in winter, but fluxes are especially high at the transitions of autumn to winter and winter to spring, as the temperature and water availability is higher (Hobbie et al., 2000). In the Damma glacier forefield 62% to 70 % of annual CO_2-effluxes are respired during the short summer

season. In a littering experiment it was shown that 15% to 63% of added litter C was lost via mineralization and dissolved organic carbon leaching during only three summer months (Gülland et al., 2012 b) (Figure 7).

The initial phase of litter decomposition is dominated by decomposition of labile C, which is mainly done by bacterial r strategists. The r phenotype permits immediate exponential growth after supply of a carbon source and other necessary nutrients (Stenstrom et al., 2001). The decomposition of complex litter compounds is later done by bacterial or fungal K strategists. The K phenotype develops as a result of carbon starvation and does permit growth at low nutrient concentrations and rather slow decomposition of complex compounds (Stenstrom et al., 2001). For both processes soil water content is a crucial factor that defines microbial activity and therefore degradation times (higher soil water content usually increases microbial activity) (Poll et al., 2008). On glacier forefields the microbes involved in soil carbon turnover are mainly decomposing fungi and mycorrhiza, gram-negative bacteria and also archaea (Esperschütz et al., 2011 a; Esperschütz et al., 2011 b). Microbe carbon degradation is mainly done by extracellular enzymes secreted by the cells. Microbial enzymes are therefore the agents of biotic carbon decomposition in soil.

1.4.4 How to Study the Carbon Cycle

Carbon has two stable isotopes (^{13}C and ^{12}C) and a radioactive isotope (^{14}C) that are useful tracers in the study of the soil C cycle. For instance, living plants exchange C with the atmosphere and it is assumed that plants have the same ^{14}C content as the contemporary atmosphere in which they live. When plants die, they no longer absorb ^{14}C from the atmosphere and their ^{14}C content declines as a result of radioactive decay. The ^{14}C content of organic matter can therefore indicate the length of time that C has resided in soils at the time of sampling and it can furthermore give an idea about carbon turnover times in soil (Levin & Hesshaimer, 2000; Garnett & Bradwell, 2010).

^{13}C is often used in litter experiments in the laboratories as well as in the field. There ^{13}C labeled litter is applied to soil and the ^{13}C can then be traced in the CO_2 respiration (with mass spectrometry) as well as in the microbial communities actively decomposing the labeled litter with molecular approaches (Evershed et al., 2006; Dumont et al., 2011; Lee, C. G. et al., 2011) (amongst others). One of the most used molecular methods to trace ^{13}C labeled compounds from a carbon source to a consumer organism is stable isotope probing (SIP), which will be introduced in more detail below (sub-chapter 1.5).

The primary way in which carbon moves from ecosystems back to the atmosphere is CO_2 respiration. CO_2 is produced in the soil by microbial decomposition (heterotrophic respiration) of organic matter and root respiration (Wang et al., 2009; Yevdokimov et al., 2010). The relative contribution of heterotrophic respiration to soil CO_2 flux can range from about 20% – 50% during the peak of the growing season to nearly 100% in the non-growing season (Hanson et al., 2000). In unvegetated soil like in recently deglaciated terrain most CO_2 originates from microbial heterotrophic respiration, making it possible to investigate the carbon cycle of microbes directly by measuring the soil CO_2 flux. For instance, after the addition of a known amount of a carbon source to soil, the carbon respired as CO_2 gives a direct indication on the amount of C actively respired (and therefore also the amount of C incorporated into cell biomass) by microorganisms.

Potential organisms involved in the carbon cycle either have to be able to fix carbon through photosynthesis or to produce enzymes that degrade organic compounds containing carbon. Carbon containing substrates are degraded by enzymes like chitinases, cellulases and glucosidases amongst others. Cellulase is probably the most important extracellular enzyme that can be studied, as cellulose is the main C source in soil (Stursová et al., 2012). Research on the carbon cycle and turnover therefore often involves the screening for extracellular activities of enzymes in either the soil environment or in pure cultures isolated from soil (Margesin et al., 2003; Koch et al., 2007).

1.5 DNA-Stable Isotope Probing

1.5.1 General Concept

Microorganisms have a vast diversity and an important role in element cycling on the globe. However, so far mainly the tiny fraction of cultivable microorganisms can be linked to specific processes. Therefore, it is an on-going challenge for microbial ecologists to identify which organisms are carrying out what metabolic activities in their natural environments (Kreuzer-Martin, 2007). Stable isotope probing (SIP) was therefore implemented by Radajewsky et al. (2000) in order to link specific microbial groups to specific functions. With SIP microorganisms involved in the metabolism of specific substrates can be identified in uncultured environmental samples directly. The general idea of SIP is that microbes incorporate stable isotope labeled substrate like ^{13}C or ^{15}N that is added to pure cultures or to environmental samples, into cellular biomass, phospholipid fatty acid, DNA and RNA. The labeled compounds can then be either selectively recovered by density-gradient centrifugation in a cesium chloride density gradient (CsCl density gradient) and identified by sequencing for nucleic acids, or in the case of lipids by separation and measurement and comparison of stable isotope ratios of individual lipids using chromatography and mass spectrometry (Dumont & Murrell, 2005; Kreuzer-Martin, 2007; Neufeld et al., 2007 b). With these approaches, phylogenetic groups actively metabolizing substrates can be identified directly in microbial communities from an environmental sample *in situ* (Morris et al., 2002; Evershed et al., 2006; Lu et al., 2007) (amongst others).

Most SIP approaches use the stable isotope ^{13}C in a substrate to label microbial cell components; others use the stable isotope ^{15}N (Cadisch et al., 2005). SIP was first used in the analysis of phospholipid fatty acids (PLFA). Groups of microorganisms that incorporated the ^{13}C-substrate like for instance fungi, gram-negative or gram-positive bacteria show signature PLFA molecules (like an unique fingerprint) that can be identified (Dumont & Murrell, 2005). However now, DNA-SIP is the method of choice, as downstream applications with the extracted DNA, like terminal restriction fragment length (T-RFLP) analysis, cloning, sequencing and phylogenetic analysis are more exact in

phylogenetic identification of microbial species metabolizing a certain substrate than PLFAs (Dumont & Murrell, 2005). A unique advantage of DNA-SIP is the possibility to identify microorganisms by targeting not only the ubiquitous SSU rRNA gene, but also functional marker genes (e.g. genes encoding key enzymes of a metabolic pathway) (Friedrich, 2006). Furthermore, RNA-SIP has been used (Manefield et al., 2002 a; 2002 b), as incorporation of the ^{13}C into RNA is faster than into DNA (over 8 hours, labeled carbon accumulated in RNA almost 10-fold more quickly than in DNA (Whitby et al., 2005)); therefore reducing incubation times. However, purification and identification after density centrifugation are more difficult, as RNA molecules are less stable than DNA molecules and not as easy to extract from an environmental sample (Manefield et al., 2002 a).

1.5.2 Tracking of ^{13}C-labelled Carbon from Source to Consumer in Soil

To track the carbon from source to consumer, a ^{13}C labeled carbon source is added to soil *in situ* or in a controlled laboratory experiment. Ideally soil samples are then taken after several different incubation times to be able to investigate different metabolization states of the added carbon. DNA is extracted from the incubated soils and a density-gradient centrifugation is performed in a cesium chloride solution. During centrifugation the cesium chloride solution forms an atomic density gradient in the centrifugation tube, increasing from top to bottom. The DNA buoyant density is higher when it contains ^{13}C compared to ^{12}C. This leads to an equilibration of the ^{13}C-DNA the denser cesium chloride fractions lower in the centrifuge tube, whereas the ^{12}C-DNA equilibrates higher in the tube (Figure 8). This allows an efficient separation of the “heavy” ^{13}C DNA from the “light” ^{12}C DNA. The DNA of the microorganisms metabolizing the heavier labeled ^{13}C substrate is therefore separated from the non-labeled DNA coming from the microorganisms not metabolizing the labeled substrate (Radajewski et al., 2000; Manefield et al., 2002 a; Lueders et al., 2004 a; Neufeld et al., 2007 b).

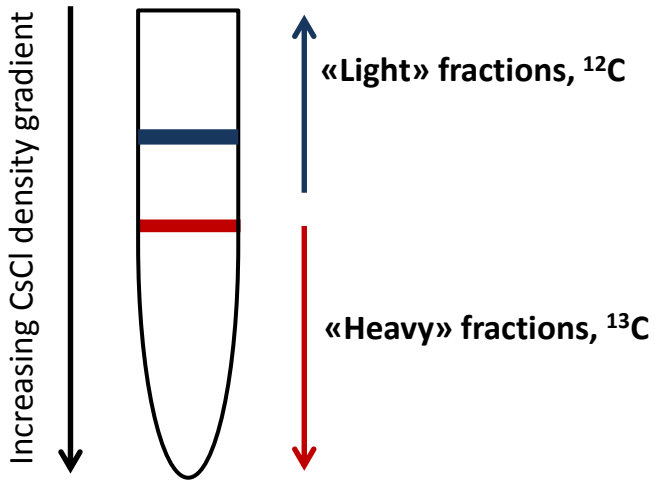


Figure 8: The "light" ^{12}C-DNA and the heavy ^{13}C-DNA are separated in a CsCl density gradient. After centrifugation the DNA is equilibrating with its respective density in the gradient, which is denser for ^{13}C-DNA, therefore it equilibrates lower in the centrifuge tube than the ^{12}C-DNA.

The SIP technique used in this thesis, which can resolve low amounts of nucleic acids, was first described by Manefield et al. performing RNA-SIP (2002 a; 2002 b). The protocol introduced fractionation of complete gradients after centrifugation by drop collection from the bottom with a syringe. This method was then adapted to DNA-SIP by Lueders et al. (2004 a). In this protocol, the centrifuge tube used for fractionation is perforated at the top and at the bottom. The different density fractions are collected at the bottom with a syringe by collecting equal amounts of drops for each fraction. This allows monitoring the gene distribution over the whole density gradient instead of only one "heavy" and one "light" fraction. The DNA is precipitated after fractionation and can be used for T-RFLP profiling, quantitative PCR, cloning and sequencing in order to characterize the microbial communities metabolizing the added carbon substrate.

The SIP technique has rarely been used to study polar or alpine ecosystems so far. On the Damma glacier forefield litter turnover and food-web dynamics were investigated using ^{13}C labeled litter and subsequent analysis of the PLFAs (Esperschütz et al., 2011 a). It was found that litter decomposition proceeded faster in older soil for the easily degradable litter fractions. Additionally, incorporation of ^{13}C into PLFAs was evident at all soil ages. The contribution of archaea, fungi and protozoa to litter

degradation was highest in recently deglaciated soil, whereas in older soil *Actinomycetes* and bacteria were the prominent litter degraders (Esperschütz et al., 2011 a). Other studies in Arctic ecosystems focused on the methanotrophs in permafrost soils, using ^{13}C labeled methane to investigate the activity and diversity of the methanotrophs and their reactions to changing temperatures. Warming of the permafrost leads to the release of methane to the atmosphere contributing to global warming. The study showed that the microorganisms can control and reduce the amount of methane which is released to the atmosphere due to warming (Martineau et al., 2010; Graef et al., 2011).

Analysis of the degradation of other carbon sources like for instance microbial cell fragments on a DNA basis are still missing in recently deglaciated terrain. But it could give a better insight into the microbial communities actively metabolizing an added substrate, as DNA is more exact in unraveling microbial community composition than PLFA analysis.

1.6 Objectives of the Thesis

The objectives of this thesis were to analyze microbial succession along the Damma glacier forefield, microbial adaptation to changing microclimates and the ability of microbes to metabolize microbial cell fragments. The main focus was on the recently deglaciated bare soil near the glacier terminus, where environmental influences are not dampened by a vegetation cover and where the nutrient content is low. The samples from the Damma glacier forefield were mainly analyzed using molecular approaches like DNA extraction, T-RFLP profiling, SIP and sequence analysis, but also using enzyme and activity measurements and diverse methods to determine soil physico-chemical parameters.

In a first part I investigated whether the three microbial groups bacteria, archaea and fungi undergo a community succession with increasing soil age and if this succession is correlated to the changing soil physico-chemical parameters along the chronosequence. There have been a number of studies on microbial succession along glacier forefields, but a simultaneous investigation on the three main microbial groups has not been done yet. In particular, studies on archaeal succession are missing, even though they are known to survive harsh environmental conditions around the world. Studies on fungi were also mostly restricted to mycorrhizal fungi, with the other fungal groups being neglected most of the times. Here I mainly used molecular approaches like DNA extraction, T-RFLP profiling and sequence analysis to investigate the microbial community structure, composition and diversity. Furthermore, a vast dataset on environmental and soil physico-chemical parameters along the Damma glacier forefield chronosequence is available through the BigLink consortium, which is not the case in such a broad range for other forefields. This made it possible to correlate my findings on microbial succession with specific parameters along the successional gradient.

Further questions investigated in a second part were if exposition, and therefore mainly temperature and soil moisture, is crucial for the microbial community structures and activities in recently deglaciated bare soil. When investigating soil microbial communities, the microclimatic differences between sites are often neglected. However, it is known that subtle changes in temperature or soil

moisture can affect microbial activities and community structures. As most research in glacier forefields was performed along a chronosequence (transect), i.e. investigating changes with increasing soil age, I decided to investigate a cross-section in parallel to the glacier terminus. Therefore, I was able to compare soil microbial communities in a similar bare soil environment but fundamentally different microclimates. The different microclimatic conditions were mainly due to shading effects of the nearby mountains, which created differing temperature and moisture regimes at the three selected sites. I used the reciprocal soil transfer technique to elucidate key environmental factors influencing the microbial communities, which were investigated using DNA extraction, T-RFLP profiling, sequence analysis, activity and enzyme measurements. Further questions to be answered were whether the microbial populations from a rather cold and moist soil transferred to a warmer and drier soil environment increased their microbial activities and if microbial community structures would change after soil transfer due to different microclimatic conditions.

In the third part, I focused on fungal and algal cell fragments as a possible carbon source for bacteria in the recently deglaciated bare soil using the DNA-SIP technique. Here I investigated whether microorganisms living in granitic sediment are able to utilize fungal and algal cell fragments as a C source; if the origin of the C (from fungi or algae) and the temperature influences respiration rates, bacterial activity and bacterial community structure and which bacterial phylogenetic groups may incorporate the fungal and algal derived C. While the degradation of labeled plant litter by microorganisms has previously been investigated in the Damma glacier forefield, other possible carbon sources for the microbes living in the recently deglaciated bare soil have not been looked at so far. Furthermore, the DNA-SIP approach has never been used in a glacier forefield soil with such low DNA and nutrient contents. With this laboratory experiment I gained new insights into the degradation of microbial cell fragments and the identity of bacterial phylotypes actively incorporating the carbon derived from the cell fragments.

1.7 THESIS OUTLINE

In the following chapters, first the microbial succession will be investigated (chapter 2):

Anita Zumsteg, Jörg Luster, Hans Göransson, Rienk H. Smittenberg, Ivano Brunner, Stefano M. Bernasconi, Josef Zeyer, Beat Frey; Bacerial, archaeal and fungal succession in the forefield of a receding glacier, „*Microbial Ecology*“, 63 (2012) 552–564

This is followed by the studies on microbial adaptation to changing microclimates (chapters 3 and 4):

Anita Zumsteg, Stefano M. Bernasconi, Josef Zeyer, Beat Frey; Microbial community and activity shifts after soil transplantation in a glacier forefield, "*Applied Geochemistry*", 26 (2011) 326-329

Anita Zumsteg, Erland Bååth, Beat Stierli, Josef Zeyer, Beat Frey; Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield, "*Soil Biology and Biochemistry"*, 61 (2013) 121 - 132

In the subsequent chapter 5 the investigation on the ability of bacteria to utilize carbon derived from fungal and algal cell fragments will be presented:

Anita Zumsteg, Stefan Schmutz, Beat Frey; Identification of biomass utilizing bacteria in a carbon-depleted glacier forefield soil by the use of ^{13}C DNA stable isotope probing, "*Environmental Microbiology Reports*", 5 (2013) 424-437

A part of my work, including general microbial community structure analysis and fungal sequence analysis, I will not present here but they have also or will also be published:

Bernasconi S. M., Bauder A., Bourdon B., Brunner I., Bünemann E., Christl I., Derungs N., Edwards P., Farinotti D., Frey B., Frossard E., Furrer G., Gierga M., Göransson H., Gülland K., Hagedorn F., Hajdas I., Hindshaw R., Ivy-Ochs S., Jansa J., Jonas T., Kiczka M., Kretzschmar R., Lemarchand E., Luster J., Magnusson J., Mitchell E. A. D., Venterink H. O., Plötze M., Reynolds B., Smittenberg R., Stähli M., Tamburini F., Tipper E. T., Wacker L., Welc M., Wiederhold J. G., Zeyer J., Zimmermann S., **Zumsteg A.**; Chemical and biological gradients along the Damma glacier soil chronosequence, Switzerland, "*Vadose Zone Journal*", 10 (2011) 867–883

Ivano Brunner, Michael Ploetze, Stefan R. Rieder, **Anita Zumsteg**, Gerhard Furrer, Beat Frey; Pioneering fungi from the Damma glacier forefield in the Swiss Alps can promote granite weathering, *"Geobiology"*, 9 (2011) 266–279

Beat Frey, Lukas Bühler, Stefan Schmutz, **Anita Zumsteg**, Gerhard Furrer; Molecular characterization of phototrophic microorganisms in the forefield of a receding glacier in the Swiss Alps, "*Environmental Research Letters*", 8.1 (2013) 015033

2. PAPER: "BACTERIAL, ARCHAEAL AND FUNGAL SUCCESSION IN THE FOREFIELD OF A RECEDING GLACIER"

With Jörg Luster, Hans Göransson, Rienk H. Smittenberg, Ivano Brunner, Stefano M. Bernasconi, Josef Zeyer and Beat Frey

Microbial Ecology, 63 (2012) 552–564

2.1 ABSTRACT

Glacier forefield chronosequences, initially composed of barren substrate after glacier retreat, are ideal locations to study primary microbial colonization and succession in a natural environment. We characterized the structure and composition of bacterial, archaeal and fungal communities in exposed rock substrates along the Damma glacier forefield in central Switzerland. Soil samples were taken along the forefield from sites ranging from fine granite sand devoid of vegetation near the glacier terminus to well-developed soils covered with vegetation. The microbial communities were studied with genetic profiling (T-RFLP) and sequencing of clone libraries.

According to the T-RFLP profiles, bacteria showed a high Shannon diversity index (H) (ranging from 2.3 – 3.4) with no trend along the forefield. The major bacterial lineages were *Proteobacteria*, *Actinobacteria*, *Acidobacteria, Firmicutes* and *Cyanobacteria*.

An interesting finding was that *Euryarchaeota* were predominantly colonizing young soils and *Crenarchaeota* mainly mature soils. Fungi shifted from an *Ascomycota*-dominated community in young soils to a more *Basidiomycota*-dominated community in old soils. Redundancy analysis indicated that base saturation, pH, soil C and N contents and plant coverage, all related to soil age, correlated with the microbial succession along the forefield.

2.2 INTRODUCTION

In the Alps glaciers have been retreating since the mid-19th century, with current rates of glacial retreat considerably higher than the long-term averages (52). Due to this glacial retreat, new terrain has become exposed as a forefield chronosequence with varying physical, chemical and biological properties (60). This is an ideal environment to study soil formation and primary microbial succession, as microorganisms like bacteria (29) and fungi (24) are the first to colonize new substrates. Their ability to interact with minerals and organic compounds through physical and chemical processes makes them contributors to rock weathering (8, 9, 20). These interactions mobilize otherwise inaccessible nutrients essential for higher organisms. This implies that fungal and bacterial colonizers could be key determinants of early ecosystem function and stability. The bacterial succession on glacier forefields has been investigated under differing climatic conditions and with changing soil parameters (48, 57, 59, 61). These studies have been able to show a bacterial succession along the forefields but they are either restricted to a few sampling sites (<8) with relatively large intervals between the different soil ages, or to laboratory experiments. Fungi have also been studied in glacier forefields, but with a focus on association with plants, and on relatively old soils (35, 36), without taking into account the complete chronosequence.

Much less information is available on the role of the archaea in glacier forefields. Nicol et al. (49, 50) found that the dominant archaeal communities in the alpine glacier forefield they studied were composed of the *Crenarchaeota*. However, it is not known to what extent the *Euryarchaeota* or other archaeal lineages are colonizing glacier forefields, or if indeed they are present at all.

We therefore studied the bacterial, archaeal and fungal community structure and composition and their simultaneous successional distribution patterns along the temperate glacier Damma forefield. The study was part of the interdisciplinary research project BigLink (4, 5), which investigates weathering, soil formation and ecosystem evolution along the Damma glacier forefield, located in the Swiss Central Alps. We took samples along a transect at 22 sampling sites, ranging from bare soils

close to the glacier terminus to densely vegetated soils up to 140 years old. In this chronosequence, increasing plant cover went along with a higher soil total organic carbon and nitrogen content, while the pH decreased (5). The bacteria (20) and fungi (8) isolated from the bare soil near the glacier terminus were shown to be phylogenetically diverse and active in weathering.

We investigated whether all the three microbial groups, bacteria, archaea and fungi, undergo a community succession with increasing soil age, plant cover and nutrient content of the soil, and whether the microbial diversity derived from SSU data changes along the chronosequence. For archaea, we also examined *Euryarchaeota* to see if they are colonizing the forefield. For fungi, we explored the community composition to find out whether it changes from a community dominated by free-living species to a more plant associated fungal community. The small subunit rRNA genes from the different samples were analyzed to determine the presence of diverse prokaryotes and eukaryotes. Soil chemical data were compared with microbial community data to identify the microbial inhabitants in the different soil ecosystems and to compare them. By simultaneously investigating the three phylogenetic groups bacteria, archaea and fungi along the chronosequence of a temperate glacier forefield, it should be possible to better understand the initial colonization and succession patterns of microorganisms in a changing environment.

2.3 Material and Methods

2.3.1 Location and sampling

The Damma glacier forefield is located in the Central Alps, within the Central Aare Granite in Switzerland, at an altitude between 1950 and 2050 m above sea level. The front of the Damma glacier has been monitored by the Swiss glacier monitoring network (http://glaciology.ethz.ch/messnetz/?locale=de) and has retreated at an average rate of approximately 10 m per year since the beginning of systematic measurements in 1921, with two short expanding periods (1920 - 1928 and 1972 - 1992), which resulted in two small moraines. The precipitation is around 2400 mm per year, and the mean annual temperature ranges from 0 to 5°C (15).

Soil samples were taken at 22 sites along the forefield, where each site was defined by an area of 4 m2 (Figure 1). From each site, 9 samples were taken randomly at 0-5 cm depth using an ethanol-cleaned shovel, and then sieved through a 2 mm sieve to remove stones and larger plant material. They were frozen at -80°C until further processing. The texture of the sieved soil was loamy sand throughout the forefield. The youngest sites sampled for this study are 2 to 13 years old, enclosed by the glacier terminus and the 1992 moraine. The intermediate sites are located between the 1992 and the 1928 moraines, and hence have soils aged between 58 and 78 years. The oldest sites are between 110 and 136 years old, and extend from the 1928 moraine onwards.

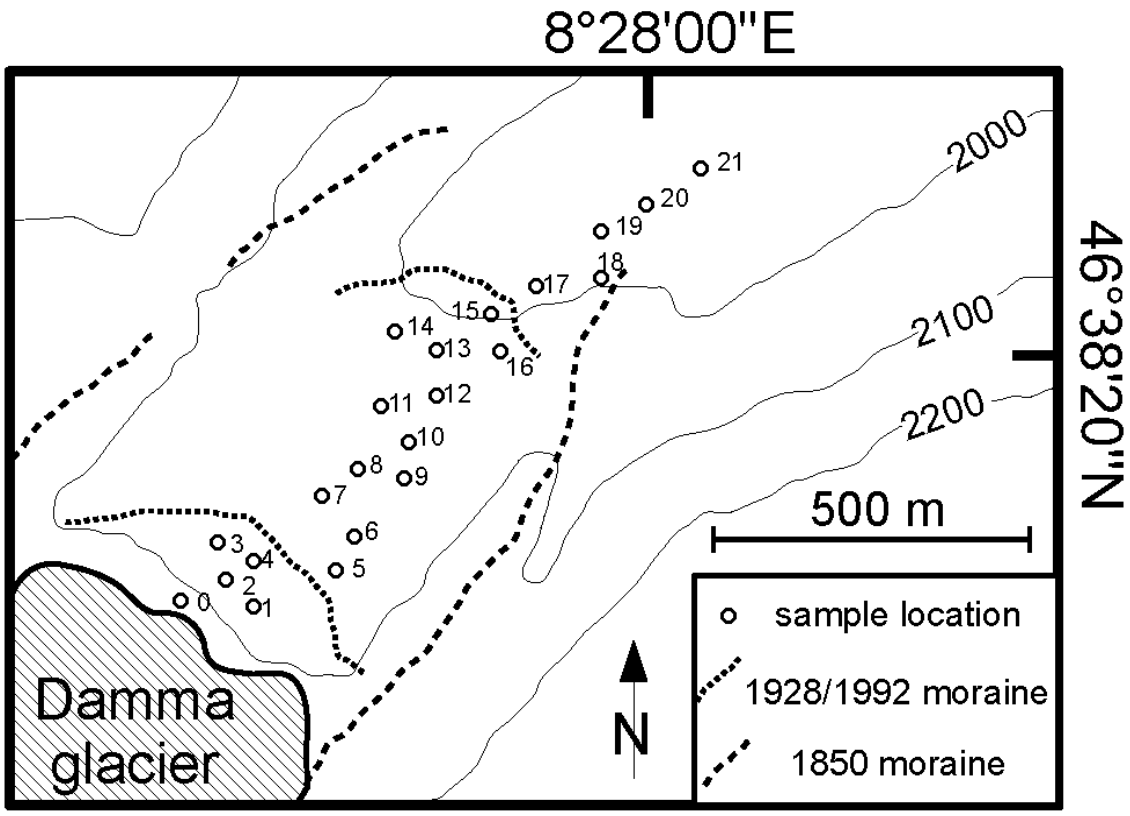


Figure 1: View of sampling sites 0 to 21 along the Damma glacier forefield, showing also the two lateral moraines from 1850 and the 1928 and 1992 moraines. Sites 0 to 4 represent young, very scarcely vegetated soils (2 to 13 years), sites 5 to 16 intermediate soils (57 to 77 years) and sites 17 to 21 the oldest, well vegetated soils (110 to 136 years). Soil age can also be approximated by the distance from the ice. The front of the glacier terminus can be noted on the lower left corner and the two end moraines are highlighted with lines, separating the age classes of the successional stages.

2.3.2 Soil Physicochemical Parameters

Soil pH was determined in 0.01 M $CaCl_2$ (65). For exchangeable metal cations, soil samples were extracted with 1 M NH_4Cl and measured using inductively-coupled plasma optical emission spectrometry, or in case of exchangeable H^+ and exchangeable Al^+, through complexation and titration (64). The cation exchange capacity (CEC) was calculated as the sum of exchangeable H^+, Al^{3+}, Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+} (mmolC kg^{-1}). The base saturation (BS) was calculated as the sum of Na^+, Mg^{2+}, K^+ and Ca^{2+} divided by CEC (%).

Total carbon (C) and nitrogen (N) content in soils were measured using 10-50 µg dried finely ground (disk mill) soil weighed into tin capsules introduced into a Flash elemental analyzer (Thermo Fisher Scientific, Wohlen, Switzerland) operated with He as a carrier gas. Samples were combusted in the presence of O_2 in an oxidation column at 1030°C and the combustion gases passed through a reduction column (650°C). The N_2 and CO_2 gases produced were separated chromatographically and the amount measured with a Thermal Conductivity Detector (TCD). Contents were calibrated by bracketing with a standard soil with known C and N.

The plant cover was determined using aerial photographs covering approximately 25 m^2 of the sites and calculating the total green plant cover of this area with Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA, USA).

2.3.3 DNA Extraction and PCR Amplification

Genomic DNA was isolated in three replicates from the pooled soil samples using the Smart Helix DNA extraction kit (Venturia, Ljubljana, Slovenia) according to the manufacturer's instructions. The extracted DNA was quantified with Pico Green (Invitrogen, Carlsbad, CA, USA) and stored at -20°C until further use. Primers for the specific PCR amplification of the bacterial and archaeal 16S rRNA gene and the fungal 18S rRNA gene are documented in Table 1. Primers were obtained from Microsynth GmbH, Balgach, Switzerland. 20 to 80 ng of DNA was added as a template for the PCR reactions, performed in three replicates for each site. For the bacterial 16S rRNA gene, the PCR conditions were as described previously (21). Archaeal specific PCR reactions were carried out according to the protocol of Chin et al. (11). Fungal-specific PCR reactions were carried out according to the protocol of Bornemann & Hartin (7).

Table 1: List of PCR primers used in this study for T-RFLP and sequencing.

Gene region	Sequence	Application in this study	Reference
Bacteria 16S rRNA gene			
27f - FAM	AGAGTTTGATCMTGGCTCAG-5'	T-RFLP, sequencing	Heuer et al. (1992)
1378r	CGGTGTGTACAAGGCCCGGGAACG-3'	T-RFLP, sequencing	Heuer et al. (1992)
341f	CCTACGGGAGGCAGCAG-5'	sequencing	Lopez-Gutierrez et al. (2004)
901rev	CCGTCAATTCCTTTRAGTTT-3'	sequencing	Lane (1991)
Archaea 16S rRNA gene			
A109f	ACKGCTCAGTAACACGT-5'	T-RFLP, sequencing	Chin et al. (1999)
A934r - FAM	CTCCCCGCCAATTCCTT TA-3'	T-RFLP, sequencing	Chin et al. (1999)
Fungi 18S rRNA gene			
nu-SSU0817 - FAM	TTAGCATGGAATAATRRAATAGGA -5'	T-RFLP, sequencing	Borneman & Hartin (2000)
nu-SSU1536	ATTGCAATGCYCTATCCCCA -3'	T-RFLP, sequencing	Borneman & Hartin (2000)
M13 Vector			
M13f	TGTAAAACGACGGCCAGT-5'	Vector primer, sequencing	Promega
M13r	CAGGAAACAGCTATGACC-3'	Vector primer, sequencing	Promega

2.3.4 T-RFLP Analysis

The three replicate PCR products were digested with 0.1 Units of either the restriction enzyme *MspI* for bacteria, *HhaI* for archaea or *AluI* for fungi according to the manufacturer's recommendations (Catalys AG, Wallisellen, Switzerland). T-RFLP analyses were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and T-RFLP profiles were analysed using Genotyper v.3.7 NT (Applied Biosystems) according to Frey et al. (21). The threshold for analysis was set to 100 fluorescence units. The profiles were analysed after transforming the raw peak heights in percentage values over the sum of the total peak heights detected in the samples, and taking the mean of the three replicates (19).

2.3.5 Clone Libraries

Three clone libraries were constructed, one for site 0 (2-year-old site), one for site 6 (62-year-old site) and one for site 17 (110-year-old site). These sites were chosen because they contained the

representative T-RFs for the soil ages “young”, “intermediate” and “old”, respectively. PCR amplifications for the clone libraries were performed on the three replicate DNA extracts of each site, which were then pooled for cloning. Reactions were performed with the same primers as described above but unlabeled. The PCR products were then ligated into the vector of the pGEM-T Easy Vector System and cloned into the competent cells JM109 (Promega Corporation, Fitchburg, WI, USA), according to the manufacturer’s instructions. A PCR reaction on the successfully transformed clones with the vector specific primers M13f and M13r was performed, as described earlier in Widmer et al. (68), to check whether the length of the insert was correct. Inserts were restricted with *MspI* for bacteria, *HhaI* for archaea and *AluI* for fungi in order to select clones to be sequenced according to unique RFLP patterns (clone operational taxonomic units, OTUs). Bacteria were sequenced with the 27F primer and the two internal primers 341f and 901rev, archaea with the primers A109f and A934r and fungi with the M13f and M13r primers (Table 1). The products were sequenced with an ABI 3730xl sequencer (Applied Biosystems), and the sequences were then sorted and aligned using the BioEdit software (www.mbio.ncsu.edu/BioEdit/bioedit.html). A chimera check was performed on the Bellerophon server (32) and manually counter-checked with NCBI BLAST (1). Chimeras were excluded from further analysis. The clones were also subjected to T-RFLP analysis, using the same primers as for the T-RFLP analysis of the environmental samples. The T-RFs of the clones were then compared to the *in silico* T-RFs of the cloned sequences. Only the T-RFs abundant in young or in old soils T-RFLP profile were compared to the T-RFs of the clones.

2.3.6 Phylogeny

The sequences of bacteria, archaea and fungi were analysed by BLAST search on NCBI. Phylogenetic trees were generated by analysing the ClustalW sequence alignment performed in BioEdit using Bayesian inference with the computer program MrBayes 3.2 (33) and the LG+I+G model. A Markov chain Monte-Carlo (MCMC) was run for 4’000’000 generations, sampling every 100th generation, until the standard deviation of the split frequency was below 0.01. The tree was then visualized with

the softwares Mesquite and FigTree. For clarity we removed similar sequences in the trees. As outgroups we chose the Spirochete *Borrelia burgdorferi* (L36160) in the bacterial tree, a *Korarchaeota* (AF255604) for the archaeal tree and a member of the *Chytridiomycota*, *Cladochytrium* sp. (AB586077) in the fungal tree. The posterior probabilities of a node are only shown when below 75%.

2.3.7 Statistical Analysis

The correlation coefficients (R) with their p-values were calculated according to Pearson with the statistical program IBM SPSS statistics (IBM Corporation, Armonk NY, USA).

From the T-RFLP profiles, the Shannon diversity index (H) of the individual terminal restriction fragments (T-RFs) was calculated according to Sigler & Zeyer (61). The mean of the three replicate DNA extracts was always taken. Redundancy analysis (RDA) using the CANOCO program for Windows (63) was chosen to analyse the T-RFLP data combined with the environmental parameters pH, BS, C,N, PC and soil age. Initial analysis by detrended correspondence analysis (DCA) revealed that the data exhibited a linear, rather than unimodal, response to the environmental variables. Furthermore, a Monte-Carlo test was performed in CANOCO within the RDA to assess the significance of the correlation of T-RFLP profiles with the environmental parameters.

The number of sequence OTUs and the Evenness were calculated from the sequence data using the open-source computer program Mothur (56). The identity for a unique OTU was set to 97%. The coverage (C) was calculated according to C = 1 - (number of sequence OTUs / number of total sequences) (2).

2.3.8 Accession Numbers

All sequences from this study were deposited in GenBank. The sequences from the bacterial libraries were assigned to the accession numbers HM065582 – HM065763; sequences from the archaeal libraries were assigned to the accession numbers HM065764 – HM065903, and the sequences from the fungal libraries to the accession numbers HM065904 – HM066004.

2.4 Results

2.4.1 Physicochemical Characteristics of the Glacier Forefield

With increasing soil age, and thus increasing distance from the ice (Figure 1), the carbon and nitrogen content in the soil increased steadily from a very low C content of 0.07% in young soils to over 2% in the oldest soils ($R = 0.72$, $p = 0.0001$), while the N content rose from 0.01% to 0.13% ($R = 0.76$, $p = 0.0001$) (Figure 2). Within the chronosequence, pH ranged from 3.8 to 4.8, with the higher values generally found at the young soil sites and the lower values at the old soil sites ($R = -0.69$, $p = 0.0001$) (Figure 2). The base saturation exhibited the same trend, with between 70% and 80% at the young sites and around 50% at the old sites ($R = -0.56$, $p = 0.007$) (Figure 2). In contrast, the plant cover increased along the forefield from no or scarce vegetation at the young sites (below 50%) to 100% plant cover at the old sites ($R = 0.92$, $p = 0.0001$) (Figure 2). The DNA content rose from 0.9 µg g-1 dry soil to 15.4 µg g-1 dry soil along the forefield ($R = 0.78$, $p = 0.0001$) (Figure 2).

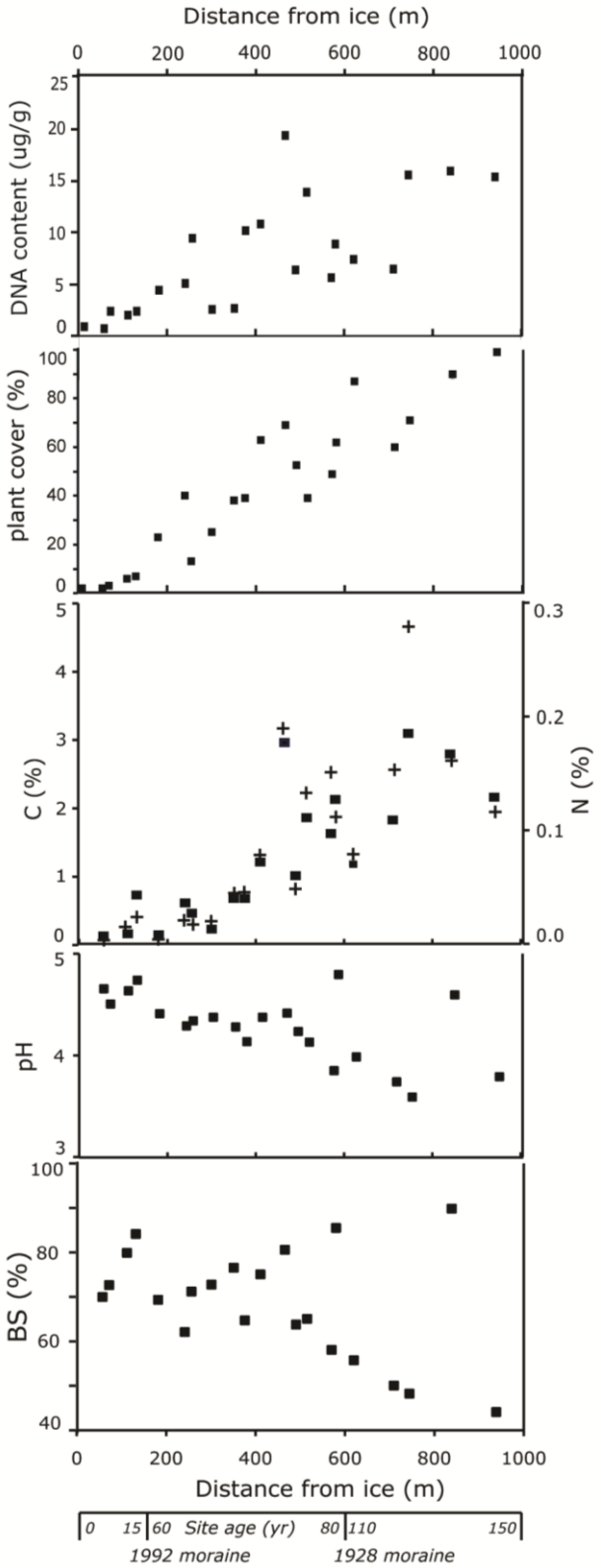


Figure 2: Environmental parameters measured along the chronosequence. DNA content, plant cover, carbon (cross), nitrogen (square), pH and base saturation (BS) are shown. The values are shown in percentages for all the values except for pH and DNA content (μg g^{-1} dry soil). The distance from the ice is marked on the X-axis, together with a scale of the three age classes.

2.4.2 BACTERIAL, ARCHAEAL AND FUNGAL COMMUNITY STRUCTURES DERIVED FROM T-RFLP PROFILING

The numbers of bacterial T-RFs (41 to 93) were higher than the numbers of archaeal and fungal T-RFs (Figure 3). The Shannon diversity index (H) based on the T-RFLP profiles was in the range of 2.3 to 3.4, with no clear trend along the forefield (Figure 3a). The numbers of archaeal T-RFs (0 to 41) decreased with soil age. Similarly, H (0.1 to 2) indicates a lower T-RF diversity of archaea in old soils compared to young soils (Figure 3b). The numbers of fungal T-RFs (2 to 12) were low throughout the chronosequence. The Shannon index based on the T-RFLP profiles ranged from 0.3 to 1.2 without any clear trend (Figure 3c).

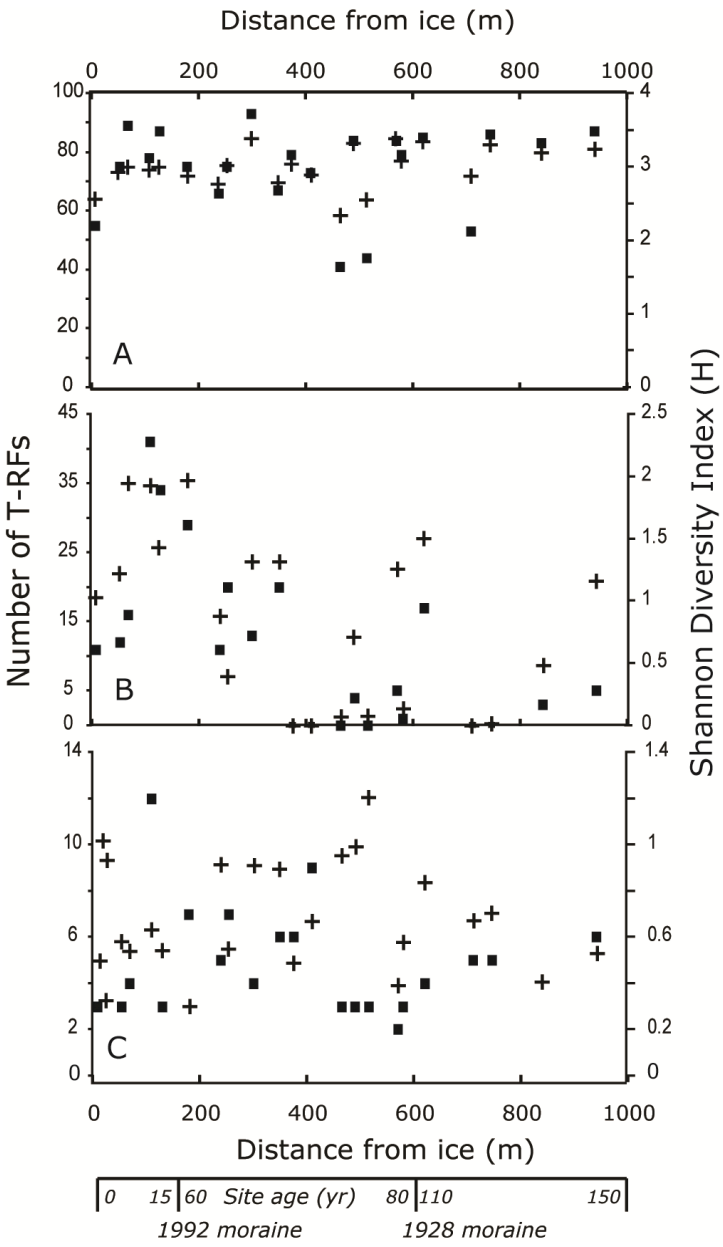


Figure 3: Number of terminal restriction fragments (cross) and the Shannon diversity index (square) are shown for bacteria (A), for archaea (B) and for fungi (C). The distance from the ice is marked on the X-axis, together with a scale of the three age classes.

The RDA axes 1 and 2 were found to explain 18.7% and 8% of the overall variance for bacteria (Figure 4a), 14.7% and 8.8% for archaea (Figure 4b) and 29% and 2.8% for fungi (Figure 4c), within the T-RFLP data correlated to the environmental data. The microbial communities of the young, sparsely vegetated soils (2 to 13 years) were separated from the old, densely vegetated soils (110 to 136 years) for all the phylogenetic groups. Species environment correlations were 0.89 for bacteria, 0.71 for archaea and 0.68 for fungi, indicating that the data were strongly correlated with environmental parameters (Table 2). Monte-Carlo significance tests revealed that the axes explain a significant amount of the variation in the bacteria data (0.001) and thus indicate a clear shift in bacterial communities. Archaeal and fungal variations, on the other hand, were not significantly explained (0.185 and 0.087) and thus only indicate a trend towards a community change along the chronosequence.

Table 2: Results of redundancy analysis (RDA) from T-RFLP profiling and corresponding environmental parameters. Values are shown for the first axes. The second axes explain less than 10% of the variance and thus do not account for a valuable part of the variance. The Eigenvalues are shown in the diagram of Figure 4.

	Bacteria	**Archaea**	**Fungi**
Cumulative percentage variance:			
Of species data	19	15	29
Of species-environment relations	51	52	79
Species-environment correlations	0.89	0.71	0.68
Monte-Carlo significance test:			
F-ratio	3.7	2.8	6.1
P-value	0.001	0.185	0.087
Correlations (100 x r):			
pH	-55	-28	-30
Base saturation	-40	-19	-13
Plant cover	84	67	48
C content	76	47	47
N content	82	58	41

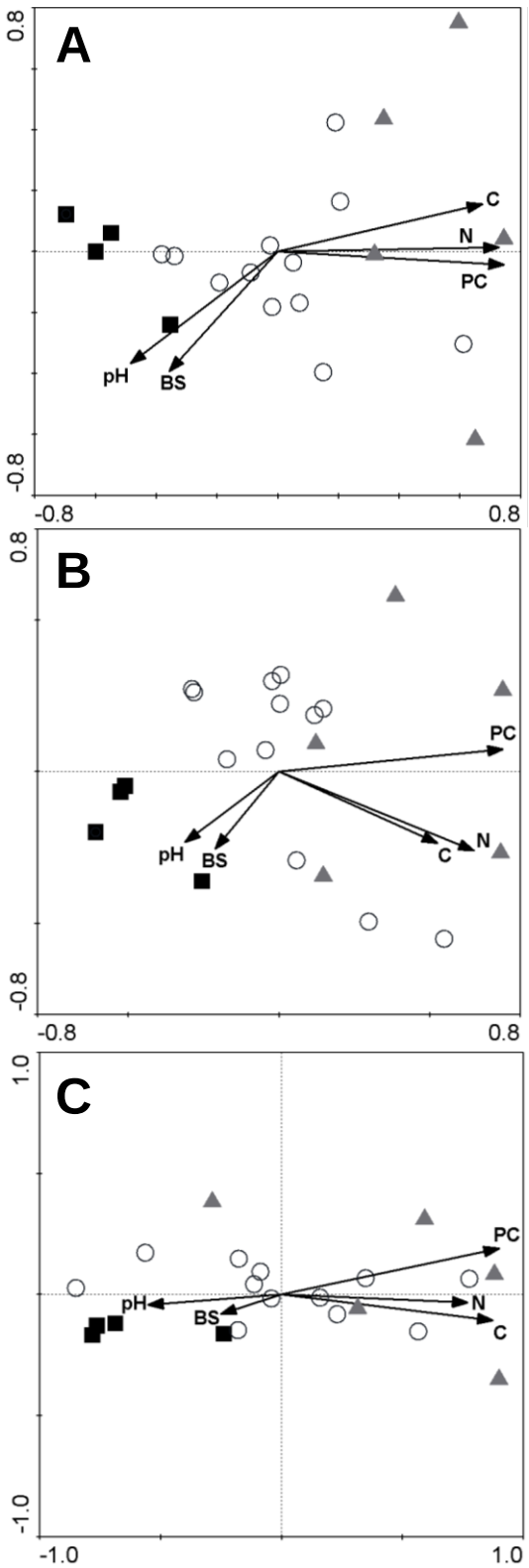


Figure 4: Ordination diagrams of redundancy analysis (RDA) of T-RFLP profiling are shown for bacteria (A), for archaea (B) and for fungi (C) from the three successional stages of the Damma glacier forefield: young soils deglaciated for 2 to 13 years (black squares), intermediate soils on sites deglaciated for 57 to 77 years (white circles) and oldest soils on sites deglaciated for 110 to 136 years (dark triangles). Arrows indicate the environmental variables (C = TC (%); N = TN (%); PC = plant cover (%); BS = base saturation (%)). Bacterial axes explain a variance of 18.7% and 8%. For archaea the axes explain 14.7% and 8.8%. Fungal axes explain 29% and 2.8%.

2.4.3 Bacterial, Archaeal and Fungal Community Compositions Derived from SSU rRNA Gene Clone Libraries

Coverage for the clone libraries was generally low (Supplementary Table T1). Only fungal sampling led to a coverage of over 50% in the older soils. The cloned sequences revealed that the dominant bacterial group in this forefield were *Proteobacteria*, with 49% of the total number of sequences belonging to this group 100% where referred to the total number of sequences of all three soil ages (Figure 5a). *Alpha-proteobacteria* were more abundant (21%) than *Beta-proteobacteria* (16%). Both classes decreased in percentage with increasing soil age. *Gamma-proteobacteria* (7%) were abundant in the young and old soils, but not in the intermediate soil. *Delta-proteobacteria* (5%) were only present in the intermediate and old soils (Figure 5a). Comparison of the *in silico* T-RFs of the sequences and the T-RFLP analysis of the environmental samples also revealed a decrease in *Alpha-proteobacteria* (T-RFs 70 bp; 125 bp and 129 bp) with increasing soil age, whereas here the T-RF specific for *Beta-proteobacteria* (135 bp) increased (Table 3). Other abundant classes were *Actinobacteria* (12%), *Acidobacteria* (9%) and *Cyanobacteria* (5%). The percentage of *Actinobacteria* and *Cyanobacteria* decreased drastically with soil age, whereas *Acidobacteria* appeared only from the intermediate site onwards. The T-RF assigned to *Cyanobacteria* (61 bp) could not be found in all the samples of the young soil, and is thus not listed in Table 3. The T-RFs for *Actinobacteria* (138 bp and 145 bp), on the other hand, were represented in both the young and old soils, but T-RF 138 bp was more dominant in the young soils. The T-RFs specific for *Acidobacteria* (93 bp and 265 bp) were dominant in the young and old soils (Table 3). *Bacteroidetes* (6%) were most abundant in the intermediate soil, *Verrucomicrobia* (5%) in the young soil, *Firmicutes* (5%) in the old soil and TM7 (5%) in the young and intermediate soils. A comparison of the T-RFLP analysis of the clones and environmental samples confirmed the results for *Bacteroidetes* (206 bp) and *Firmicutes* (140 bp; 154 bp and 290 bp) (Table 3). *Gemmatimonadetes* (2%) and *Chloroflexi* (1%) were minor classes each representing less than 2% of the total clone numbers (Figure 5a). In the bacterial phylogenetic tree, the distribution of our clones in the classes mentioned above can be seen. It also indicates that our

cloned bacterial sequences were highly similar to cloned sequences from other cold habitats (Supplementary Figure S1).

The fraction of *Crenarchaeota* increased with soil age (51%) (Figure 5b). They belonged mainly to the group 1.1c *Crenarchaeota* (62%), which were only present in the old site. The group 1.1a (37%) appeared in both the young and intermediate soils, whereas the group 1.1b (1%) was only in the intermediate soil (Figure 5b). Most cloned sequences retrieved from the young and intermediate soils were *Euryarchaeota* (49%) of which only 4% could be assigned to a taxon, *Methanomicrobia*. The remaining 96% could be assigned to *Euryarchaeota*, but not to specific taxa (Supplementary Figure S2). This clearly indicates a community shift from *Euryarchaeota* in bare soils to *Crenarchaeota* in densely vegetated soils. A comparison of the in silico T-RFs of the sequences and the T-RFLP analysis of the environmental samples confirmed this shift, as the abundance of T-RFs assigned to *Euryarchaeota* (73 bp; 135 bp and 157 bp) was higher in the young soils and the abundance of T-RFs assigned to *Crenarchaeota* (120 bp and 162 bp) was higher in the old soils (Table 3).

The fungal community was mainly composed of the phyla *Ascomycota* (60%) and *Basidiomycota* (37%). The other 3% are from the basal fungal lineages (Figure 5c). Cloned sequences retrieved from the young soil belonged mostly to *Ascomycota*, and the sequences retrieved from the old soil to *Basidiomycota* (Supplementary Figure S3). *Leotiomycetes* (49%) and *Eurotiomycetes* (18%) were the most abundant Ascomycota classes (Figure 5c). They were found in the clone libraries of all three soil ages. The proportion of cloned sequences belonging to *Eurotiomycetes* increased with soil age, whereas *Leotiomycetes* were the most abundant genera in the intermediate soil. The classes *Dothideomycetes* (11%) and *Taphrinomycetes* (9%) were only present near the glacier terminus. *Pezizomycetes* (7%), *Sordariomycetes* (2%) and *Orbiliomycetes* (4%) were other fungal classes found (Figure 5c). On the other hand, *Basidiomycota* were dominated by *Agaricomycetes* (97%), which rose in numbers of cloned sequences with soil age. In the fungal T-RFLP analysis of the clones, one T-RF represented many phylogenetic classes (450 bp). The T-RF for *Eurotiomycetes* (448 bp) confirmed the increase in the class along the gradient, whereas the T-RF for *Agaricomycetes* (271 bp) did not.

Phylogenetic affiliations of the fungal specific T-RFs from the environmental samples proved to be difficult to assign, as the T-RFs were all of a similar size without great variation (Table 3).

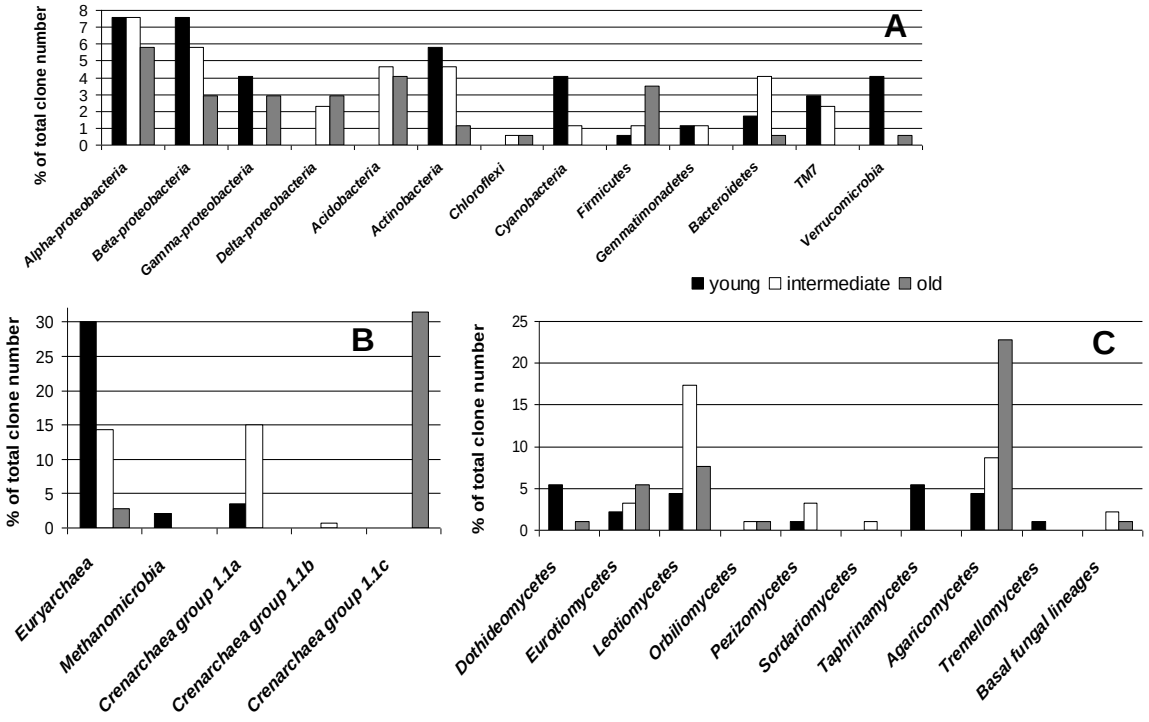


Figure 5: Comparison of phylogenetic classes over the three successional stages, the 2-year-old site (young soil, black), 62-year-old site (intermediate soil, white) and 110-year-old site (old soil, dark). Sequences were obtained from 16S rRNA gene clone libraries of bacteria (A); archaea (B) and from the 18S rRNA gene clone library of fungi (C). Phylogenetic classes are shown in percentages of the total clone number.

Table 3: Comparison of the T-RFs of the cloned sequences (*in silico* T-RF) with the T-RFLP profiles of the environmental samples (predicted T-RF). Only T-RFs from the environmental T-RFLP profiles present in either all the young (sites 0-4) or in all the old soils (sites 17-21) are shown.

Predicted T-RF	Young soils (site 0-4)	Old soils (site 17-21)	T-RF *in silico* from sequence	Clone name	Closest relative clone	Accession number (NCBI)	Corresponding phylogenetic class
				Bacteria			
68	++	++	63	1-b44	*ncd390d05c1*	HM321946	*Gemmatimonadetes*
70	++	+	69	1-b49	*Bas-7-52*	GQ495410	*Alpha-proteobacteria*
74	++	+	60	18-b135	*WT15*	HQ738646	*Gamma-proteobacteria*
93	+	++	96	18-b203	*Acidobacteria Amb_16S_1563*	EF019024	*Acidobacteria*
125	++	+	128	18-b70	*Elev_16S_841*	EF019683	*Alpha-proteobacteria*
129	++	+	135	1-b69	*hfmB137*	AB600435	*Alpha-proteobacteria*
135	+	++	141	18-b192	*Eubacterium WD2102*	AJ292626	*Beta-proteobacteria*
138	++	+	132	1-b14	*Pseudonocardiaceae Elev_16S_454*	EF019272	*Actinobacteria*
141	+	++	147	18-b199	*ncd1274h02c1*	JF086927	*Firmicutes*
146	++	++	149	18-b73; 18-b88	*ncd1276f09c1; Frankineae MWM1-27*	JF107313; HQ674868	*Firmicutes; Actinobacteria*
153	++	+	152	18-b186	*Gamma-proteobacterium AKYG891*	AY922074	*Gamma-proteobacteria*
206	++	++	212	1-b25	*FFCH12595*	EU133712	*Bacteroidetes*
265	++	+	269	18-b103	*Ellin5095*	AY234512	*Acidobacteria*
291	++	+	297	18-b38	*RABS_A82*	HQ660787	*Firmicutes*
				Archaea			
73	++	+	72	18-a37	*LAa02.18*	EU782009	*Euryarchaeota*
120	+	++	114	18-a111	*B51*	HQ233425	*Crenarchaeota 1.1c*
135	++	-	135	1-a48	*CaR3b.h03*	EU244277	*Euryarchaeota*
157	++	+	156; 154	1-a56; 18-a118	*SDG-195; mrR1.11*	EU365243; FJ746506	*Euryarchaeota*
162	+	++	168	18-a17	*Crenarchaeote PsRTC_28*	GU815318	*Crenarchaeota 1.1c*
				Fungi			
100	++	++	101	18-f27	*Soil.06.17*	AY099411	*Basal fungal lineages*
271	++	+	276	18-f102, 18-f123	*S_Canopy_450_01_07; fungus 26_16*	AY382458	*Agaricomycetes*
448	+	++	452; 451; 455	1-f177; 1-f185; 18-f155	*Cladophialophora minutissima voucher UAMH 10710*	EF016375	*Eurotiomycetes*
450	++	++	456; 452; 455; 455	1-f138, 18-f26, 18-f189, 18-f133	*M228; Ceraceomyces borealis CFMR:L-8014; soil fungus 849; 6034RhFu*	EU940063; GU187624; GU568164; GU201458	*Leotiomycetes; Agaricomycetes; Agaricomycetes; Eurotiomycetes*

2.5 Discussion

This is the first simultaneous investigation of the bacteria, archaea, and fungi along the chronosequence of a temperate glacier forefield. The Shannon diversity indices from T-RFLP profiling (Figure 3) for bacteria were remaining constant along the chronosequence. This finding is in accordance with that of Schutte et al. (58), but in contradiction to Nemergut et al. (48) who detected an increase in diversity with soil age in an alpine forefield. These different results suggest that the factors influencing bacterial diversity are not solely related to the soil age, but also to other factors such as climatic conditions, bedrock composition, soil texture and pH (40, 44).

The RDA and clone library analyses indicated a change in the community structure from bare to densely vegetated soils for bacteria, archaea and fungi, and thus a microbial succession along this forefield (Figs. 4 and 5 and Table 3). Besides physical factors, in the initial phase of colonizing bare soils, the bacterial community composition is influenced by the chemical composition of the rock. Thus different minerals are colonized by different bacteria (23). It has also been shown that plant root exudates have a strong influence on cell counts even 10cm away from the roots (46), which implies that plant colonization is important for microbial communities (15), and that the bacterial community structures therefore change with altering soil parameters and plant colonization. Limited C and N sources on bare soil select for a specific bacterial community. Mainly C and N fixers (12, 62) and weathering associated microorganisms can survive here (20). Some microorganisms that utilize C and N from atmospheric deposition and from plants and mosses can also be expected to be present. The N fixing diazotrophic community was shown to be abundant and diverse in the Damma glacier forefield and is a potentially important contributor to the N input in this environment (14). In the older, vegetated soils, decomposers of plant material and nitrate reducers were thriving, and outcompeted those bacteria that are successful in bare soils (13, 58). This is also indicated in the RDA, as the change in bacterial community structure is clearly correlated to the soil parameters (Figure 4a).

The most frequently clone sequences and T-RFs found in environmental samples belonged to *Proteobacteria* (Figure 5a and Supplementary Figure S1, Table 3). This is consistent with the findings of Kersters et al. (39), who demonstrated that this phylum is phenotypically very versatile. *Proteobacteria* consist of numerous phototrophs, photoheterotrophs and chemolithotrophs, which are advantageous traits in this initial ecosystem with limited nutrient resources (30). Within *Actinobacteria*, *Actinomycetales* were the dominant order (56.3%) (data not shown). *Actinomycetales* are active in the decomposition of organic materials in soil, including lignin and other recalcitrant polymers (27). As they were mainly present near the glacier terminus, they may decompose materials from dead microbes, arthropods, fungal spores and pollen (25, 34), and other organics deposited by air on the forefield (53).

Cyanobacteria are photoautotrophic organisms that survive on bare rocks as the first colonizers (16), and in lichens they have a symbiotic relationship with fungi. Initial colonization of barren soils by *Cyanobacteria* is known to raise the nutrient status as their photosynthetic activities provide a significant input of C and N into the upper soil layers, which is important for the further succession of heterotrophs (18, 57). *Cyanobacteria* were also shown to form the dominant ground cover for 60 years on an Arctic glacier chronosequence, before they declined (29). This was also the case in our clone libraries. However, the T-RF specific for *Cyanobacteria* was only present in a few of the young soils. In general however, the T-RF for *Cyanobacteria* was less abundant in the old soils than in the young soils (data not shown).

Acidobacteria are ubiquitous in soil samples around the world in various habitats (42), including endolithically in granite rock (31), which implies they should be capable of surviving on this forefield. Interestingly though, they were not found in the clone library of the bare soil, but the T-RFs assigned to *Acidobacteria* were present in the T-RFLP analysis from the young soils. The low coverage of the clone libraries could account for these contrasting results. Thus autotrophs and chemolithotrophs as *Cyanobacteria* and *Proteobacteria* declined with rising soil age.

Archaea are known to be able to live under extreme conditions, but recently archaea that only adapted to moderate environmental conditions have also been found (6, 10). Unlike bacteria and fungi, the Shannon diversity index of archaeal T-RFLP profiles declined along the chronosequence (Figure 3b), which is contradictory to the observations made by Nicol et al. (50) who reported an increase in diversity along a successional gradient. The environmental conditions occurring in bare soils are extreme compared to those in vegetated soils, where there are fewer moisture and temperature fluctuations and more organic matter. Our findings suggest that bare soils provide a large range of microenvironments where different archaeal species can survive, especially favoring those adapted to more extreme conditions. Indeed the RDA demonstrated that the archaeal community structure in the young soils differed from that in the old soils (Figure 4b). Sequence analysis of archaeal clone libraries and comparison of the in silico T-RFs of the sequences with the predicted T-RFs of the environmental samples confirmed this finding, showing a shift from a *Euryarchaeota*-dominated archaeal community in the young soil to a *Crenarchaeota*-dominated archaeal community in the old soil (Figure 5, Table 3 and Supplementary Figure S2). To the best of our knowledge, this is the first time the dominance of *Euryarchaeota* sequences on bare soil close to a glacier terminus has been shown. Contrary to our findings, Nicol et al. (50) reported a dominance of *Crenarchaeota* over the whole forefield. Their finding might be explained by the fact that they used different primers. Archaea in general are known to be influenced mainly by soil properties and not plant cover (51). *Crenarchaeota* are often found in plant rhizospheres (66), but can also particularly adapt to soils with a low nutrient status (3). They have also been found to be resistant to freeze-thaw cycles (54). *Crenarchaeota* are also influenced by soil organic matter (69), which is barely present in the young soils on this forefield, thus the *Euryarchaeota* found here are better adapted to the harsh environment with such low nutrient conditions. We suggest that, in the Damma glacier forefield, the rhizosphere-inhabiting *Crenarchaeota* species predominantly colonize the later successional stages of the forefield, whilst *Euryarchaeota* species predominantly colonize the bare soils. Alternatively, the *Euryarchaeota* might be outcompeted by *Crenarchaeota*, and/or other microorganisms, in rhizosphere soils rich in organic matter.

We studied fungi using primers detecting the 18S rRNA gene (36). As with bacteria and archaea, we also found distinctly different fungal communities in the bare and the vegetated soils. This may be related to the establishment of vegetation along the chronosequence, as fungi are closely associated with plants (51). Here the fungi found were either saprophytic or mycorrhizal, with some exceptions like *Taphrinamycetes* which are either plant parasites or pathogens. Thus a change in the community structure can be expected as the nutrient supply and plant cover changes along the gradient. The clone libraries show that the fungal community changed from *Ascomycota* which are able to live on rocks (22) or as dark septate endophytes like some *Dothidiomycetes*, to the *Basidiomycota*, which colonized the vegetated soils (Figure 5c and Supplementary Figure S3). These findings corroborate those of Jumpponen (36) on the Lyman glacier forefield. The RDA also supports this interpretation, as the fungal community structure in bare soils is negatively correlated to the C and N content in the soil and to plant cover, whereas the fungal community structures in old soils correlated positively with these parameters (Figure 4c). We are aware that the plant community also strongly influences the soil microbial communities (15, 46), at least in the older soils (67), but we did not specifically investigate plant-microbial interactions. Rather, we consider the changing plant community to be part of the environmental conditions, and thus an external factor for the microbial community in the same sense as C and N.

Of the *Ascomycota* detected, most species belonged to the subphylum *Pezizomycotina*. This includes the *Lecanoromycetes*, to which most of the lichen-forming fungal species belong (45). Lichens are known to colonize rocks and stones and are important in bioweathering (26, 41). Dark septate endophytes, as some *Dothidiomycetes*, can enhance plant growth by improving nutrient and water acquisition, especially in unfavourable environments, which could explain why *Dothidiomycetes* were found mainly in the young soil (37). The dominance of *Basidiomycota* in the older soils can be explained by the mycorrhizal associations of the *Agaricomycetes* with *Pinaceae* and angiosperms, including first colonizing plants on glacier forefields (28, 47), and the plant-litter decomposing activities of other *Basidiomycota* classes (43).

It has been suggested that fungi are more influenced by plant growth than prokaryotes, which are more influenced by soil properties (51). This would explain the successional trend in community structure and composition of archaea and fungi, as both plant colonization and soil properties change along the forefield. Soil pH has also been suggested as one of the main factors influencing microbial communities in soil (17). Since bacteria are the most competitive regarding the availability of simple organic substrates and this is probably reflected in the pH (69). Bacteria may be able to outcompete the other groups for resources, thus maintaining a constant SSU diversity and having the community structure that changed the least along the forefield.

Very few fungal T-RFs present in the environmental T-RFLP profiles could be found in the *in silico* analysis of the clones. The restriction site seems to be located in close proximity in all phylogenetic classes found here. Thus many T-RFs included multiple phylogenetic groups. Most likely, this is due to high sequence conservation of the fungal 18S rRNA gene (2). With this close proximity of the fungal *AluI* restriction site, we may have underestimated the fungal T-RF diversity. In all analyses it should be noted, that differences can occur between the size of theoretical digest (*in silico* T-RF) and the actual size from the capillary sequencer (predicted T-RF) (Table 3) (38, 55). Despite such differences we were still able to confirm the increase or decrease in certain bacterial, archaeal or fungal phylogenetic classes in the clone library by comparing *in silico* T-RFs of the clones to the environmental T-RFLP profiles and assigning them to specific classes.

Overall we were able to successfully link the two approaches, T-RFLP analyses and clone libraries, and to show that bacteria, archaea and fungi all exhibit successional patterns along a glacier forefield chronosequence that ranges from bare soils to >100-year-old vegetated soils with a high organic matter content. The autotrophic *Cyanobacteria* and the versatile *Proteobacteria* appeared to decline along the gradient, but a higher number of sequences from heterotrophs were found at the older sites. The archaea underwent a distinct community shift from a dominance of *Euryarchaeota* in the bare soils, to a dominance of *Crenarchaeota* in vegetated soils. The fungi showed a community change from an *Ascomycota* to a *Basidiomycota* dominated community along the chronosequence.

RDA analysis showed that the environmental factors pH, base saturation, carbon and nitrogen content and plant cover can explain 25-30% of the community changes. Further research is needed to determine other potential drivers of the community shifts, such as microclimatic conditions, and other nutrients. However, an inherent variability in the nature of glacier forefields may lie behind the majority of the observed differences.

2.6 Acknowledgements

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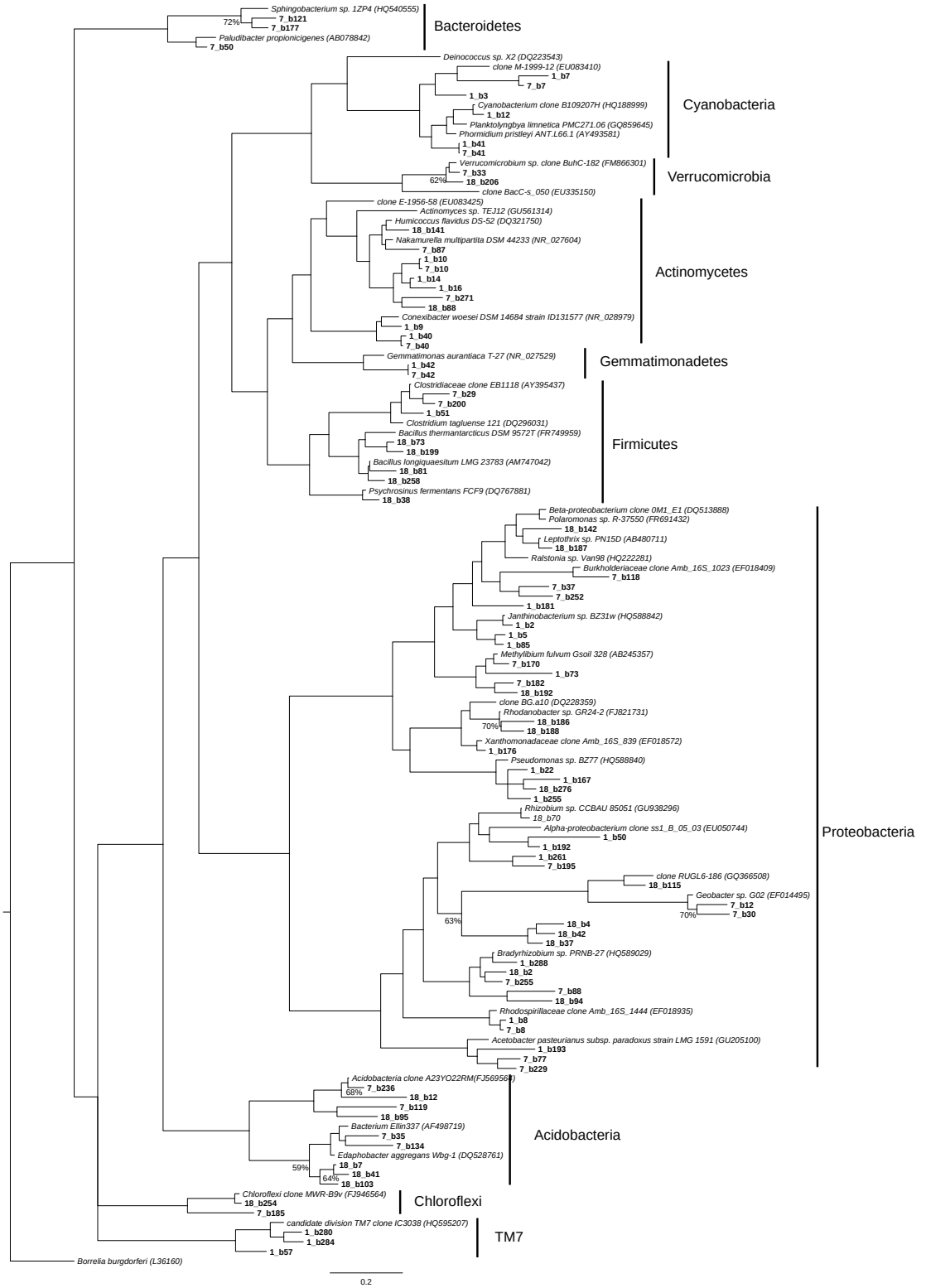
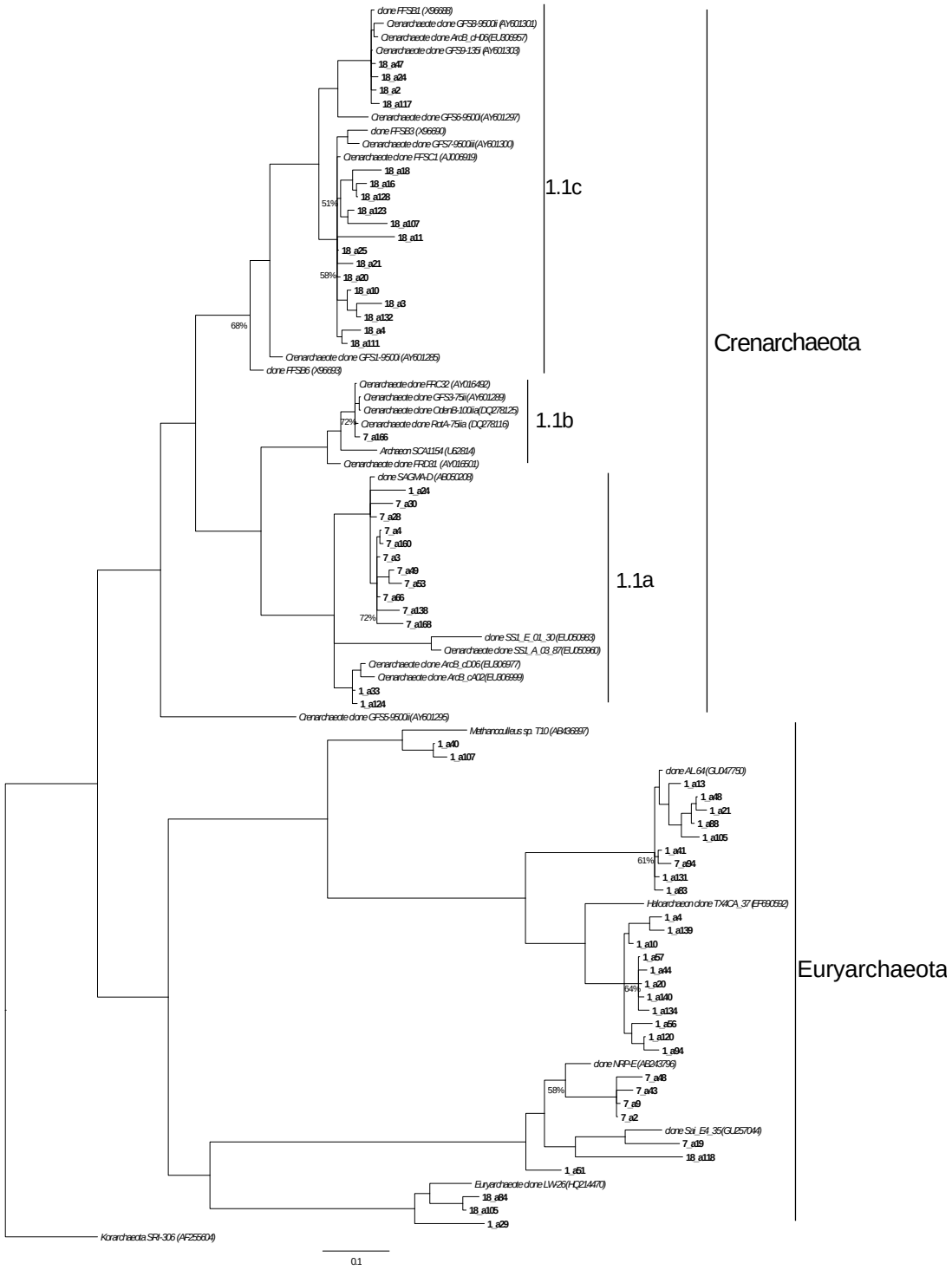
2.8 SUPPLEMENTARY MATERIAL

Supplementary Table ST1. Results of sequence analysis of bacteria, archaea and fungi for the young (site 0, 2 year-old soil), intermediate (site 6, 62 year-old soil) and old soil (site 17, 110 year-old soil), calculated with Mothur with a 97% identity for a unique genus. A total of 192 clones were recovered for each phylogenetic group and site. After RFLP pattern analysis for selecting the clones with unique OTU's, 100 bacterial clones, 64 archaeal clones and 50 fungal clones were sequenced.

	Total clones	Clone OTU's	Number of clones sequenced	Total sequences	Sequence OTU's	Coverage (%)	E
				Bacteria			
young	192	42	100	72	69	4.2	0.996
intermediate	192	41	100	72	69	4.2	0.997
old	192	38	100	58	57	1.7	0.998
				Archaea			
young	192	35	64	53	47	11.3	0.988
intermediate	192	31	64	42	32	23.8	0.966
old	192	34	64	48	43	10.4	0.985
				Fungi			
young	192	22	50	24	13	45.8	0.901
intermediate	192	17	50	39	15	61.5	0.855
old	192	20	50	38	18	52.6	0.887



Supplementary Figure S1: Bacterial phylogenetic tree calculated by Bayesian inference using 748bp, showing affiliation of clones to closest related sequences of different cold and soil habitats and other reference sequences. The *Spirochaete Borrelia burgdorferi* (L36160) was chosen as outgroup. Young 2 year-old site clones (named 1_b); intermediate 62 year-old site clones (7_b) and old 110 year-old site clones (18_b) are shown. For clarity we removed similar sequences in the tree. The accession numbers of the reference sequences are given in brackets. Posterior probabilities from the Bayesian analysis are only shown when below 75%.



Supplementary Figure S2: Archaeal phylogenetic tree calculated by Bayesian inference using 570bp, showing affiliation of clones to closest related sequences of different cold and soil habitats and other reference sequences. The *Korarchaeota* (AF255604) was chosen as outgroup. Young 2 year-old site clones (named 1_a); intermediate 62 year-old site clones (7_a) and old 110 year-old site clones (18_a) are shown. For clarity we removed similar sequences in the tree. The accession numbers of the reference sequences are given in brackets. Posterior probabilities from the Bayesian analysis are only shown when below 75%.

Supplementary Figure S3: Fungal phylogenetic tree calculated by Bayesian inference using 751bp, showing affiliation of clones to closest related sequences of different cold and soil habitats and other reference sequences. A member of the *Chytridiomycota, Cladochytrium* sp. (AB586077) was chosen as outgroup. Young 2 year-old site clones (named 1_f); intermediate 62 year-old site clones (7_f) and old 110 year-old site clones (18_f) are shown. For clarity we removed similar sequences in the tree. The accession numbers of the reference sequences are given in brackets. Posterior probabilities from the Bayesian analysis are only shown when below 75%.

3. PAPER: "MICROBIAL COMMUNITY AND ACTIVITY SHIFTS AFTER SOIL TRANSPLANTATION IN A GLACIER FOREFIELD"

With Stefano M. Bernasconi, Josef Zeyer and Beat Frey

Applied Geochemistry, 26 (2011) 326-329

3.1 ABSTRACT

The majority of Alpine glaciers are currently receding because of global warming. Their forefields have become interesting sites to study primary microbial colonization and microbial adaptation. Here, we discuss the structure and enzyme activity of microbial communities in exposed rock substrates and their changes in a gradient of temperature and soil moisture conditions within the forefield of the Damma glacier in the Swiss Central Alps.

The temperature at the sites differed in the course of a day and also showed differing mean temperatures over the summer. Distinct bacterial communities inhabit the differing sites at the beginning of the experiment and even after transplantation they stay distinct. But a seasonal shift in the communities could be observed, which followed the same pattern for all the samples. Interestingly, microbial enzyme activity was highest at the site with the smallest temperature shifts.

3.2 INTRODUCTION

Most of the glaciers in the Alps are currently retreating due to climate change. As a consequence, they leave behind a visible soil age chronosequence. This chronosequence starts near the glacier terminus where the soil is composed of granitic sand and rocks. This very young soil is of prime interest due to the sparse carbon and nitrogen contents (lack of plants; (Ruf et al., 2006). Microorganisms which live near glaciers, have to withstand harsh environmental conditions, e.g., high UV radiation, large temperature shifts, and large humidity fluctuations (Ley et al., 2004). All these conditions make these environments ideal investigation sites for microbial adaptation and stress resistance.

Seasonal changes in soil microbial populations in alpine environments are common (Lipson & Schmidt, 2004). Microbes even seem to increase their activity with higher soil temperatures (Loffler et al., 2008). Although it is known that altering microclimatic conditions influences microbial biomass and activity, it was the aim of the present study to investigate the extent of changes in the microbial community and activity in dependence on temperature and moisture shifts. This was achieved by soil transplantation, which is a technique to determine key environmental parameters, which can alter microbial structure and activity in soils (Bottomley et al., 2006; Boyle et al., 2006; Waldrop & Firestone, 2006).

3.3 METHODS

We selected three sites with a similar soil age but differing expositions in front of the glacier terminus at the Damma glacier, Switzerland (Figure 1A). We choose the sites primarily due to the differing amount of sunlight hours which they receive because of the shading effects of the nearby mountains. The sites are called 'shadow', 'intermediate' and 'sun'. At these 3 sites, 15 pots per site were filled with local granitic sand and 5 transplanted to each of the other two sites, 5 pots remained at the original site. The experiment started at the beginning of summer 2010 (Figure 1B).

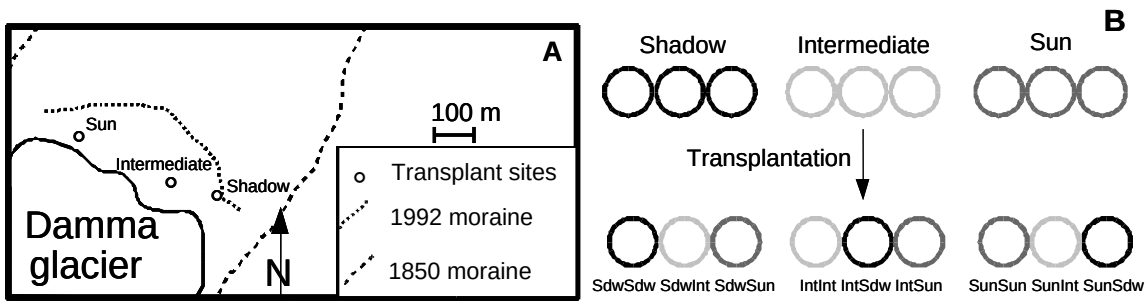


Figure 1: (A) Transplantation sites in the Damma glacier forefield, showing the two lateral moraines from 1850 and the 1992 terminal moraine with the glacier terminus in the lower left corner. (B) Schematic of the experimental setup. Each circle represents 5 replicate pots filled with granitic sand. From each site 5 pots were transplanted to the two other locations and vice versa. The name given to each sample is specified in the lower part of the graph. The first part of the name is the site where the sample is now located: Sdw for shadow, Int for intermediate and Sun for sun. The second part of the name tells the origin of the sample (e.g., SunSdw is the shadow sample located at the sun site after transplantation).

During the vegetation period, we monitored continuously environmental conditions such as soil moisture and temperature with 8 probes for temperature and 5 probes for soil moisture at each site. The values shown here are the mean values of these probes for a given time period or time point respectively. At the starting point, after 8 weeks, and after 16 weeks of transplantation we determined the bacterial community structure with terminal restriction fragment analysis (T-RFLP) followed by multivariate statistics (PCA) according to Frey et al. (2009) using the CANOCO program for windows (Ter Braak & Smilauer, 2002). We measured the microbial enzyme activity of beta-

glucosidase, N-acetyl-glucosaminidase, L-Leucine-aminopeptidase and phosphatase (Marx et al., 2001). The total enzyme activity was then calculated summing-up the results to give the total enzymatic activity in nmol g^{-1} h^{-1} with the cumulative standard deviation.

3.4 Results and Discussion

3.4.1 Temperature and soil moisture

Fluctuations of the soil moisture were fairly high, most likely due to rainfall (Figure 2A). The sharp increase in July is due to complete dryness of the soil after transplanting, followed by rainfall for several days. The pots did not dry out completely again but rather retained some water. All plots showed a similar moisture regime with corresponding maxima and minima as well as comparable seasonal means but overall with few differences between sites (Table 1). Interestingly, the intermediate site showed slightly higher soil moisture content and the highest difference between the maximum and minimum compared to the other sites.

The temperature gradually dropped towards autumn at all sites (Figure 2B). The mean temperature during the season was 7.67°C for the shadow site, 8.99°C for the intermediate site and 9.47 °C for the sun site (Table 1). The temperatures fluctuated strongly, up to 20°C in the course of each day (data not shown). Also over the whole monitoring period (July-October) the temperature difference between maximum and minimum was as much as 38.47°C for the intermediate site and 32.92°C for the sun site. At the shadow site the temperature was more stable with the difference being only 20.93°C. This data confirm the expected temperature fluctuations and the temperature peak differences between the sites.

Table 1: Soil Moisture and Temperature in comparison for all the three sites. The seasonal mean, the maximum and minimum and the difference between the two extremes are shown. The temperature was measured with 8 probes at each site, the soil moisture with 5 probes at each site. The means of these probes for one time point are shown here.

	Soil Moisture (m^3/m^3 VWC)				Temperature (°C)			
Site	**Seasonal Mean**	**Maximum**	**Minimum**	**Max-Min**	**Seasonal Mean**	**Maximum**	**Minimum**	**Max-Min**
Shadow	0.11	0.17	0.00	0.17	7.67	21.18	0.25	20.93
Intermediate	0.14	0.31	0.00	0.31	8.99	35.26	-3.21	38.47
Sun	0.12	0.28	0.00	0.27	9.47	30.23	-2.69	32.92

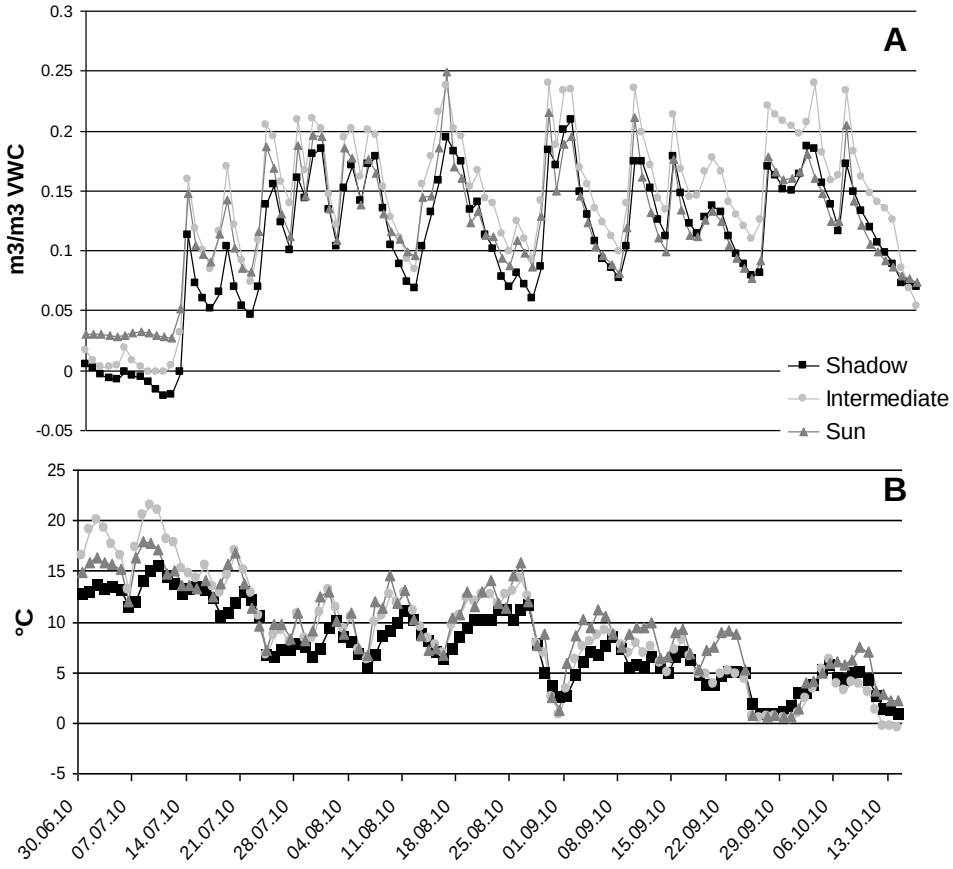


Figure 2: Soil moisture (A) and temperature (B) fluctuations during the 16 week experiment in 2010 at the three experimental sites. The means of all the measurements during one day are shown.

3.4.2 Enzyme Activity

Enzyme activity can be used to estimate the activity of the soil microbes and their nutrient demand (Sinsabaugh et al., 2008). The results in this study revealed that the microbial communities originating from the shadow site (black bars) showed the highest enzyme activity and variability (Figure 3), from the beginning of the experiment until week 16, independent of the soil transplantation procedure. Conversely, the samples originating from the sun site (dark grey bars) showed the least activity during the whole experiment, indicating a microbial population which was less active. It even seemed that there was a substantial decline of the active microbial population after 8 weeks, as the enzymatic activity dropped drastically. This could be due to the fact that fewer microorganisms were able to be active in the harsher conditions at the sun site, where a stronger adaptation to temperature fluctuation is needed. As a consequence, activity was much lower from the start and the microbes could not adapt to the new conditions during the timescale of our measurements (16 weeks). However, the samples originating from the shadow did not decrease in activity, thus the microbes did not seem to encounter adaptation problems after transplantation. This indicates that the microbes were more active already from the start, and were not influenced by the transplantation during this timescale.

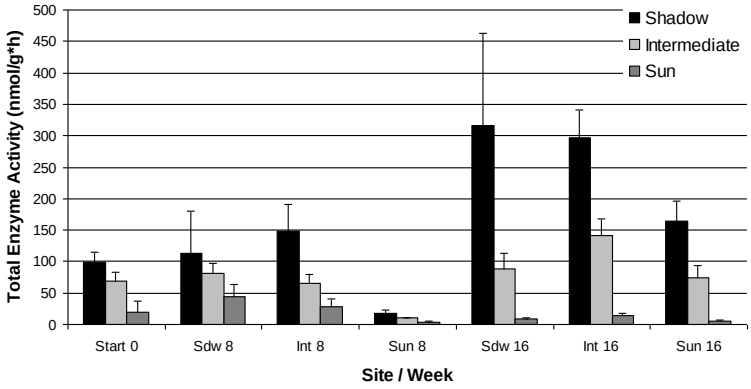


Figure 3: Total enzymatic activity at the starting point, 8 and 16 weeks after transplantation.

3.4.3 T-RFLP

After the T-RFLP analysis a covariance principal component analysis (PCA) of the peaks was performed in order to classify bacterial community shifts during the experiment (Fig. 4). The data showed that the community structure was indeed different between the sites already at the start of the experiment. After 8 weeks of transplantation the three samples from the same origin clustered together, independently on the transplantation procedure (e.g., all sites clustered to the left in the PCA plot). However, after 16 weeks they seemed to be more distinct from each other, which indicates a better separation in community composition after a longer timescale.

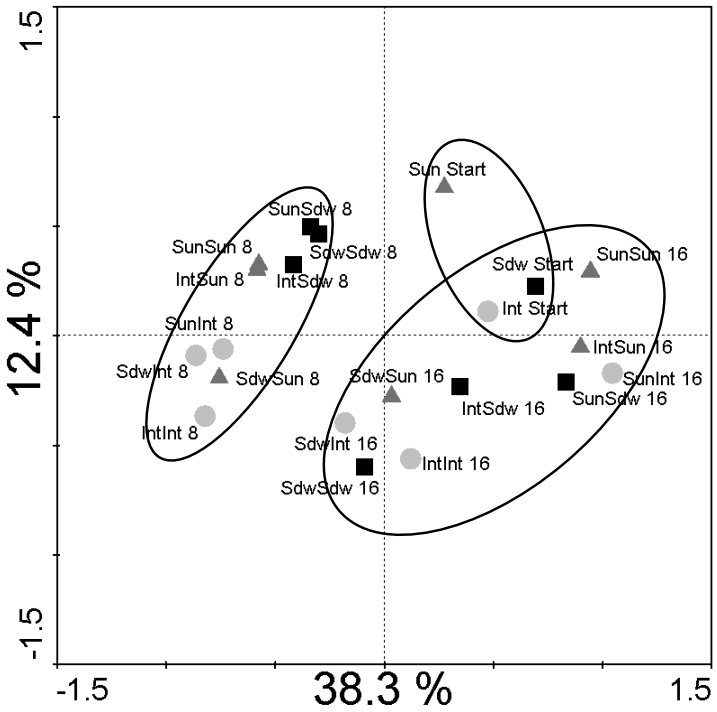


Figure 4: Terminal restriction fragment polymorphism shown after covariance principal component analysis (PCA). The samples from the start and after 8 and 16 weeks after transplantation are shown (week marked after sample name; stages circled). The first axis is accounting for 38.3% of the variance, the second axis for 12.4%.

Our results so far are in accordance to Stres et al. (2008), who did not observe any community shift at differing temperatures after a 12 week microcosm experiment. However, Bell et al. (2008) detected community responses to higher temperature and moisture in a desert but in a 3 years experiment. Such shifts could be probably detected in our study as well after a longer timescale. It is interesting that all samples in the PCA tended to shift in the same direction after 8 and after 16 weeks, no matter where the starting point was. Thus, one can assume that the seasonal variation is always the same in each sample, and that not even transplantation could interrupt this pattern, implying a greater influence of the season than of the transplantation on the community.

3.5 Conclusion

With the present results we can say, that the temperature was indeed different at our three selected locations with the same soil age. Also a differing bacterial community was present at the differing sites before transplantation. The communities of the site with the least temperature fluctuation were the most active, whereas the ones with a greater fluctuation were less active. Interestingly, it seemed that the seasonal influence on the communities was more pronounced than the temperature and moisture fluctuations, as the communities' evolution followed a similar pattern over the 16 weeks of experiment regardless of the site.

Overall, our results show the need for a longer time series in order to be able to more accurately distinguish for the evolution in community structure and to be able to do better statistical analyses. There is also a need to determine the temporal fluctuations of the biogeochemical composition of the granitic sand of the three sites in detail, including microbial biomass and nutrient composition, in order to gain insights into the environmental drivers responsible for the observed microbial community variations.

3.6 Acknowledgements

Financial support for this study was provided by the "Biosphere-Geosphere interactions: Linking climate change, weathering, soil formation and ecosystem evolution (BigLink)" project of the Competence Centre Environment and Sustainability (CCES) of the ETH Domain (Bernasconi & BigLink, 2008).

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4. PAPER: "BACTERIAL AND FUNGAL COMMUNITY RESPONSES TO RECIPROCAL SOIL TRANSFER ALONG A TEMPERATURE AND SOIL MOISTURE GRADIENT IN A GLACIER FOREFIELD"

With Erland Bååth, Beat Stierli, Josef Zeyer and Beat Frey

Soil Biology and Biochemistry, 61 (2013) 121-132

4.1 ABSTRACT

The influence of soil physicochemical properties on microbial communities can be large, especially in developing soils of glacier forefield chronosequences. However, small-scale expositional differences in bare soils and their impacts on soil microbial communities have so far been largely neglected. Here we studied the changes of microbial communities in three deglaciated unvegetated sites along a soil moisture and temperature gradient near a glacier terminus. In order to elucidate the driving forces for these changes, fine granite sediment was reciprocally transferred and regularly sampled during 16 months to determine microbial activities and the bacterial and fungal community structures and compositions using T-RFLP profiling and sequence analysis. Microbial activities only responded to soil transfer from the warmer and drier site to the colder and moister site, whereas the bacterial and fungal community structures responded to transfer in both directions. Bacterial phylotypes found to react to soil transfer were mainly the *Acidobacteria*, *Actinobacteria*, *alpha-* and *beta-Proteobacteria*. The common fungal phylogenetic groups *Pezizomycetes* and mitosporic *Ascomycetes* also reacted to soil transfer. It seemed that the soil moisture was the limiting factor for the microbial activities. We concluded that for the microbial community structures transferring soil from a colder to a warmer site induced a higher rate of change due to a higher microbial activity and faster species turnover than the reverse transfer.

4.2 INTRODUCTION

Temperate Alpine glaciers are retreating rapidly due to climate change (Paul, et al. 2004). The soil close to the glacier terminus is therefore composed of recently exposed sediment and stones. With increasing time since deglaciation, and therefore increasing distance to the glacier terminus, this bare sediment evolves into a developed soil through physical and chemical weathering, colonization of plants, microbes and animals (Bernasconi, et al. 2011). Several studies have shown that bacteria, archaea and also fungi undergo a community succession along such soil age chronosequences in glacier forefields (Jumpponen 2003; Nicol, et al. 2005; Schutte, et al. 2009; Zumsteg, et al. 2012). These studies also revealed how soil carbon, nitrogen and vegetation cover affect the microbial community composition and thus might drive the changes in microbial community structures along glacier forefield chronosequences. But microclimatic factors, such as soil moisture, high temperature fluctuations, freeze-thaw cycles and UV-radiation can also have a large impact on soil microbial communities (Lipson 2007; Albert, et al. 2008; Bell, et al. 2008). At higher altitudes, e.g. the Alps, the microclimate in barren soils is very variable, in particular in valleys where the number of daily sunlight hours might differ greatly from one site to another due to their exposure to the sun. Furthermore, large stones and glacial streams add to the heterogeneity, which might also considerably influence the microclimate in soil. However, such studies have been neglected in temperate glacier forefields so far.

Bare soils provide an ideal investigation site to elucidate the effects of different microclimatic conditions on the soil microbial communities. Additionally, reciprocal soil-transfer experiments often are a valuable way of evaluating the adaptational capabilities of microbial communities (Bottomley, et al. 2006; Hart 2006; Waldrop and Firestone 2006; Lazzaro, et al. 2011; Zumsteg, et al. 2011). Performing a reciprocal soil transfer in an unvegetated glacier forefield soil could, therefore, answer the question of whether soil microbial communities might change in response to small-scale microclimatic differences, but under comparable soil physicochemical conditions.

The Damma glacier in the Swiss Alps lies in a valley surrounded by mountains, and the microclimate is, as expected, highly variable (Bernasconi, et al. 2011). In a preliminary evaluation we found significant differences in soil microbial activity and community structures comparing different sites along a soil moisture and temperature gradient in bare soils but within the same distance from the Damma glacier terminus (Zumsteg, et al. 2011). In the present study we extended this reciprocal soil transfer experiment to elucidate the driving forces for these variations between the sites. We hypothesized that (1) the exposure of a site to the sun, which may affect soil temperature and soil moisture, is a crucial variable influencing microbial community structures and activities in soils; (2) the microbial activity will be temperature dependent, therefore it can be expected that the microbial activity will increase when transferred from a rather cold soil environment to a warmer soil environment (3) also the microbial community structure will be dependent on the microclimatic conditions and therefore specific species will react to soil transfer by either increasing or decreasing in abundance.

DNA extracted from soil samples along a microclimatic gradient at different sampling times after soil transfer were subjected to terminal restriction fragment length polymorphism (T-RFLP) profiling to characterize the bacterial and fungal community structures in the soils followed by cloning and Sanger sequencing to evaluate which phylogenetic groups reacted most to the soil transfer. The most responsive operational taxonomic units (OTUs) were grouped into “resistant” (their abundance increased with transfer) and “sensitive” (their abundance decreased with transfer) (Lankau 2010). The characterization of the bacterial and fungal community composition was complemented by assessing various microbial activity parameters like bacterial and fungal activity, fluorescein diacetate (FDA) hydrolytic activity and selected enzyme activities.

4.3 Materials and Methods

4.3.1 Location and study sites

The Damma glacier forefield is located in the Central Alps in Switzerland (N 46°38 E 08°27), within the Central Aar Massif at an altitude between 1950 and 2050 m a.s.l.. The front of the Damma glacier has been monitored by the Swiss glacier monitoring network (http://glaciology.ethz.ch/messnetz/?locale=en) since 1921 and has since retreated at an average rate of approximately 10 m per year since the beginning of systematic measurements. The forefield has been the subject of extensive interdisciplinary studies (Bernasconi, et al. 2011). Climatic data were obtained from meteo-stations installed within the glacier forefield. The mean precipitation in 2011 was 1900 mm and the mean annual temperature 1.8°C.

We selected three sites along the cross-section in front of the Damma glacier characterized by unvegetated bare soil, each approximately 40 m away from the forefront (Figure 1). These sites were primarily chosen because they are exposed to different hours of sunshine, depending on how shaded they are by the nearby mountains. The north-facing site (N) receives a mean of 7.7 h of sunshine during the summer period from June to October, the middle site (M) 8.5 h and the south-facing site (S) 9.1 h. Thus each site has a different temperature regime.

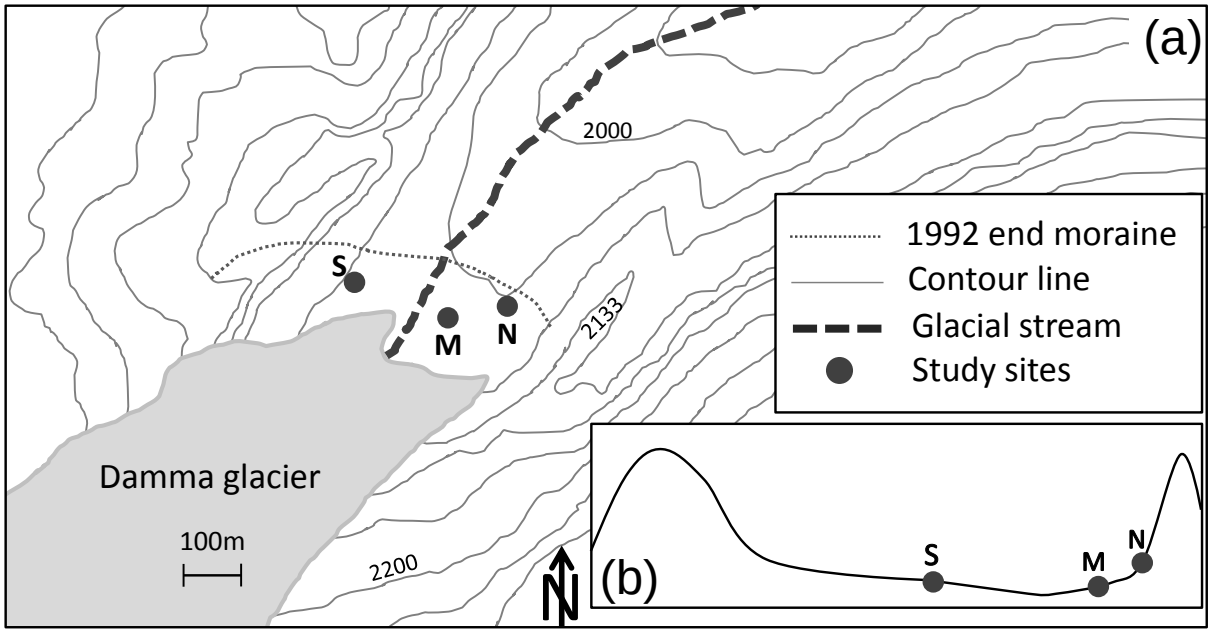

Figure 1: Map of the Damma glacier forefield with the three study sites near the glacier terminus along a microclimatic gradient. a) The glacier has formed a valley which is surrounded by mountains. The sites are located along a cross-section. The north-facing site (N) is cold and moist, the middle site (M) is less cold and moist and the south-facing site (S) is warm and dry as could be expected from their exposition. b) Cross-section of the gradient where the three sampling sites are located. The side moraine in the north-facing site is steeper than that in the south-facing site, but both have the same elevation of approximately 150 m.

4.3.2 Climatic and soil physico-chemical parameters

Temperature was continuously monitored at the three study sites with eight HOBO loggers for temperature (Onset Computer Corporation, Pocasset, MA, USA) at a depth of 5 cm. The loggers were all removed before winter and replaced in spring. The temperature difference between day (3 pm) and night (3 am) was calculated to illustrate the climatic variation between the N and the S sites only (Figure 3a), as the temperature at the M site was fairly similar to the S site. These time points were chosen because they represented the minimum and maximum temperatures during the day. Additionally the gravimetric water content was determined at every sampling date by pooling the soils from the five replicate samples at each site (n = 1) (Figure 3b). To measure the gravimetric water content was the best compromise, as measuring the soil moisture content with sensors proved to be difficult in these sandy soils.

Sieved soils (2 mm mesh size) after soil transfer (see below) of all the respective sites were taken to the laboratory where they were water-saturated to evaluate the porosity and then desorbed to a water potential of -6 kPa (field capacity) by applying a hanging water column and weighed before and after drainage. The drained pore volume represents the coarse pore fraction (>50 µm). The samples were then dried at 105°C for 6 days and weighed again. The pore volume desorbed between -6 kPa and the oven-dry state represents the fine-to-intermediate pore fraction (<50 µm) (Schaffer, et al. 2007). The water holding-capacity was determined by calculating the proportion of water the soil is able to retain of the fully saturated amount at field capacity (-6 kPa).

The soil texture was analyzed according to Gee and Bauder (1986) after acid digestion with H2O2.

The soil pH in water was determined as described in Zumsteg et al. (2012). 10 g of soil were extracted with 50 ml of water and the total dissolved macro- and micronutrients were and measured with inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6000; Perkin-Elmer, Boston, MA) and the concentrations of major anions were analyzed with ion chromatography (DX-120; Dionex, Thermo Scientific, Sunnyvale CA, USA). Additionally 10 g of soil were extracted with 50 ml 1 M ammonium-chloride solution to analyze the total dissolved cation concentrations (without ammonium) with ICP-AES (ARL 3580 OPTIMA 3000; Perkin-Elmer). Ammonium concentrations were measured using flow-injection analysis (FIA, FIAS 300; Perkin-Elmer) from 30 g of soil extracted with 60 ml of water. The total carbon and total nitrogen in the fine sediment were measured in 1 mg of soil (NA 2500; CE Instruments, Wigan, UK) after drying the soils at 60°C over-night and grinding in a Retsch MM 400 mill (Verder Catag AG, Basel, Switzerland).

4.3.3 Reciprocal transfer setup and sampling

Fine granitic sediments collected at each of the three study sites along the cross-section (Figure 1) were sieved (2 mm mesh size) and pooled for each site. The pooled granitic sediment was put in 15 pots at each of the three sites, and five of them were transferred to each of the other two sites. Five

pots remained at each original location (Figure 2). The pots were 12 cm in diameter and 25 cm in height, with holes (1 cm in diameter) at the bottom for water drainage. Approximately 3 kg of dry sand was filled into each pot. The experiment started on 30 June 2010, and the five replicate pots at each site were sampled 2, 4, 12, 14 and 16 months later. For each replicate pot, soil samples were taken with an ethanol cleaned shovel at a depth of 0 to 7 cm in the middle of the pots. The samples were stored at -20°C until further processing.

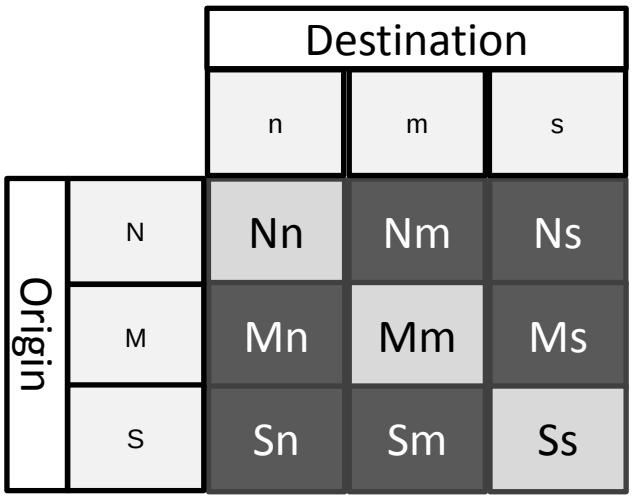

Figure 2: Conceptual representation of the transfer setup along the microclimatic gradient. From each of the three sites in front of the Damma glacier terminus, five pots with granite sediment were transferred to each of the two other sites and five pots remained at the original location. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters.

4.3.4 Measurements of microbial activity

Right after sampling the fluorescein diacetate (3´,6´-diacetylfluorescein (FDA)) hydrolytic activity (colorimetric assay) was measured according to Green et al. (2006) in the five replicate samples (one per pot). FDA hydrolysis is a proxy for total microbial activity as both bacteria and fungi can hydrolyze FDA in soil (Schnurer and Rosswall 1982). The activity is given as pmol g^{-1} h^{-1}.

Leucine incorporation was used as a proxy for the bacterial activity, and acetate-in-ergosterol incorporation as a proxy for the fungal activity, measured according to Bååth (Bååth 1998) and

(Bååth 2001), respectively. Both methods use radiolabeled carbons (L-^{3}H leucine and 1-^{14}C acetic acid respectively) for the measurement of the incorporated radioactivity and take this as a proxy for the microbial activity. As the soil samples were frozen at -20°C they were thawed at room temperature for 24 h prior to the measurement. The activities were measured at room temperature because during the summer months, 20°C is not an uncommon temperature in the forefield and because activities were too low at the specific mean temperatures of the sites (which were below 10°C, see below). In contrast to the original protocols, 2 g of soil was processed instead of 1 g, and the incubation time for the leucine incorporation was extended from 2 h to 2.5 h and for the acetate-in-ergosterol incorporation from 2 h to 6 h. The activity is given as incorporated pmol leucine g^{-1} h^{-1} and pmol acetate g^{-1} h^{-1}.

Activities of β-glucosidase and phosphatase were measured using the MUF assay according to Marx et al. (2001) (change in fluorescence over time) after thawing the samples at room temperature for 24 h. In contrast to the original protocol, the samples were incubated at 15°C instead of 37°C for 3 h. The activity is given as nmol g^{-1} h^{-1}. All the activities were measured using chemicals from Sigma-Aldrich (St. Louis, MO, USA).

4.3.5 Analysis of bacterial and fungal communities

Genomic DNA was isolated from the soil samples from each of the five replicate pots using the soil DNA extraction kit “Ultra Clean Soil DNA” (Mo-Bio Laboratories Inc., Carlsbad, CA, USA), with 1 g of granite sand according to the manufacturers’ instructions. The extracted DNA was quantified with Pico Green (Invitrogen, Carlsbad, CA, USA), and stored at -20°C until further use. Primers 27F and 1378r were used to amplify the bacterial 16S rRNA genes, and the primers nu-SSU0817 and nu-SSU1536 to amplify the fungal 18S rRNA genes. PCR conditions were the same as previously described (Zumsteg, et al. 2012). Primers for the PCR amplification were obtained from Microsynth GmbH (Balgach, Switzerland). For the T-RFLP analysis, the bacterial 16S rRNA amplicons were

digested with the restriction enzyme *MspI*, and the fungal 18S rRNA amplicons with *AluI*. The T-RFLP analysis was performed as previously described using FAM-labeled primers (Frey, et al. 2011; Zumsteg, et al. 2012). We also performed T-RFLP analyses on undisturbed soil samples in the vicinity of the experimental pots 12 and 16 months after transfer to ensure that the samples in the pots were comparable to the undisturbed soils as controls.

To evaluate the bacterial community composition, five clone libraries were prepared, and three clone libraries for the evaluation of the fungal community composition, with the same but unlabeled primers as described above. PCR for the clone libraries was performed on the pooled DNA replicates from each soil at all three locations after transfer (Nn, Nm, Ns, Mn, Mm, Ms, Sn, Sm and Ss) at five time points (start and 2, 4, 12 and 16 months after transfer) for bacteria, and at three time points (start and 4 and 16 months after transfer) for fungi. The PCR products of the corresponding time points were then pooled together for transformation (thus 9 samples were pooled for one clone library) giving five clone libraries for bacteria (start and 2, 4, 12 and 16 months after transfer) and three clone libraries for fungi (start and 4 and 16 months after transfer). T-RFLP profiling was performed on DNA from all individual samples (soils and sampling times) whereas for the clone libraries the individual DNA from the same sampling times were pooled for PCRs and cloning was then performed separating the individual sampling times but not the soils.

PCR products were ligated into the vector of the pGEM-T Easy Vector System and cloned into the competent cells JM109 (Promega Corporation, Fitchburg, WI, USA), according to the manufacturers' instructions. The clones were subjected to T-RFLP analysis to compare the clone T-RFs with the T-RFLP profiles of the environmental samples. The M13 PCR products were bi-directionally sequenced with the primers 27f and 907rev (bacteria), or with the primers T7 and SP6 (fungi), using an ABI prism sequencer 3730XL (Applied Biosystems, Carlsbad, CA, USA). The sequences were then sorted, aligned, chimera-checked and analyzed as previously described (Zumsteg, et al. 2012).

One T-RF found through the T-RFLP profiles was considered to be one operational taxonomic unit (OTU). The difference between the mean normalized abundance of the OTUs of the transferred samples after 16 months and at the starting point was calculated from the T-RFLP profiles. The OTUs with positive values were considered to be “resistant” OTUs, as their abundance increased with transfer. The OTUs with negative values were considered to be “sensitive” OTUs, as their normalized abundance decreased after soil transfer (Lankau 2010). The classified OTUs could then be assigned to phylogenetic groups according to the sequences and the corresponding T-RFLP profile.

The sequences were deposited in Genbank under the accession numbers JQ480456 to JQ480609 for the bacterial, and JQ805724 to JQ805779 for the fungal sequences.

4.3.6 Statistical analysis

The activity measurements from the three sites were compared statistically using the program IBM SPSS Statistics (Armonk, NY, USA). After log transformation, the activity parameters from the start (t0) were analyzed with one-way ANOVA to determine significant differences between the three sites. After transfer the mean of each soil at the three sites was taken over all the time points (t0 – t5) and again compared by one-way ANOVA to determine significant differences between the mean of non-transferred samples and the transferred soil samples. In general disturbance effects should be minimal, as all the samples were treated in the same way, both transferred and non-transferred soil samples.

The activity parameters after transfer were also analyzed with one- and two-way repeated measures ANOVA (time as the repeated measure variable) according to time (one-way) in the non-transferred samples, and according to transfer and time (transferred samples compared to the non-transferred samples; two-way) to evaluate the effect of time and transfer on the microbial activities. Here the confidence intervals were adjusted for the test of significance according to Bonferroni (reducing the probability of making one or more false discoveries, rejecting a true null hypothesis when performing

multiple hypotheses tests (Dunn 1961)) and the degrees of freedom according to Greenhouse-Geisser (alters the degrees of freedom and produces an F-ratio where the type I error rate is reduced (incorrect rejection of a true null-hypothesis) (Greenhouse and Geisser 1959)).

The T-RFLP profiling data were analyzed with the statistics program Primer E (Primer E Ltd, Ivybridge, GB). The normalized T-RFLP data were subjected to a square root transformation, followed by a hierarchical cluster analysis on the Bray-Curtis similarities (Hartmann, et al. 2012), combined with a simprof test, which is a similarity profile routine to test for evidences of structure in a unstructured set of samples (Clarke, et al. 2008), to show the significance of the branches in the cluster. The T-RFLP data were subjected to a PERMANOVA (distance-based permutational multivariable analysis of variance (Anderson 2001)) analysis to investigate the influence of time and transfer on all samples. A Monte-Carlo test was performed with 9999 unrestricted permutations. The time effect represents the change in soil microbial communities in the non-transferred samples within the particular time period, i.e. without any change in location or microclimate. The transfer effect represents the change in soil microbial communities when transferred to another site compared to the non-transferred samples. To evaluate the influence of the temperature and the soil moisture on the microbial community structures in both the non-transferred and the transferred samples, a DistLM analysis was performed on the square root transformed T-RFLP data, with temperature and soil moisture as factors. DISTLM calculates a multivariate multiple regression analysis of symmetric distance matrices (Anderson 2004). The principal coordinate analysis (PCO) was calculated on the T-RFLP data of both bacteria and fungi, showing the community distribution according to time and site.

4.4 RESULTS

4.4.1 PHYSICO-CHEMICAL AND MICROCLIMATIC CHARACTERIZATION OF THE CROSS-SECTION

The soil characteristics at the three study sites on the Damma glacier forefield were similar. The pH varied between 5.1 at the N site and 5.6 at the M site (Table 1). The nutrient contents in all three soils were very low and did not change during the experiment (Supplementary Table T1) and also the C and N contents were low at all three sites (Table 1). Measurements of nitrite, phosphate, Fe, Pb and Zn were at the detection limit and are therefore not shown. The N site was characterized by a higher silt content (14.5%) and a higher fine-to-intermediate pore volume (< 50 µm) than the other two sites, which had a silt content of 7.9% (M site) and 5.6% (S site), respectively. This meant that the N soil could hold more water than the other two soils (Table 1).

Table 1: Soil physico-chemical parameters at the three study sites (N = north-facing site, M = middle site, S = south-facing site) along the microclimatic gradient. The soil porosity was measured as the pore volume in the corresponding soil volume (m^3/m^3).

	C	**N**	**pH**	**Sand**	**Silt**	**Clay**	**Soil porosity**		**Water-holding capacity**
	(%)	(%)	(in H_2O)	(%)	(%)	(%)	> 50µm	< 50µm	(%)
N	0.09	0.008	5.1	84.8	14.5	0.7	0.171	0.258	60
M	0.12	0.007	5.6	91.0	7.9	1.1	0.276	0.120	30
S	0.08	0.006	5.3	93.1	5.6	1.3	0.225	0.173	43

The mean temperature in 2010 during the summer months was 7.7°C at the N site, 9°C at the M site and 9.5°C at the S site (Zumsteg, et al. 2011). The mean for the 2011 summer months was 8.2°C at the N site, 9.5°C at the M site and 11.6°C at the S site. Temperature fluctuations between day and night were highly variable at the three sites; with the largest fluctuations at the S site (Figure 3a). The maximum temperature at the N site was 20°C, at the S site it was 36°C (data not shown). The gravimetric water content of the N soil was always higher than the water content of the M and the S soil when comparing the soils at the same sites (Figure 3b).

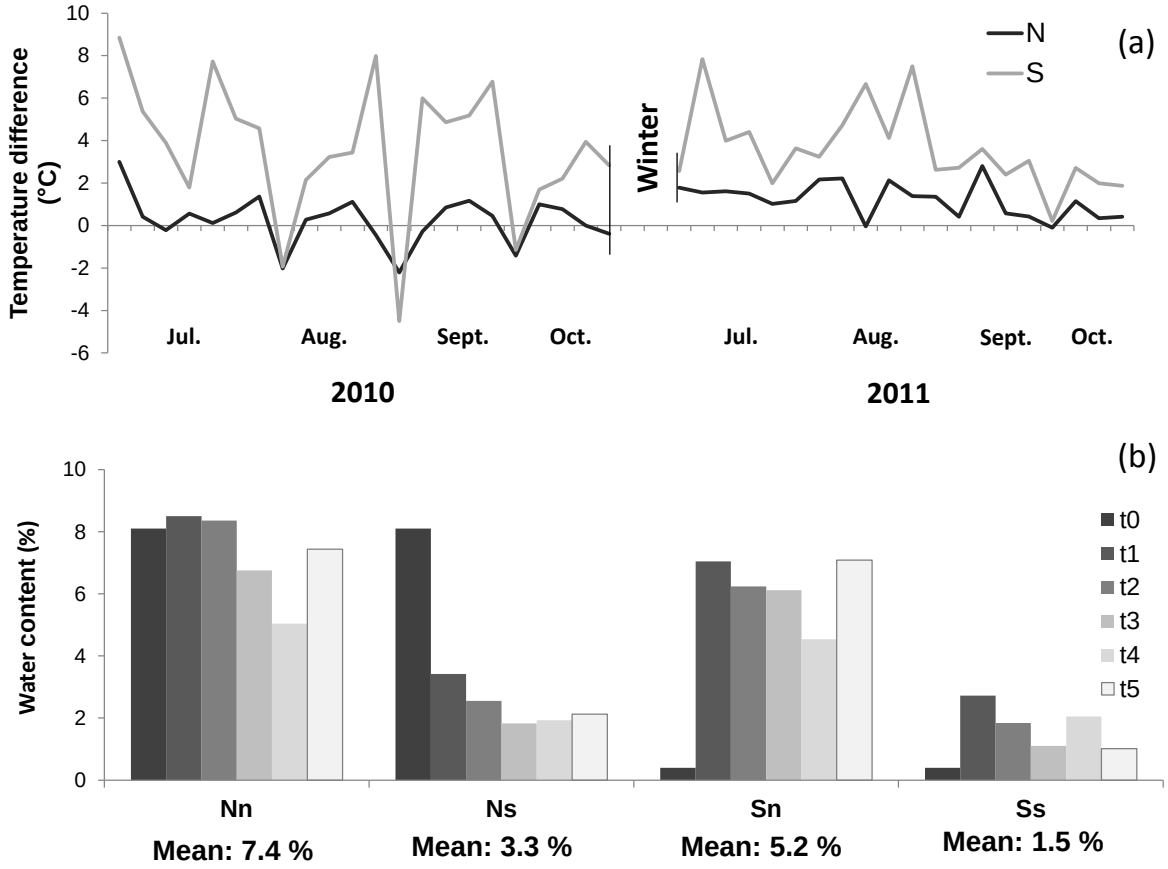

Figure 3: a) Soil temperature difference between day and night at a depth of 5 cm at the north-facing site (N) and the south-facing site (S) for the two vegetation periods 2010 and 2011. A value below 0 indicates that the night was warmer than the day. The measurements were stopped during the winter months. b) Soil moisture as the gravimetric water content at the N site and at the S site for the N soil (Nn and Ns) and the S soil (Sn and Ss) at the five sampling dates (t0 – t5). The mean over all the sampling dates for each soil is given bellow the graph, showing that the N soil was always more moist than the S soil at the same site.

4.4.2 Microbial parameters along the microclimatic gradient (cross-section)

In general, microbial activities were highest in the cold and moist N soil, and significantly ($P < 0.05$) different from those in the other soil environments in most cases. FDA was around 3 times as high as in the other soil environments, leucine incorporation (bacterial activity) 7 times, acetate-in-ergosterol incorporation (fungal activity) 3 times and the phosphatase activity 1.7 times (Figure 4). β–glucosidase activity did not differ between soil environments.

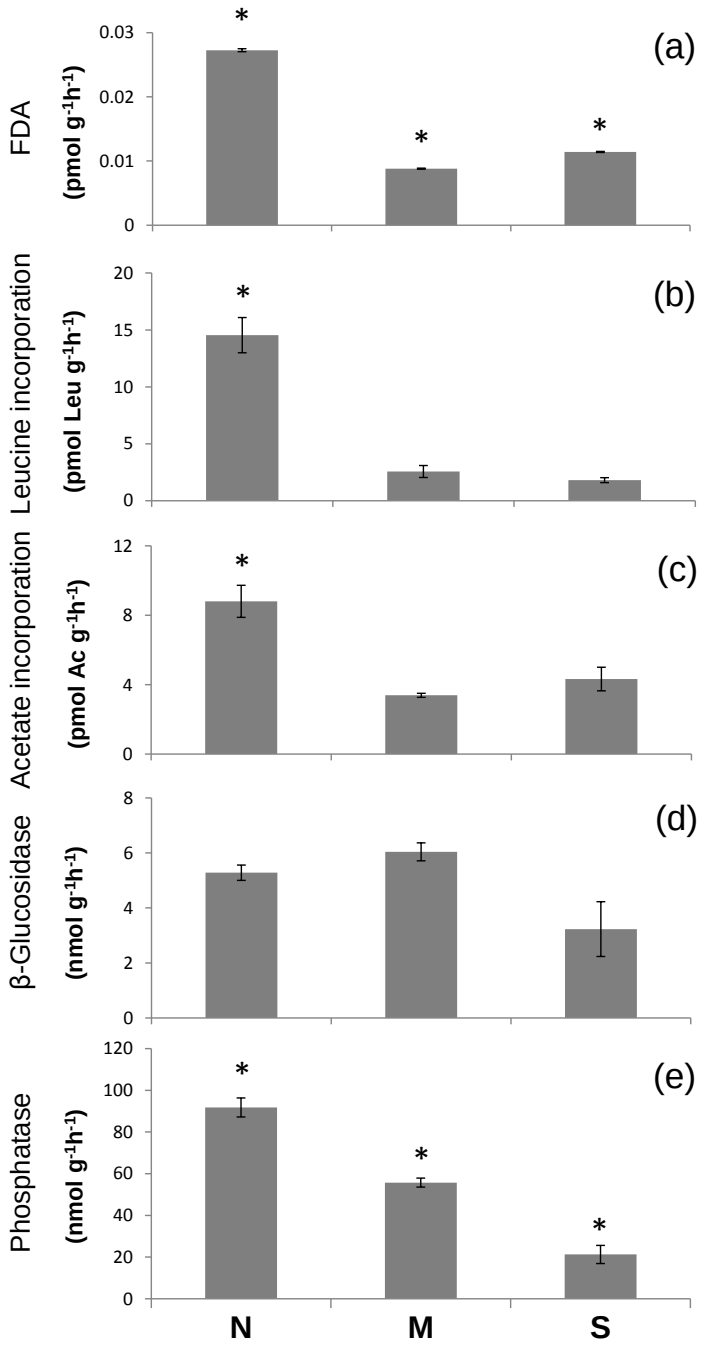

Figure 4: Microbial activities at the three study sites along the microclimatic gradient in June 2010 at the start of the experiment (N = north-facing site, M = middle site, S = south-facing site). a) FDA, b) leucine incorporation (bacterial activity), c) acetate-in-ergosterol incorporation (fungal activity), d) β-glucosidase activity, and e) phosphatase activity. The values are given for one gram of dry soil (n = 3 ± SE).

* Significantly different from the other two sites ($P < 0.05$) according to one-way ANOVA.

The bacterial and fungal community structures in the three sites differed significantly ($P < 0.05$) between the soil environments (Figure 5). In particular, the bacterial and fungal community structures of the warmer S site clustered differently from those of the other two sites.

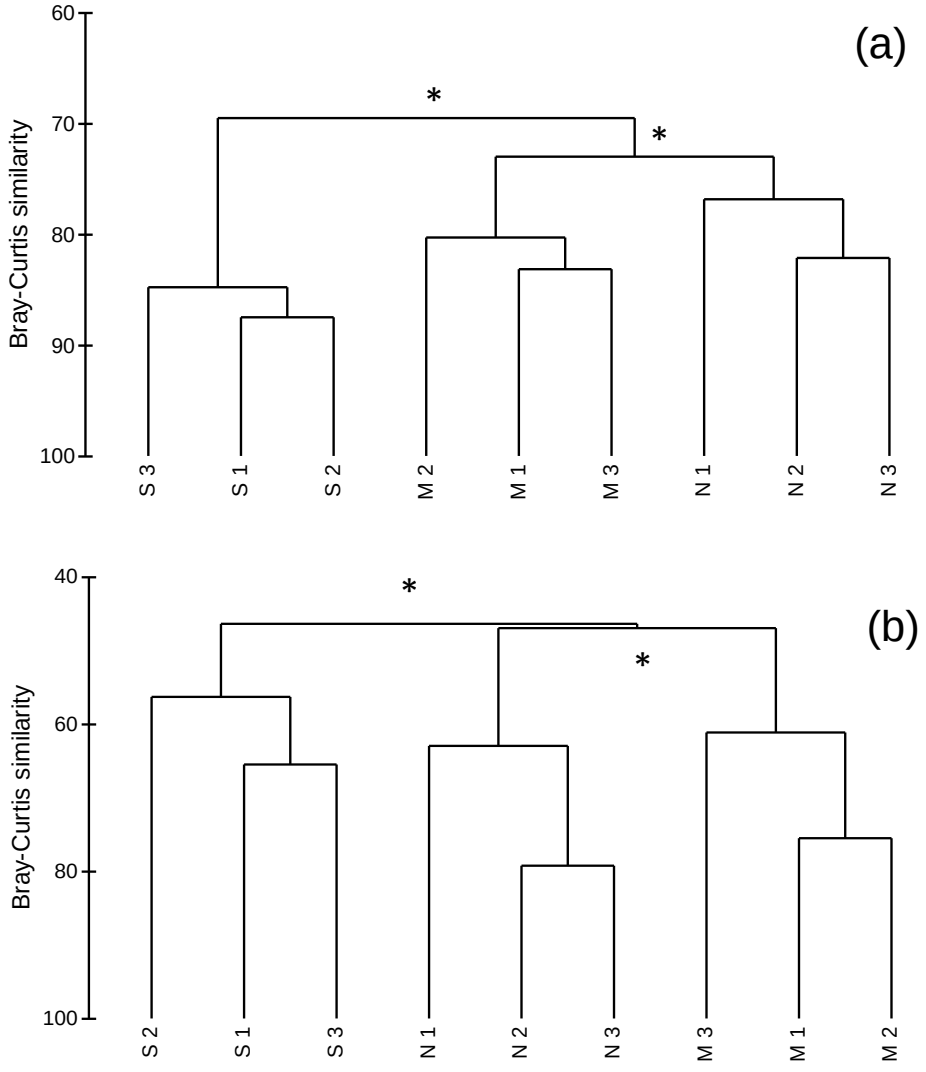

Figure 5: Hierarchical cluster analysis of the bacterial and fungal T-RFLP profiles at the three study sites along the microclimatic gradient in June 2010 at the start of the experiment (N = north-facing site, M = middle site, S = south-facing site). a) Bacterial community structure (three replicas per site), b) fungal community structure (three replicate samples per site).

* Significant difference between the branches ($P < 0.05$).

4.4.3 Reaction of microbial activities to transfer and time

The effect of soil transfer on the microbial activities (comparing the non-transferred samples with the transferred samples for all the time points (two-way repeated measures ANOVA)) was significant ($P < 0.01$) in the Sn soil for leucine incorporation and phosphatase activity, and in the Sm soil for β–glucosidase and phosphatase activity (Table 2). Significant effects ($P < 0.05$) were also found for β–glucosidase in Sn. The effect of soil transfer was also significant ($P < 0.01$) for the phosphatase activity and leucine incorporation ($P < 0.05$) in the Ms soil, with lower significance ($P < 0.05$) for leucine and acetate incorporation in the Mn soil and for leucine incorporation in the Ms soil. There were no significant effects of transfer for the N soil. Generally, the activities in the S soil tended to increase after soil transfer, while the activities in the N soil did not change much after soil transfer (Figure 6; Supplementary Figures S1 – S5). Comparing the average activities over the whole experiment (average of t0 – t5) only the phosphatase activity between Ms and Mn and Mm did significantly differ ($P < 0.05$) (Figure 6), while the other transferred soil activities did not differ significantly from the activity at their original location. The three soils (N, M and S) always differed significantly when compared with each other (with one exception: the leucine incorporation of the M and the S soil did not differ significantly).

Table 2: Effects of time and soil transfer on the soil microbial activities along the microclimatic gradient. The time effect was calculated from the non-transferred samples for all the time points at each site (one-way repeated measures ANOVA). The transfer effect was calculated by comparing the non-transferred samples with the transferred samples for all the time points (two-way repeated measures ANOVA). The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site), the destination of the soil in the corresponding lower-case letters.

		Time	**Transfer**		
			n	m	s
FDA	N	**		n.s.	n.s.
	M	n.s.	n.s.		n.s.
	S	**	n.s.	n.s.	
Leucine	N	n.s.		n.s.	n.s.
incorporation	M	n.s.	*		*
	S	**	**	n.s.	
Acetate	N	n.s.		n.s.	n.s.
incorporation	M	*	*		n.s.
	S	n.s.	n.s.	n.s.	
β-Glucosidase	N	**		n.s.	n.s.
	M	**	n.s.		n.s.
	S	**	*	**	
Phosphatase	N	n.s.		n.s.	n.s.
	M	*	n.s.		**
	S	**	**	**	

FDA = fluorescein diacetate incorporation; leucine incorporation = bacterial activity; acetate incorporation = fungal activity.

** $P < 0.01$

* $P < 0.05$

n.s. = not significant

Microbial activities not only reacted to soil transfer (change of location), but also changed significantly ($P < 0.05$) with time (duration of the experiment) (non-transferred samples for all the time points at each site (one-way repeated measures ANOVA)). In particular, the microbial activities in the S soils changed with time in most cases (Table 2). Generally, the microbial activities fluctuated seasonally, but overall increased with time (Supplementary Figures S1 – S5).

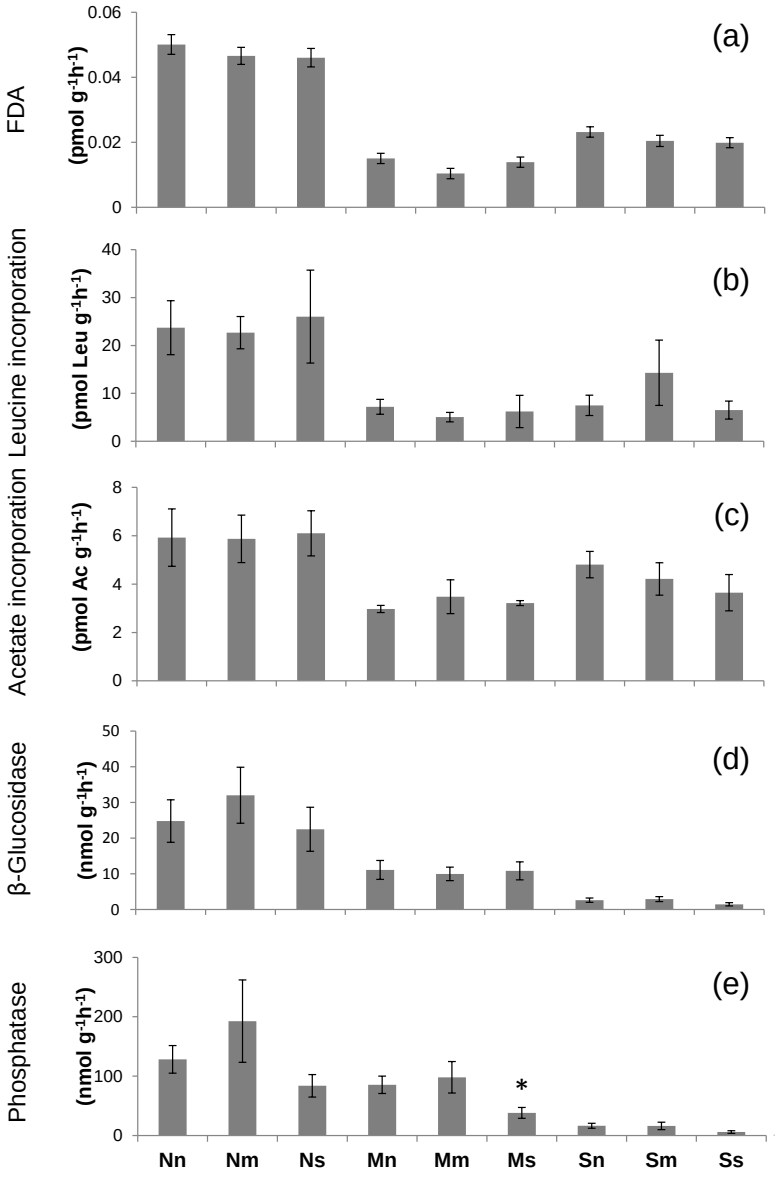

Figure 6: Average of the microbial activities after soil transfer at the three study sites along the microclimatic gradient during the whole experiment (t0 – t5; n = 6 ± SE) (N = north-facing site, M = middle site, S = south-facing site). a) FDA, b) leucine incorporation (bacterial activity), c) acetate-in-ergosterol incorporation (fungal activity), d) β-glucosidase activity, and e) phosphatase activity.

* Significantly different from the other two sites (when comparing transferred with non-transferred samples of the same soil) (P < 0.05) according to one-way ANOVA.

4.4.4 Reaction of the microbial community structure to transfer, time and microclimatic factors

Soil transfer had a highly significant effect on the bacterial community structures from T-RFLP profiling (P = 0.0001) in all the soils (Table 3), and also on the fungal community structures apart from those in the M soil, which did not react to transfer (Table 3). The effect of time on the bacterial and fungal community structures was significant (P = 0.001) in all soil environments. Overall the effect of time on the bacterial and fungal community structures was higher than the effect of soil transfer, as the higher F-values and the higher explained variance (EV) indicate (Table 3). This can also be seen in the PCO plots, where the bacterial as well as fungal communities clusters more according to time than according to site (Supplementary Figures S6 and S7).

Table 3: Effects of time and soil transfer on the bacterial and fungal community structures from T-RFLP profiling along the microclimatic gradient. The time effect was calculated on the non-transferred samples from each site for all the time points. The transfer effect was calculated by comparing the non-transferred samples with the transferred samples for all the time points at the three sites (N = north-facing site, M = middle site, S = south-facing site). The significance level was set to $P < 0.05$ and F was considered to imply a high influence of time or soil transfer on the microbial communities when F > 5. Significant results ($P < 0.05$) and F > 5 are shown in bold.

	Bacteria			**Fungi**		
Time						
	P	F	EV*	*P*	F	EV
N	**0.0001**	**23.7**	19.2	**0.0001**	**10.7**	26.1
M	**0.0001**	**33.1**	23.1	**0.0001**	**9.0**	29.6
S	**0.0001**	**27.6**	19.5	**0.0001**	**10.2**	29.2
Transfer						
	P	F	EV	*P*	F	EV
N	**0.0001**	**5.3**	6.0	**0.017**	2.3	6.7
M	**0.0001**	**7.2**	7.3	0.060	1.8	6.7
S	**0.0001**	**6.0**	6.1	**0.007**	2.7	9.1

* EV = Explained Variance (%)

Multivariate statistics of the T-RFLP of the control samples and the undisturbed soil samples from the vicinity of the pots indicated that their T-RFLP profiles were not separated from the T-RFLP profiles of the control soils in the pots. Therefore we assume that the non-transferred control samples did evolve in a similar way as the undisturbed soils over the course of the experiment (data not shown). The bacterial community structures in the N and M soil correlated significantly ($P < 0.05$) to temperature and soil moisture (Table 4) whereas in the S soil it was not. The fungal community structure in the N soil correlated significantly ($P < 0.05$) to temperature and soil moisture whereas in the S soil only ($P < 0.01$) to temperature (Table 4).

Table 4: Influence of temperature and soil moisture on the bacterial and fungal community structures along the microclimatic gradient. The three soils originating from each of the three sites (N = north-facing site, M = middle site, S = south-facing site) were investigated separately and both the non-transferred and the transferred samples were included. The mean values of the temperature and soil moisture in 2010 and 2011 of the respective samples and sites were used. The significance level was set to $P < 0.05$ and F was considered to imply a high influence of temperature or soil moisture on the microbial communities when F > 5. Significant results ($P < 0.05$) and F > 5 are shown in bold.

	Bacteria			**Fungi**		
Temperature						
	P	F	EV*	*P*	F	EV
N	**0.0001**	**27.9**	26.9	**0.001**	**8.4**	10.0
M	**0.05**	3.5	4.4	0.07	3.1	4.0
S	0.29	1.1	1.5	**0.001**	**11.2**	13.0
Soil moisture						
	P	F	EV	*P*	F	EV
N	**0.01**	**6.4**	7.8	**0.03**	3.7	4.6
M	0.18	1.9	2.4	**0.0001**	**16.5**	18.3
S	0.85	0.04	0.1	0.09	2.7	3.5

*EV = explained Variance (%)

4.4.5 REACTION OF THE BACTERIAL COMMUNITY COMPOSITION TO TRANSFER

In the Ns soil, 16 of the bacterial OTUs (n = 178) had changed significantly ($P < 0.05$) 16 months after soil transfer, whereas in the Sn soil, 9 OTUs had changed significantly (n = 178). In order to evaluate the character of the reaction to transfer, the significant changing OTUs were classified as "sensitive" or "resistant" OTUs according to the difference in the OTUs normalized abundance (from the mean of the five replicate samples at each site) 16 months after and before soil transfer. Of the total (n = 17) significantly changing bacterial OTUs, 9 OTUs reacted sensitively and 8 OTUs with resistance to transfer for both soils (data not shown).

We did perform a linear regression on the OTUs abundance over the 6 time points and found that the OTUs which were classified as sensitive to transfer showed a gradual reduction of abundance over the different time points. And also the OTUs classified as resistant to transfer showed a gradual increase in abundance. Only 4 out of 17 OTUs showed a chaotic reaction, indicating that most of the OTUs were changing directionally rather than fluctuating randomly.

Seven of the significantly changing bacterial phylotypes (OTUs 65, 73, 76, 86, 144, 161 and 170) were classified as sensitive to transfer in the N and S soils (Figure 7). Cloned sequences of OTUs 73 and 76 were phylogenetically affiliated to *delta-Proteobacteria* (92% and 99% similarity) (Table 5) and the three OTUs (OTUs 65, 144 and 161) were phylogenetically affiliated to *Actinobacteria* (96% and 98% similarity). The OTU 170 was closely related to an environmental clone within *alpha-Proteobacteria* (Table 5).

Six bacterial phylotypes (OTUs 91, 121, 124, 133, 399 and 401) were classified as resistant to transfer in both soils (Figure 7). The clone sequence of OTU 91 was closely related (98% similarity) to an environmental clone within *Acidobacteria*, the sequence of OTU 121 was closely related (96% similarity) to an environmental clone within *Actinobacteria*, that of OTU 124 was closely related (93% similarity) to an environmental clone within *delta-Proteobacteria* and that of OTU 133 was closely related (99% similarity) to an environmental clone within *beta-Proteobacteria* (Table 5). The clone

sequences of the OTUs 399 and 401 were phylogenetically affiliated to *alpha-Proteobacteria* (97% similarity).

Two bacterial phylotypes (OTUs 93 and 278) showed a sensitive reaction to transfer from the N site to the S site, but a resistant reaction to the reverse transfer (Figure 7). The clone sequence of the OTU 93 was closely related (98% similarity) to an environmental clone within *Bacteroidetes*, and that of OTU 278 closely related (96% similarity) to an environmental clone within *Acidobacteria* (Table 5).

Two bacterial phylotypes (OTUs 118 and 153) had a resistant reaction to transfer from the N site to the S site, but a sensitive reaction to the reverse transfer (Figure 7). The clone sequence of OTU 118 was most similar (98% similarity) to an environmental clone within *Firmicutes* and that of OTU 153 was most similar (99% similarity) to an environmental clone within *Cyanobacteria* (Table 5).

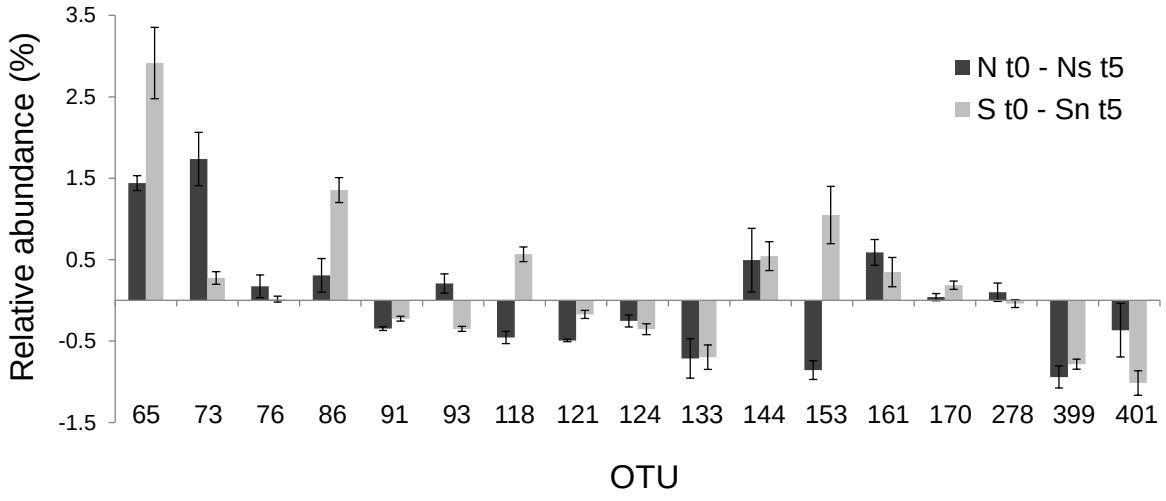

Figure 7: Resistant and sensitive bacterial OTUs (T-RFs in bp). The difference in OTU abundances in June 2010 at the start of the experiment (t0) at the original location (N = north-facing site, S = south-facing site), and 16 months (t5) after transfer along the microclimatic gradient is given (N soil transferred to S = Ns, S soil transferred to N = Sn). Negative values represent the "resistant" OTUs (increase after transfer) and positive values the "sensitive" OTUs (decrease after transfer). The corresponding phylogenetic analysis of the bacterial OTUs is shown in Table 5.

Table 5: Bacterial OTUs with the *MspI* terminal restriction fragment length (bp) of the environmental samples (environmental OTU) and the clones (clone OTU). The OTUs correspond to the ”resistant“ and ”sensitive“ OTUs shown in Figure 6.

Environmental OTU	Clone OTU	Name	NCBI Accession no.	NCBI match		Phylogenetic Group	Habitat	Identity
				accession no.	clone name			%
65	65	transfer_3D12	JQ480577	EU132932	FFCH16325	*Actinobacteria*	prarie soil	96
73	73	transfer_3H02	JQ480602	EF516185	FCPN515	*delta-Proteobacteria*	grassland soil	92
76	76	transfer_3G06	JQ480598	AB630491	MPB1-109	*delta-Proteobacteria*	Antarctic fresh-water lake	99
86	85	transfer_1C11	JQ480469	EF516468	FCPT450	*Bacteroidetes*	grassland soil	99
91	91	transfer_3F02	JQ480587	EU861864	G08_bac_con	*Acidobacteria*	Alpine tundra soil	98
93	93	transfer_1D01	JQ480470	DQ450749	C08_WMSP1	*Bacteroidetes*	Alpine wet-meadow soil	98
118	117	transfer_3B09	JQ480553	EF663868	GASP-MA3W3_E01	*Firmicutes*	primary succession soil	98
121	121	transfer_3F04	JQ480589	GQ339193	IS-119	*Actinobacteria*	Fresh-water pond	96
124	124	transfer_3E02	JQ480579	DQ129588	AKIW600	*delta-Proteobacteria*	aerosols	93
133	133	transfer_1A07	JQ480459	DQ450777	G09_WMSP1	*beta-Proteobacteria*	Alpine wet-meadow soil	99
144	144	transfer_3D07	JQ480573	FR732246	1813K157	*Actinobacteria*	anoxic fen soil	98
153	153	transfer_3E03	JQ480580	HQ189103	B90202H	*Cyanobacteria*	Hymalaya and Antarctica soil	99
161	160	transfer_2B09	JQ480499	AB489859	*Longispora fulva*	*Actinobacteria*	isolated from forest soil	98
170	170	transfer_1F04	JQ480476	AM935865	CMJC5	*alpha-Proteobacteria*	contaminated soil	98
278	278	transfer_2A01	JQ480488	EF072059	GASP-WA1S1_E10	*Acidobacteria*	agricultural soil	96
399	399	transfer_3E06	JQ480583	HQ118897	1112851587149	*alpha-Proteobacteria*	loami sand soil	97
401	401	transfer_3D08	JQ480574	AF395032	*Sphingomonas* sp. strain SIA181-1A1	*alpha-Proteobacteria*	isolated from Antarctic ice	97

4.4.6 Reaction of the fungal community composition to transfer

In the Ns soil, 2 of the fungal OTUs (n = 86) had changed significantly ($P < 0.05$) 16 months after soil transfer, whereas in the Sn soil, 7 had changed significantly. Of the total (n = 9) significantly changing fungal OTUs, 7 OTUs showed a sensitive reaction and 2 OTUs a resistant reaction to transfer in the Ns soil, and 4 OTUs showed a sensitive reaction and 5 OTUs a resistant reaction to transfer in the Sn soil (data not shown). Most of the sequences found were closely related to environmental clones obtained from glacier forefield soils.

Of the 9 OTUs that significantly changed after transfer, one fungal phylotype (OTU 450) showed a sensitive reaction to transfer in both the N and S soils (Figure 8). The clone sequence of OTU 450 was closely related (99% similarity) to a *Hymenoscyphus ericae* strain (Table 6).

One fungal phylotype (OTU 399) reacted with resistance to transfer in both soils. The clone sequence of OTU 399 was closely related (99% similarity) to a *Hyaloscypha vitreola* isolate.

Two fungal phylotypes (OTUs 448 and 449) reacted sensitively to transfer from the S to the N site (Figure 8), and were phylogenetically affiliated to the *Pezizomycotina*. The clone sequence of OTU 448 was closely related (99% similarity) to a *Helicoon fuscosporum* strain and the clone sequence of OTU 449 was most similar (99% similarity) to a *Lasiobolidium orbiculoides* strain.

Two fungal phylotypes (OTUs 50 and 283) reacted sensitively to soil transfer from the N to the S site, but with resistance to the reverse transfer (Figure 8). The clone sequence of OTU 50 was closely related (96% similarity) to a *Nidula niveotomentosa* isolate and the clone sequence of OTU 283 was mostly similar to a *Lecophagus longispora* isolate.

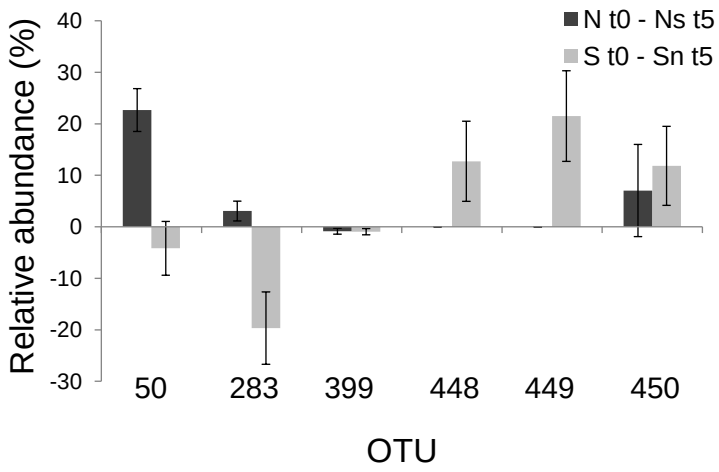

Figure 8: Resistant and sensitive fungal OTUs (T-RFs in bp). The difference in OTU abundances in June 2010 at the start of the experiment (t0) at the original location (N = north-facing site, S = south-facing site), and 16 months (t5) after transfer along the microclimatic gradient is given (N soil transferred to S = Ns, S soil transferred to N = Sn). Negative values represent the "resistant" OTUs (increase after transfer) and positive values the "sensitive" OTUs (decrease after transfer). The corresponding phylogenetic analysis of the fungal OTUs is shown in Table 6.

Table 6: Fungal 18S rRNA sequence OTUs with the *AluI* terminal restriction fragment length (bp) of the environmental samples and the clones. The OTUs correspond to the "resistant" and "sensitive" OTUs shown in Figure 7.

Environmental OTU	Clone OTU	Name	NCBI Accession no.	NCBI closest cultured species		Identity (%)	NCBI closest match		Phylogenetic Group	Habitat	Identity (%)
				accession no.	strain name		accession Nr.	clone name			
50	51	Fungi_transfer_F12	JQ805775	GU296099	*Nidula niveotomentosa* isolate AFTOL-ID 1945	96	AF504766	B25	*Agaricomycotina*	Grassland soil	96
283	282	Fungi_transfer_A6	JQ805726	AB014400	*Lecophagus longispora* strain CBS 845.91	99	GU201417	5062RhFu	mitosporic *Ascomycota*	desert soil	98
399	397	Fungi_transfer_F2	JQ805767	EU940080	*Hyaloscypha vitreola* isolate M236	99	HM065929	7-f2	*Pezizomycotina*	glacier forefield soil	99
448	448	Fungi_transfer_B2	JQ805734	AY856946	*Helicoon fuscosporum* strain UAMH 8757	99	HM065922	1-f152	*Pezizomycotina*	glacier forefield soil	99
449	449	Fungi_transfer_A3	JQ805724	DQ063000	*Lasiobolidium orbiculoides* strain CBS 344.73	97	HM065908	1-f28	*Pezizomycotina*	glacier forefield soil	99
450	450	Fungi_transfer_D7	JQ805753	AY524847	*Hymenoscyphus ericae* strain UAMH 8873	99	HM065966	7-f190	*Pezizomycotina*	glacier forefield soil	99

4.5 Discussion

4.5.1 Environmental parameters and microbial activity

The main difference between the three sites investigated was the temperature and the soil moisture regime, with the mean temperature during the summer months being higher with larger daily fluctuations at the S site than at the N site and the soil of the N site being generally moister. The M site proved to be very similar to the S site in both environmental parameters. Additionally, the N soil had slightly higher silt content than the other two soils. Since glacier grinding is assumed to play a major role in the formation of silt particles (Wright, et al. 1998), it could be that the advancing and retreating of the glacier deposited varying amounts of silt across the forefield, leading to differences in the silt content at the three sites. To reduce the complexity of our data set and for a better overview we will only focus on the N and S soils for the discussion, as the results from the M soils proved to be similar to the S soils.

We showed that microbial activities were highest at the cold and moist N site, and lowest at the warm and dry S site. The microbial activities in the N soil furthermore did not change when the soil was transferred to the warmer and drier microclimate at the S site. The microbial activities in the S soil, on the other hand, mostly increased after soil transfer under the colder temperatures and higher soil moisture at the N site. As the microbial activities were all measured at standardized temperatures, this can be expected, as with higher temperatures more substrates will be used and therefore the substrate availability will decrease (Kirschbaum 2004; Rousk, et al. 2012). Therefore the substrate availability in the S soil will increase after transfer to the N site, since the lower temperature will result in a lower consumption of the available substrate. On the other hand, the activity in the N soil transferred to the S site should decrease, as the substrate availability decreases with higher temperatures. This we did not observe during the time investigated. But the N soil, with its greater water-holding capacity due to its higher silt content, contained more moisture as was confirmed by the higher gravimetric water content. This can be advantageous (Beyer, et al. 2000) as

it improves microbes' access to nutrients and also enhances their motility (Long and Or 2009). As a consequence, the more favorable soil moisture conditions in the N soil could have allowed the microbes to maintain their activities after transfer, even when the soil was transferred to the warmer S site. Such favorable soil moisture conditions could also be responsible for the higher activities in the S soil after transfer to the N site (besides an increase in substrate availability), as the remaining nutrients are more accessible after transfer, even if the build-up of carbon during the experimental time was not large.

Microbial activities also changed with time. Some fluctuated seasonally, as has been well described (Loffler, et al. 2008; Bell, et al. 2010), but most activity parameters increased during the experiment. The longer newly deglaciated terrain is exposed to wind and rain, the more particles (pollen, dust and sand) are deposited on the ground (Hawes 2008). These can then be used as nutrient sources by microbes, as it has been shown that soil bacteria were limited by both C and N in these recently deglaciated soils (Göransson, et al. 2011). Snow melt in spring furthermore deposits nitrogen and carbon on the underlying soil (Brankatschk, et al. 2011). This nutrient input on the bare soil was too small to be detected in our soil analysis, but it could nevertheless have induced the observed increase in the microbial activities over time.

That the fungal activities seemed to be less affected by soil transfer than the bacterial activities could be due to the findings that fungi in general are more tolerant to water stress than prokaryotes and furthermore can tolerate dry conditions better due to their hyphae structures, being able to grow towards water resources more easily than prokaryotes (Torsvik and Øvreås 2008).

Generally our findings imply that in bare soils moderate conditions favor colonization of microbes and a higher activity compared to more extreme conditions.

4.5.2 BACTERIAL AND FUNGAL COMMUNITY STRUCTURE AND COMPOSITION

In general, we observed that soil transfer has changed the microbial community composition more vigorously than the microbial activities. One reason might be that the species composition could be considered to integrate changes over a longer time span, while activities change more rapidly and therefore reflect rather the conditions at a certain time point. The effect of the soil transfer on the microbial communities was more pronounced in the N soil. Transferring soil from the N site to the warmer S site could have induced a higher rate of change in the community patterns due to a faster species turnover at warmer temperatures. This is in accordance to other studies showing that changes in microbial community structures were faster after a shift from lower temperatures to higher temperatures than vice versa (Pettersson and Bååth 2003). Besides of a competitive advantage of certain populations over others at higher temperatures, it might be possible that the extreme temperature conditions (> 36°C) at the S site have also led to the death of certain microbial populations.

The bacterial OTUs were generally found to show small changes in abundance after soil transfer, fungal OTUs showed larger changes in abundance than bacterial OTUs. This is surprising, as fungi were found to show a higher resistance to changes in the water availability and in temperature in general (Barcenas-Moreno, et al. 2009; Yuste, et al. 2011), which would therefore mean their abundance should not change to such a large extent after soil transfer. Therefore the reason for this strong response of the fungal population to soil transfer could lie in the possible difference in nutrient availability of the different soils. It was proposed that there are two functional groups of fungi, one feeding only on fresh-C and the other on soil organic matter (Fontaine, et al. 2011). Therefore one could assume that the fungal community composition changes if the availability of the soil organic matter changes, which is likely with the observed change in the microclimate.

Overall, the bacterial phylogenetic groups found here are known to be abundant in glacier forefields (Philippot, et al. 2011; Zumsteg, et al. 2012) and some were shown to be active in weathering (Frey, et al. 2010; Lapanje, et al. 2011).

Of the bacterial phylotypes sensitive to soil transfer in both directions, OTUs 65, 144 and 161 were affiliated to *Actinobacteria*. Members of this phylum have been found to react strongly to seasonal changes in a glacier forefield (Lazzaro, et al. 2012), indicating that this phylogenetic group is sensitive to changes in environmental parameters.

One cultured close relative to a bacterial phylotype resistant to soil transfer (OTU 401) was isolated from Antarctic ice (*Sphingomonas* sp., AF395032) within *alpha-Proteobacteria* (Christner, et al. 2001), illustrating the high survival capabilities of this phylotype. Another bacterial phylotype resistant to transfer (OTU 133) was closely related to a clone from Alpine tundra wet-meadow soil (DQ450777) (Costello and Schmidt 2006) within *beta-Proteobacteria*. *Alpha-* and *beta-Proteobacteria* are both known to be abundant in bare glacier forefield soils (Sattin, et al. 2009; Schloss, et al. 2010; Zumsteg, et al. 2012).

The bacterial OTU 153 reacted sensitively to soil transfer from the S to the N site, but with resistance to the reverse transfer. The closest relative clone (HQ189103) was obtained from the dry valleys of the Himalayan mountains and Antarctica (Schmidt, et al. 2011), and is affiliated to the phototrophic *Cyanobacteria*. These are known to be the first colonizers on barren substrate (Brinkmann, et al. 2007; Fermani, et al. 2007). They produce compounds that protect them against UV light (Vincent, et al. 2004), and were therefore better protected against the higher UV radiation at the S site compared to the N site. The occurrence of *Cyanobacteria* largely depends on the combined conditions of light and temperature, with different phylotypes adapting to different conditions (Oberhaus, et al. 2007). Assuming the *Cyanobacteria* found here are generally adapted to high light intensities and rather high temperatures, the decrease in their abundance after transfer to the N site could therefore be due to the N site having less sunshine hours and colder temperatures.

An interesting observation was that two clones belonging to the *Acidobacteria* (OTUs 91 and 278) were found to be resistant to transfer. *Acidobacteria* are generally slow growing (Davis, et al. 2011), which makes their resistant reaction to transfer surprising, as one would think the fast growing

opportunists would be more successful after a disturbance. But they were found to be active at low temperatures, withstand freeze-thaw cycles (Mannisto, et al. 2009) and to thrive in nutrient depleted environments (Ward, et al. 2009). This and their resistance to fluctuating conditions in the soil (Eichorst, et al. 2011) support our findings.

Most of the fungal phylotypes (OTUs 283, 399, 448, 449 and 450) found to react to soil transfer along this microclimatic gradient in a glacier forefield were affiliated to *Ascomycetes*. They are known to outnumber the *Basidiomycetes* in these recently deglaciated soils (Brunner, et al. 2011; Zumsteg, et al. 2012). The reason for this is the absence of plants. It was for instance shown that ectomycorrhizal propagules were scarce in bare soils close to the glacier terminus but increased with time since deglaciation occurred as soon as the first host plants were present (Jumpponen, et al. 2002).

The three fungal OTUs 448, 449 and 450, whose closest relatives were found in the Damma glacier forefield soil during an earlier study (Zumsteg, et al. 2012), were sensitive to soil transfer, especially in the Sn soil. This implies that varying expositions can induce a change in community composition even if these communities are adapted to the glacier forefield ecosystem. We observed these OTUs in far greater abundance at the S site in general, implying that these fungal species are well adapted to the hot and dry environment at the S site, and therefore are not able to adapt to the colder and wetter conditions at the N site.

One fungal phylotype (OTU 399), whose closest relative was also obtained from glacier forefield soil (HM065929), reacted with resistance to soil transfer in both directions. As the closest relative was also found on the Damma glacier forefield during an earlier study and it was found in the N and S soil to a similar extent, this particular species seems to be adapted to different environmental parameters in general.

Many of the closest relatives of the fungal phylotypes found to react to transfer here (OTUs 399, 448, 449 and 450) were obtained from the Damma glacier forefield during an earlier study (Zumsteg, et al.

2012). This indicates that the most dominant fungal phylotypes found are homogeneously distributed over the recently deglaciated terrain.

4.5.3 Conclusions

We conclude that the small-scale differences in a sites' exposure to the sun along a cross-section at the front of the Damma glacier influenced the soil temperature and soil moisture directly and the microbial communities indirectly. Interestingly, we observed an increase in microbial activities when soil was transferred from the warmer S site to the colder and moister N site suggesting that more substrate was available at lower temperatures and at higher soil moisture levels. In contrast, microbial activities did not change when soil was transferred from the colder and moister N site to the warmer S site indicating that the higher soil moisture content enabled the microbes to maintain a certain activity level as the nutrients remained available to the microbes. Overall, the effect of the soil transfer on the microbial communities was more pronounced in the N soil. Transferring soil from the colder N site to the warmer S site induced a higher rate of change in the community patterns due to a higher microbial activity and faster species turnover than the reverse transfer. Besides of a competitive advantage of certain populations over others at higher temperatures we suggest that the extreme temperature conditions (>36°C) in bare soils have led to the death of sensitive populations. In fact, we showed that certain bacterial phylotypes reacted either sensitively (*Actinobacteria*) or with resistance (*Acidobacteria*) or both (*Cyanobacteria*, *Proteobacteria*) to soil transfer, depending of the direction of transfer. Fungal phylotypes found to react to soil transfer mainly belonged to the *Ascomycetes*. An interesting finding was that many bacterial phylotypes showed a moderate change in abundance after soil transfer, but the few fungal phylotypes which reacted to soil transfer showed large changes in abundance.

4.6 Acknowledgements

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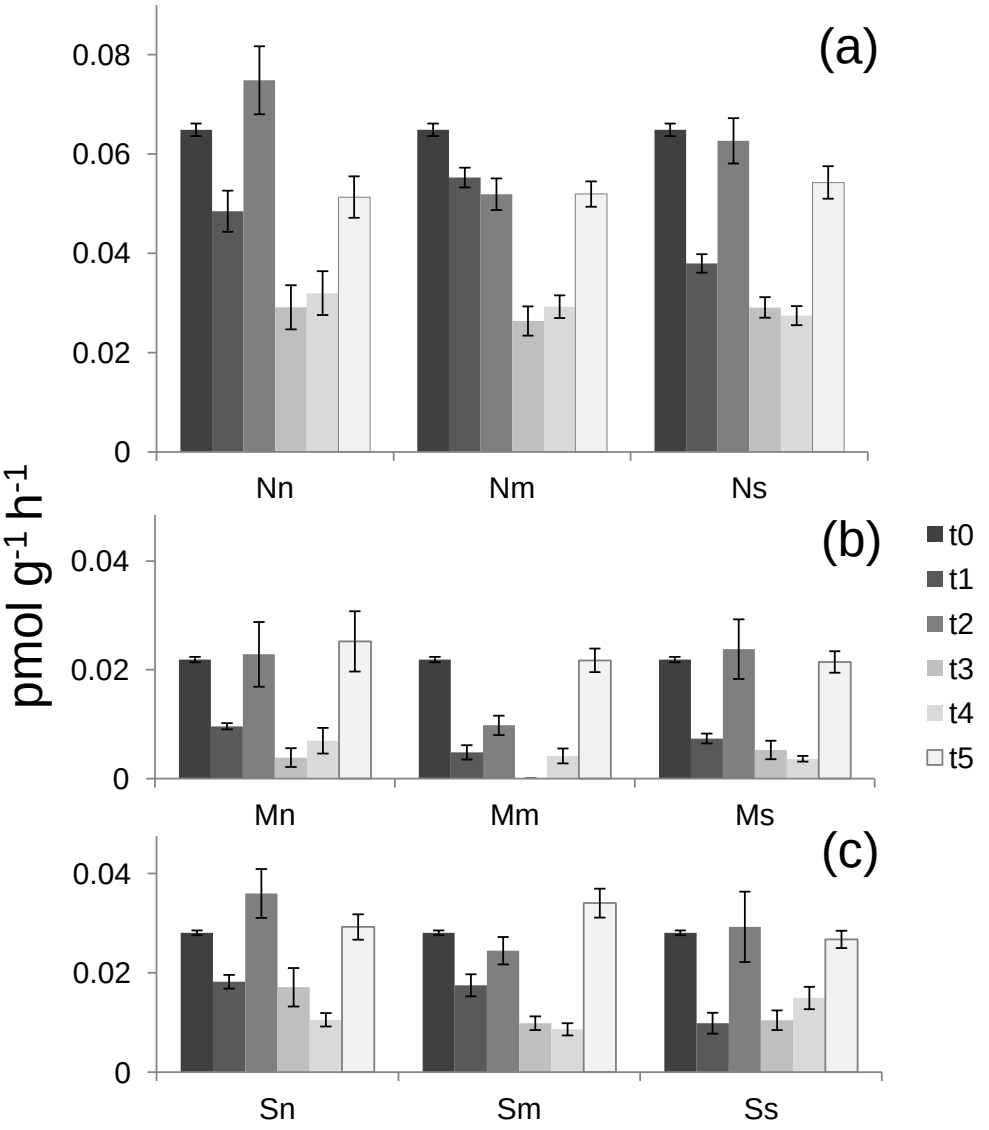
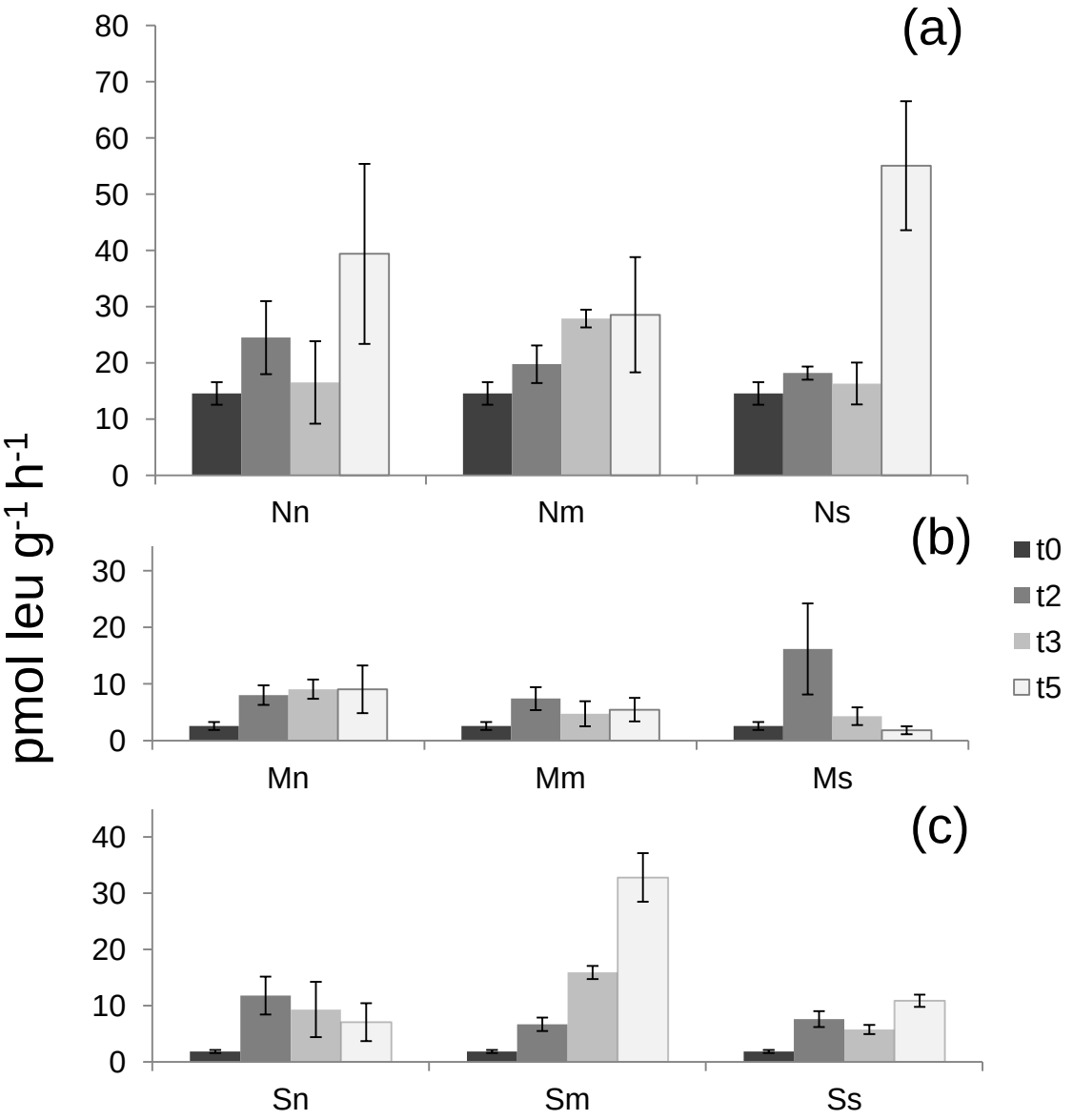
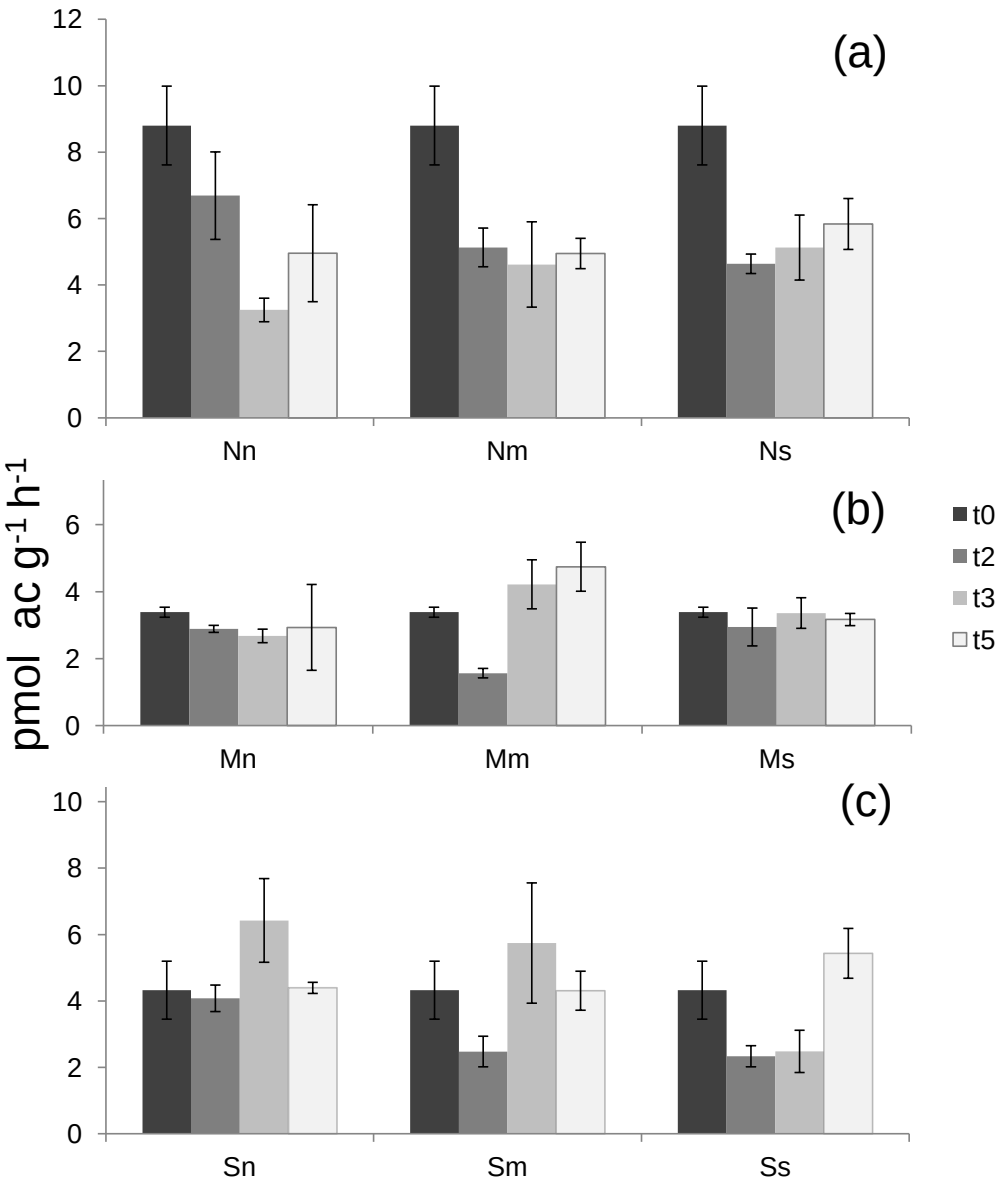
4.8 Supplementary Material



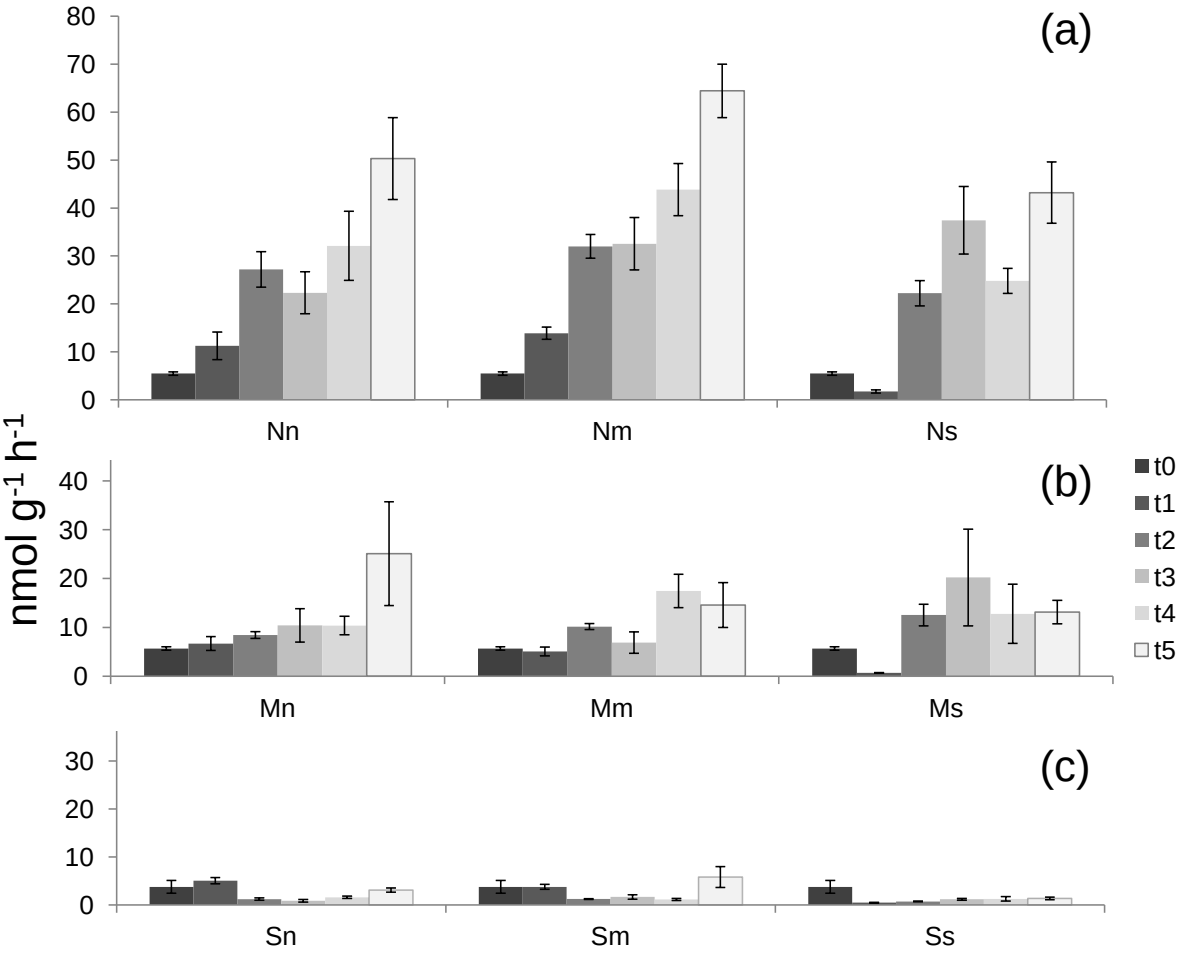
Supplementary Figure S1: Fluorescein diacetate hydrolization (FDA) in pmol g^{-1} h^{-1} (mean ± se; n = 5) for the microbial activity at different sampling times after soil transfer t0 – t5 (t0 = start in June 2010, t1 = after 2 months, t2 = after 4 months, t3 = after 12 months, t4 = after 14 months and t5 = after 16 months) along the microclimatic gradient. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters. a) In N soil, b) in M soil, and c) in S soil (Mm t3 is missing).



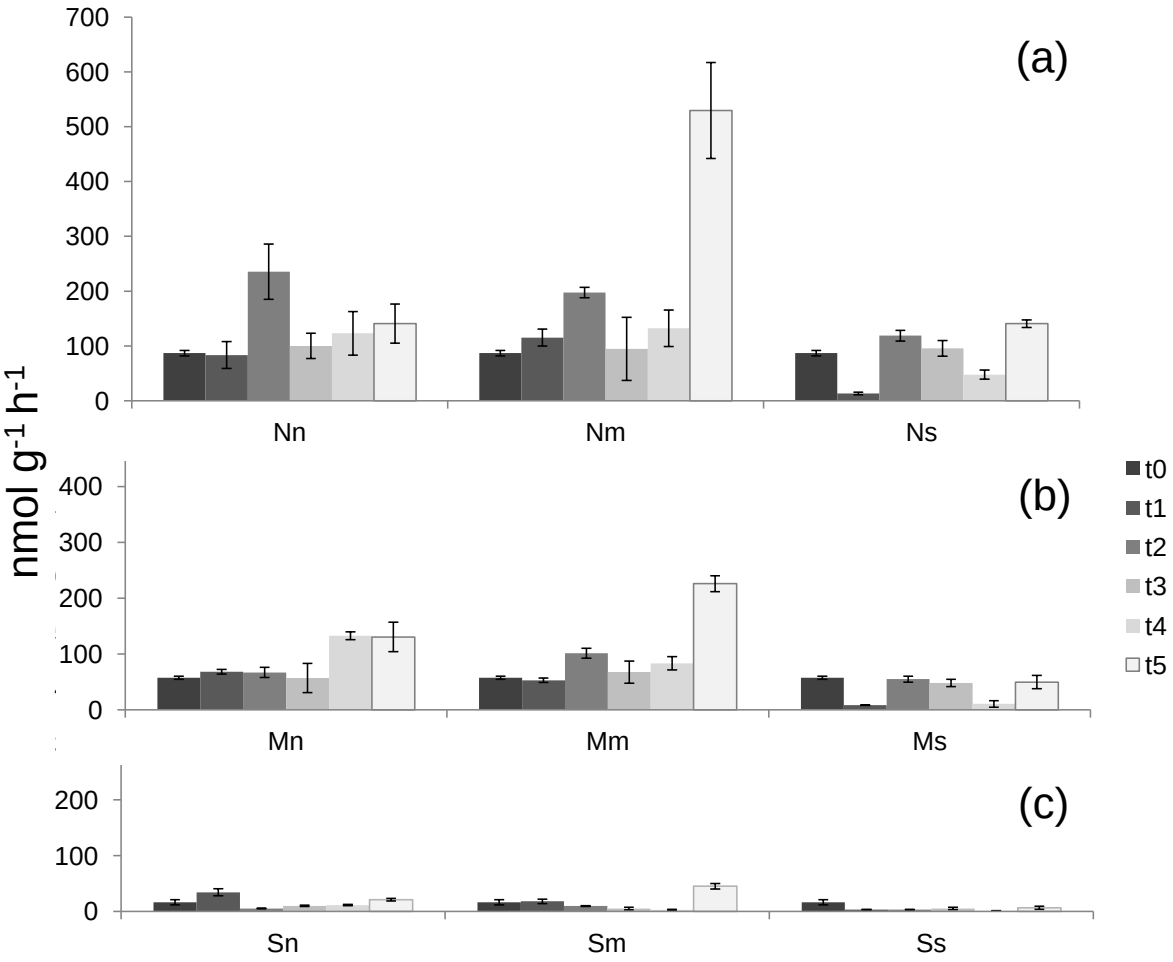
Supplementary Figure S2: Leucine incorporation for the bacterial activity in pmol g^{-1} h^{-1} (mean ± se; n = 5) at different sampling times after soil transfer t0 – t5 (t0 = start in June 2010, t1 = after 2 months, t2 = after 4 months, t3 = after 12 months, t4 = after 14 months and t5 = after 16 months) along the microclimatic gradient. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters. a) in N soil, b) in M soil, and c) in S soil.



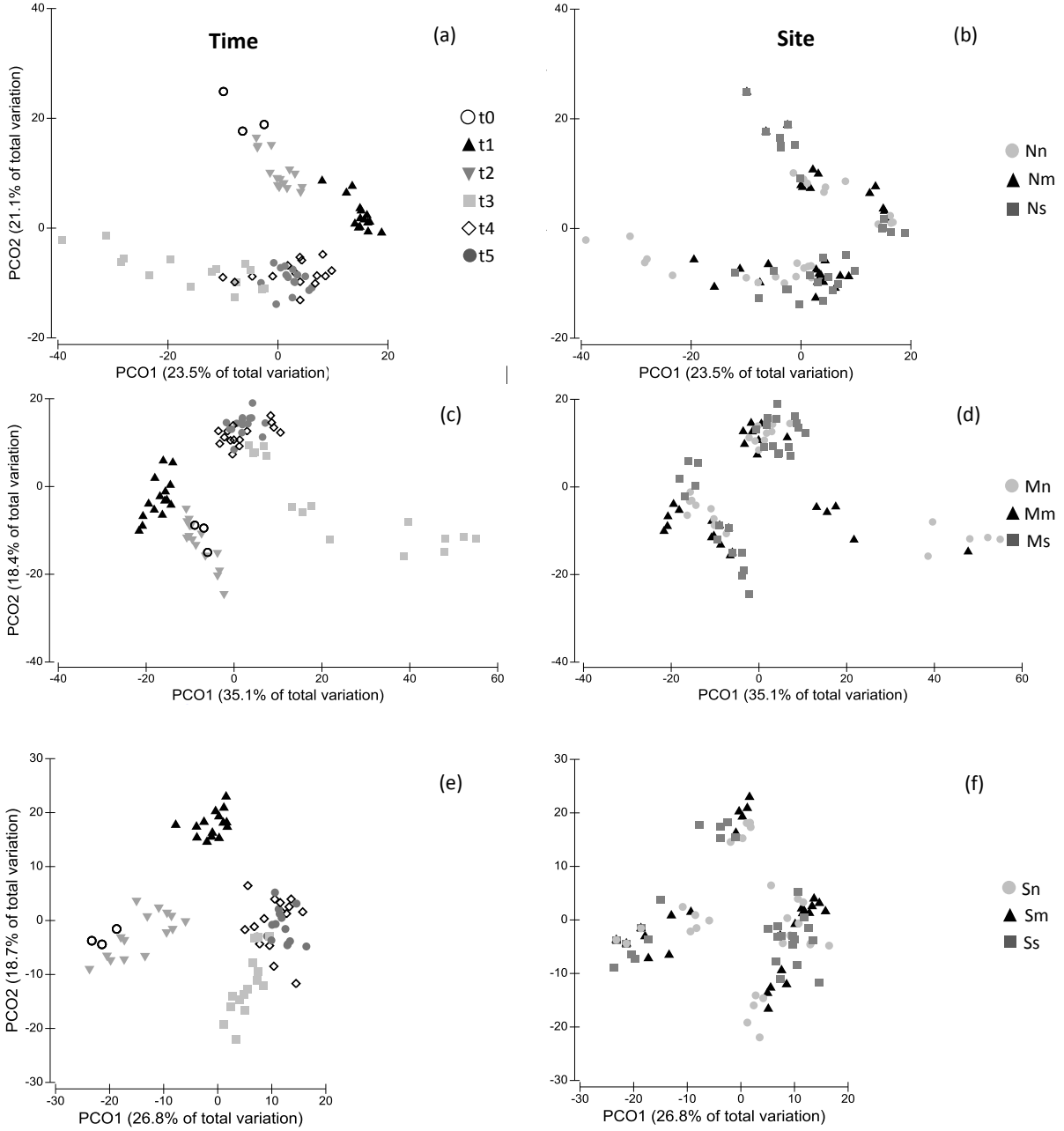
Supplementary Figure S3: Acetate-in-ergosterol incorporation in pmol g^{-1} h^{-1} (mean ± se; n = 5) for the fungal activity at different sampling times after soil transfer t0 – t5 (t0 = start in June 2010, t1 = after 2 months, t2 = after 4 months, t3 = after 12 months, t4 = after 14 months and t5 = after 16 months) along the microclimatic gradient. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters. a) in N soil, b) in M soil, and c) in S soil.



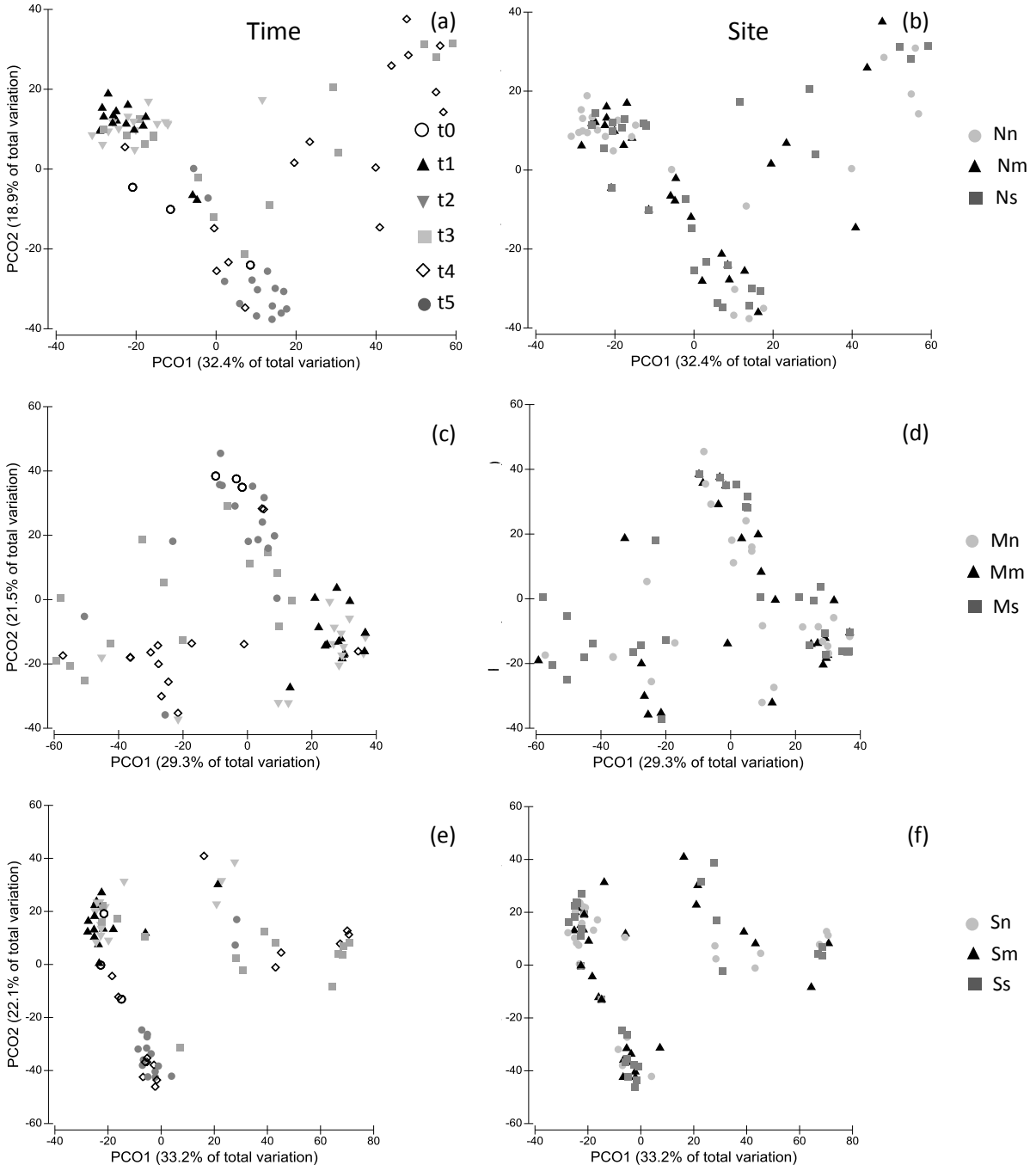
Supplementary Figure S4: β-glucosidase activity in nmol g^{-1} h^{-1} (mean ± se; n = 5) at different sampling times after soil transfer t0 – t5 (t0 = start in June 2010, t1 = after 2 months, t2 = after 4 months, t3 = after 12 months, t4 = after 14 months and t5 = after 16 months) along the microclimatic gradient. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters. a) in N soil, b) in M soil, and c) in S soil.



Supplementary Figure S5: Phosphatase activity in nmol g^{-1} h^{-1} (mean ± se; n = 5) at different sampling times after soil transfer t0 – t5 (t0 = start in June 2010, t1 = after 2 months, t2 = after 4 months, t3 = after 12 months, t4 = after 14 months and t5 = after 16 months) along the microclimatic gradient. The origin of the soil is given in capital letters (N = north-facing site, M = middle site, S = south-facing site) and the destination of the soil in the corresponding lower-case letters. a) in N soil, b) in M soil, and c) in S soil.



Supplementary Figure S6: Principal coordinate analysis of the bacterial T-RFLP profiles (restricted with *MspI*) of non-transferred and transferred soils over all time points (t0 – t5) of all three soils (n = 5 at each time point for each soil and site). For clarity, the analysis was split into graphs according to time (a), (c) and (d) and to site (b), (d) and (f). PCO analyses for the soil originating from the N site are shown in (a) and (b), PCO analyses for the soil originating from the M site are shown in (c) and (d) and PCO analyses for the soil originating from the S site are shown in (e) and (f). The two axes indicate the explained variance between the samples.



Supplementary Figure S7: Principal coordinate analysis of the fungal T-RFLP profiles (restricted with *AluI*) of non-transferred and transferred soils over all time points (t0 – t5) of all three soils (n = 5 at each time point for each soil and site). For clarity, the analysis was split into graphs according to time (a), (c) and (d) and to site (b), (d) and (f). PCO analyses for the soil originating from the N site are shown in (a) and (b), PCO analyses for the soil originating from the M site are shown in (c) and (d) and PCO analyses for the soil originating from the S site are shown in (e) and (f). The two axes indicate the explained variance between the samples.

Supplementary Table T1: Soil chemistry data at the three study sites in June 2010 at the start of the experiment (t0) and after 16 months (t5) along the microclimatic gradient. N = north-facing soil, M = middle soil and S = south-facing soil. All values are given in µmol g^{-1} soil.

	Ammonium	Chloride	Nitrate	Sulfate	Al	Ca	K	Mg	Mn	Na	P	S	Si
N t0	0.018	0.040	0.131	0.039	0.156	1.370	0.190	0.133	0.009	0.234	0.017	0.056	0.536
N t5	0.030	0.163	0.095	0.028	0.184	1.325	0.265	0.128	0.005	0.236	0.010	0.041	0.362
M t0	0.016	0.025	0.019	0.025	0.144	0.327	0.140	0.048	0.007	0.239	0.013	0.037	0.574
M t5	0.013	0.082	0.012	0.023	0.162	0.132	0.119	0.020	0.004	0.240	0.012	0.032	0.322
S t0	0.016	0.028	0.020	0.035	0.218	1.028	0.168	0.149	0.015	0.235	0.014	0.049	0.401
S t5	0.019	0.058	0.035	0.036	0.228	0.647	0.198	0.098	0.019	0.237	0.012	0.048	0.327

5. PAPER: "IDENTIFICATION OF BIOMASS UTILIZING BACTERIA IN A CARBON-DEPLETED GLACIER FOREFIELD SOIL BY THE USE OF ^{13}C DNA STABLE ISOTOPE PROBING"

With Stefan Schmutz and Beat Frey

Environmental Microbiology Reports 5 (2013) 424-437

5.1 SUMMARY

As Alpine glaciers are retreating rapidly, bare soils with low organic C and N contents are becoming exposed. Carbon availability is a key factor regulating microbial diversity and ecosystem functioning in these soils. The aim of this study was to investigate how bacterial activity, community structure and composition are influenced organic carbon availability. Bare soils were supplied with ^{13}C-labelled fungal (*Pencillium* sp.) and green algal (*Chlorella* sp.) biomass and the CO_2 evolution and its $\delta^{13}C$ signature were monitored up to 60 days. These organisms have previously been isolated near the glacier terminus. DNA stable isotope probing followed by T-RFLP profiling and sequencing of 16S rRNA genes was employed to identify consumers able to assimilate carbon from these biomass amendments. Higher respiration and higher bacterial activity indicated a more efficient utilization of algal cells than fungal cells. *Flavobacterium sp.* predominantly incorporated fungal-derived C, whereas the algal-derived C was mainly incorporated by *Acidobacteria* and *Proteobacteria*. This study emphasizes the important role of both fungal and algal biomass in increasing the carbon pool in recently deglaciated bare soils, as only 20% of the added C was respired as CO_2, and the rest, we presume, remained in the soil.

5.2 INTRODUCTION

Due to climate change, the Alpine glaciers are retreating rapidly (Paul et al., 2004). The recently deglaciated terrain close to the glacier terminus is mainly composed of sand, gravel and stones. These bare soils are generally nutrient poor, with little organic carbon (C) or nitrogen (N) (Matthews, 1992; Bardgett et al., 2004; Bernasconi et al., 2011). Nevertheless, heterotrophic organisms, which rely on organic C sources for growth, are colonizing these recently deglaciated soils along with autotrophs (Jumpponen et al., 2002; Nicol et al., 2006; Zumsteg et al., 2012). The organic C sources available for heterotrophic organisms have different origins. They are either ancient, i.e. buried under the ice (Bardgett et al., 2007), or modern, i.e. deposited from the atmosphere or introduced through photosynthetic organisms (Barker et al., 2006; Hawes, 2008; Jurado et al., 2008; Freeman et al., 2009). In developed soils, microbial biomass C corresponds to only 1 – 2% of the soil organic C, but in bare glacier forefield soils, this proportion could be much higher, as there is a lack of other sources of soil organic matter (like plant residues and animal detritus) (Kindler et al., 2006). Therefore microbial cells could become an important C source for heterotrophic organisms after their death in glacier forefield ecosystems.

While the fate of microbial biomass has been investigated in agricultural soils (Kindler et al., 2006; Kindler et al., 2009; Miltner et al., 2009), little is known about such biomass in bare soil ecosystems, where the addition of biomass could greatly influence microbial activities and community structures. Generally, a substantial C addition to soil increases soil respiration and induces a change in the microbial community structure from a slow growing state to a fast growing state, which can efficiently use the C source available (Stenstrom et al., 2001; Cleveland et al., 2007). Furthermore, different microbial communities prefer different C substrates. Thus a change in the C substrate can induce a shift in the microbial community structures (Wagner et al., 2009). It is unknown whether the addition of microbial biomass induces similar reactions in bare glacier forefield soils and whether they are influenced by temperature.

To study potential shifts in microbial community structures after the addition of isotopically labelled nutrients (^{13}C- or ^{15}N-labelled) and to identify the phylogenetic groups actively incorporating specific labelled substrates into cell components, stable isotope probing (SIP) is used (Radajewski et al., 2000; Padmanabhan et al., 2003; Neufeld et al., 2007). The SIP technique has rarely been applied to study polar or alpine ecosystems so far. On the Damma glacier forefield, litter turnover and food-web dynamics were investigated using ^{13}C-labelled litter and subsequent analysis of phospholipid fatty acids (PLFAs) (Esperschütz et al., 2011). They found an increase of fungi and Gram-negative bacteria shortly after litter addition. The more recalcitrant litter components were later degraded by Gram-positive bacteria. To analyse soil bacteria actively metabolizing labelled C substrates, DNA SIP is more suitable for unravelling microbial community composition in terms of taxonomic resolution than PLFA analysis (Dumont and Murrell, 2005).

We therefore performed DNA-SIP combined with respiration and activity measurements to address the following questions: (i) are bacteria living in bare glacier forefield soils able to utilize fungal and algal biomasss as a C source, (ii) does the origin of the C (fungi or algae) and temperature influence CO_2 respiration, bacterial activity and bacterial community structure and (iii) which bacterial phylotypes are likely to predominantly incorporate the fungal and algal derived C.

Penicillium sp. (Brunner et al., 2011) and *Chlorella* sp. (Frey et al., 2010) were isolated from the Damma glacier forefield, located in the Swiss Central Alps. They were ^{13}C-labelled and added to recently deglaciated bare soil. The soils supplied with biomass were incubated for 60 days at either 4°C or 18°C and were sampled at different time points. CO_2 respiration and its $\delta^{13}C$ signature were monitored continuously. *Chlorella* sp. as photoautotrophs are proposed to have a role in generating pioneering organic carbon inputs in these carbon-limited environments, whereas the spore-forming *Penicillium* sp. represented 'ancient' and more recalcitrant carbon sources. We hypothesized that the resource quality influences the consumers with distinct temporal succession of consumer communities between the two substrates.

To characterize the bacterial community structure, DNA was extracted, subjected to SIP and bacterial 16S rRNA gene T-RFLP profiling. Cloning and Sanger sequencing were performed to assess the phylogenetic groups that were able to incorporate the labelled ^{13}C. Molecular analyses were complemented by assessing the bacterial activity using the leucine incorporation technique (Bååth, 1998).

5.3 RESULTS

5.3.1 CO_2 EVOLUTION

Generally, respired CO_2 was higher at 18°C than at 4°C, and was higher in soils supplied with algal biomass (Figure 1). At 4°C, the highest CO_2 respiration values (100 - 200 µmol CO_2 h^{-1}) were measured 4 days after the addition of biomass. At 18°C, the highest values (200 - 500 µmol CO_2 h^{-1}) were measured already 2 days after the addition of biomass (Figure 1). The CO_2 respiration decreased after the first days with both temperatures and carbon sources, reaching a constant level 13 days after the addition of biomass (Figure 1). Without the addition of a carbon source (control), the CO_2 respiration remained low throughout the experiment.

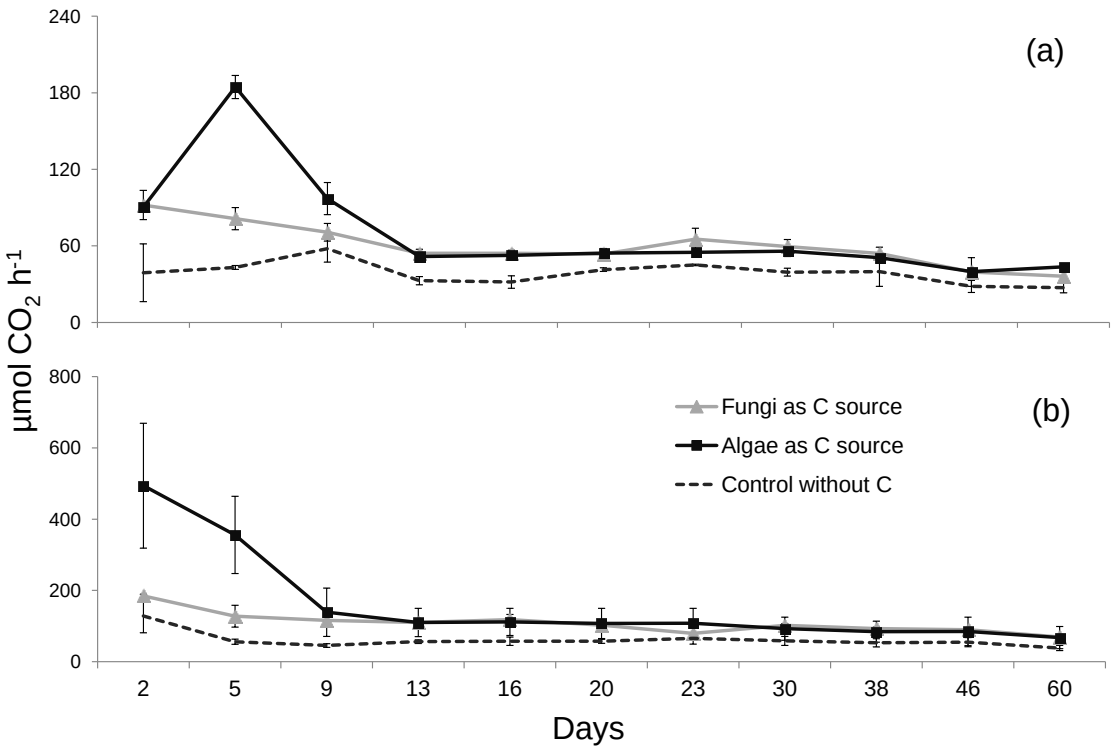


Figure 1: CO_2 evolution from soils over time (mean ± SE; n = 3). CO_2 was measured in bottles containing bare soil supplied with ^{13}C labeled fungal or algal biomass (0.18 mgC g^{-1} soil), or in soils without biomass (control) incubated at either 4°C (a) or 18°C (b).

About 20% of the added carbon sources were respired as CO_2 after 60 days (data not shown). Furthermore, the addition of fungal and algal biomass was clearly reflected by an increase in $\delta^{13}C$ in the respired CO_2, indicating that the utilization of the microbial biomass significantly contributed to soil CO_2 respiration (data not shown).

5.3.2 Bacterial activity

The addition of fungal and algal biomass to soils induced an increase (from 20 up to 120 pmol leu h^{-1} g^{-1}) in bacterial activity measured as leucine incorporation compared with the activity right after biomass addition (Figure 2), whereas the activity in the control samples without biomass addition remained low (between 5 and 10 pmol leu h^{-1} g^{-1}) (data not shown). Generally, algal biomass applied to the soils led to a higher bacterial activity than the addition of fungal biomass (Figure 2). After 4 and 22 days the bacterial activities in samples incubated at 4°C and supplied with fungal biomass were significantly ($P < 0.05$) different from those in samples supplied with algal biomass. The bacterial activities in the samples supplied with fungal biomass were significantly different from the samples supplied with algal biomass after 8 days of incubation at 18°C. Depending on incubation temperature, bacterial activities were highest after 4 days (18°C) and after 8 days (4°C), which reflects rather the difference in substrate availability (which is higher at higher temperatures) than the temperature effect itself, as the activities were measured at room temperature.

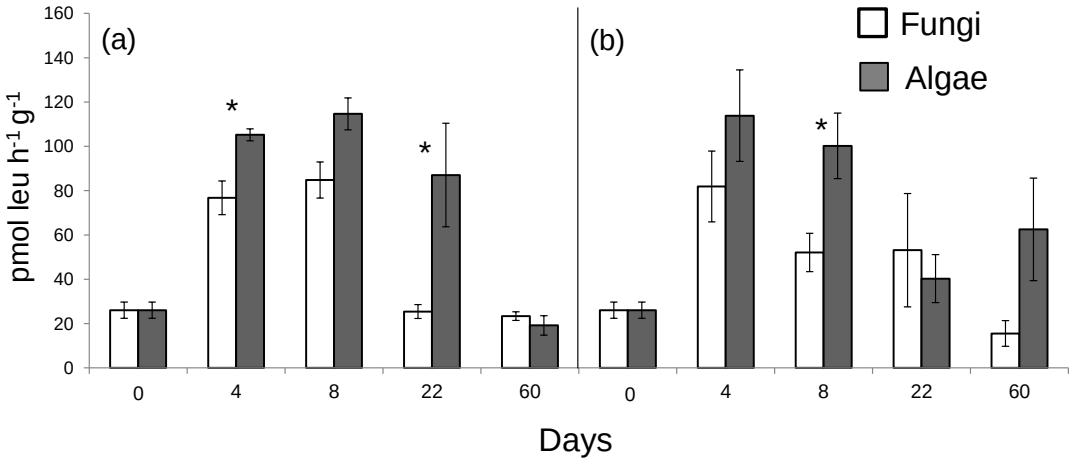


Figure 2: Bacterial activity measured as leucine incorporation (pmol leu h^{-1} g^{-1} soil). White bars represent the bare soils supplied with fungal biomass, and dark bars soils supplied with algal biomass. (a) Bacterial activities at the five sampling times (0, 4, 8, 22 and 60 days after biomass addition) in soils incubated at 4°C, (b) activities in soils incubated at 18°C (mean ± SE; n = 3). Asterisk (*) mark significant differences between bacterial activities in the soils supplied with fungal biomass and soils supplied with algal biomass ($P < 0.05$).

5.3.3 Effects of carbon source, temperature and time on the bacterial community structure

Statistical analyses were performed on the T-RFLP profiles from all the time points before density gradient centrifugation. The bacterial community structures of soils supplied with either ^{13}C-labelled fungal or algal biomass were significantly different ($P < 0.001$) from the control samples without biomass at both incubation temperatures (data not shown). This was confirmed by principal coordinate analysis (PCO), where the control samples clustered away from the samples supplied with biomass (Figure 3a and 3c). Furthermore, the effect of the carbon source (the supply of fungal compared of algal biomass) on the bacterial community structures was significant ($P < 0.01$) at 18°C, but not at 4°C ($P > 0.05$) (Table 1).

The effect of temperature on the bacterial community structures derived from T-RFLP data was highly significant ($P < 0.001$) for the samples supplied with either fungal or algal biomass (Table 1). The effect of time on the bacterial community structures in the samples supplied with fungal or algal biomass was also highly significant ($P < 0.01$) at both temperatures (Table 1). This can also be

observed in the PCO, where a shift in the bacterial community structures with time is visible for the samples with the addition of biomass at both incubation temperatures (Figure 3b and 3d). The effect of time on the control samples was not significant ($P > 0.05$, data not shown).

Table 1: Multivariate statistics (PERMANOVA) performed on 16S rRNA T-RFLP profiles from samples supplied with ^{13}C-labelled biomass. Effect of C source on the soil bacterial community structure (fungal biomass versus algal biomass), effect of temperature on the soil bacterial community structure (4°C versus 18°C incubation) and effect of time (change in the bacterial community structure over the duration of the experiment) were determined over all time points.

Effect of C-source

4°C		**18°C**	
F[a]	*P*[b]	F	*P*
1.7018	0.093	3.2201	**0.0026**

Effect of temperature

Fungi		**Algae**	
F	*P*	F	*P*
6.1888	**0.0001**	**5.6703**	**0.0001**

Effect of time

Fungi				**Algae**			
4°C		**18°C**		**4°C**		**18°C**	
F	*P*	F	*P*	F	*P*	F	*P*
5.7647	**0.0001**	**10.553**	**0.0001**	**8.0072**	**0.0001**	4.3109	**0.0001**

[a] Influence of either C-source, temperature or time on the bacterial community structure; effect is high if F > 5, shown in **bold**

[b] Significance value of the investigated effect on the bacterial community structure; significant if $P < 0.05$, shown in **bold**

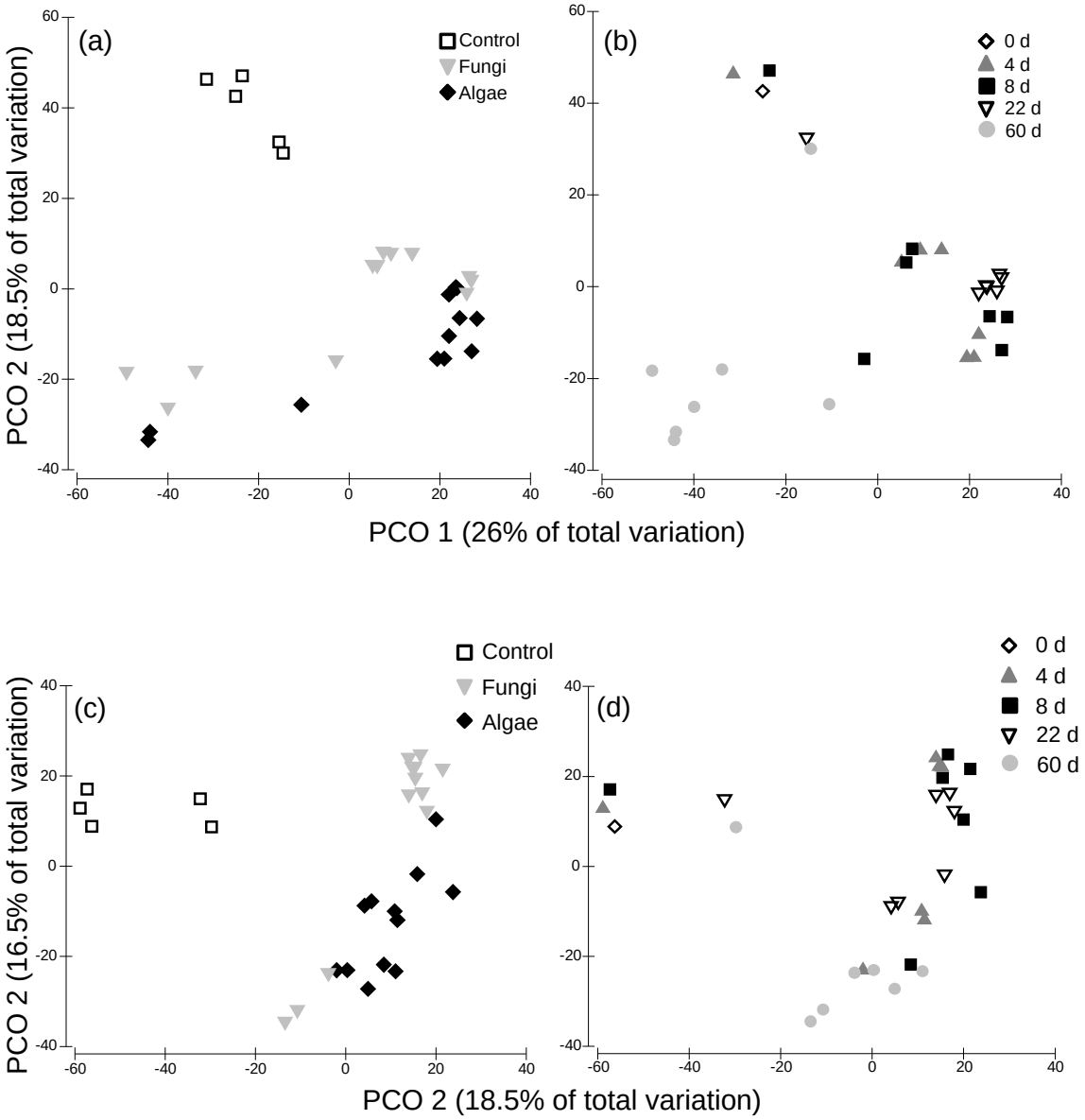
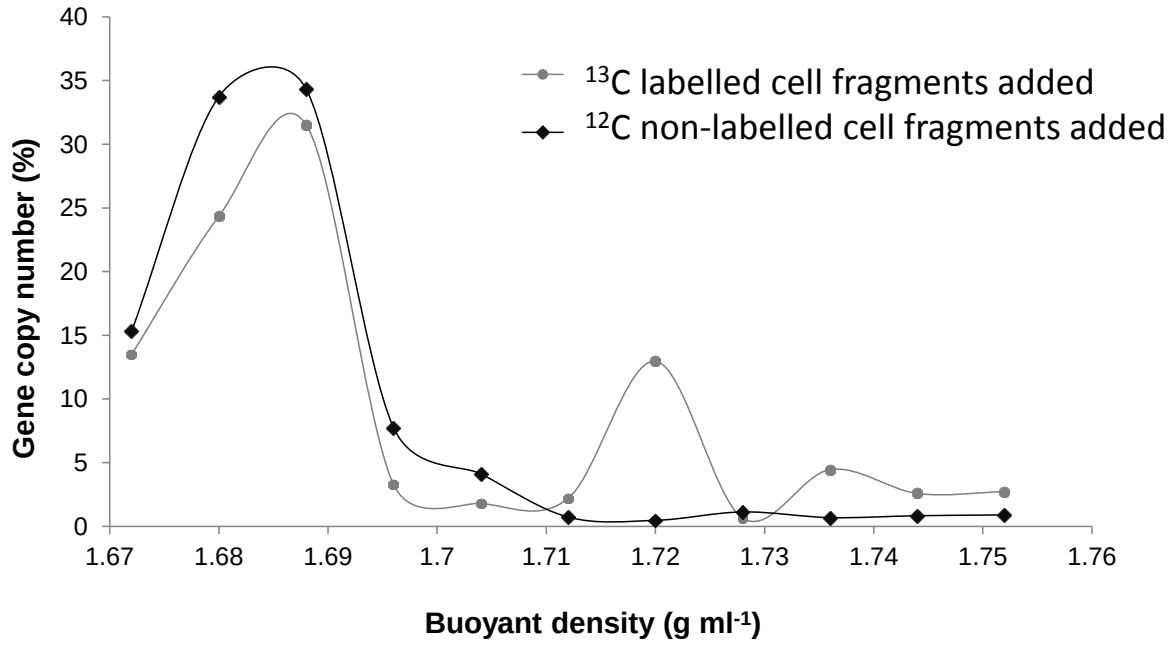


Figure 3: Principal coordinate analysis of the bacterial T-RFLP profiles (restricted with MspI) of bare soils supplied with ^{13}C labeled fungal or algal biomass (n = 3), and soils without biomass (control; mean of n= 3) before density gradient centrifugation. For clarity, the analysis was split into graphs according to soils incubated at 4°C (a and b) or 18°C (c and d). PCO analyses according to carbon source (fungal, algal or no biomass) are shown in (a) and (c) PCO analyses according to time (0, 4, 8, 22 and 60 days) are shown in (b) and (d). The two axes indicate the explained variance between the samples.

5.3.4 Distribution of ^{12}C- and ^{13}C-labelled DNA in CsCl gradients

To differentiate between ^{13}C-labelled and non-labelled fractions, the 16S rRNA copy numbers were plotted versus the buoyant density of the individual fractions. To unequivocally identify the density at which ^{12}C- and ^{13}C-DNA occurred, we used ^{12}C-labelled control samples that were treated similarly as the ^{13}C-labelled microbial cells. Analysis of 16S rRNA copy numbers using real-time PCR demonstrated only a single peak at a buoyant density of 1.68 g ml^{-1} in the unlabelled control samples (data not shown). The labelled ^{13}C-DNA and non-labelled ^{12}C-DNA were clearly separated and formed two distinct peaks (highest 16S rRNA copy numbers) at 1.68 g ml^{-1} and 1.72 g ml^{-1} (Figure S1) We therefore considered the fractions corresponding to buoyant densities at 1.675 - 1.685 g ml^{-1} (3 out of 18 fractions) as the “light” non-labelled ^{12}C-DNA and those fractions at buoyant densities of 1.715 – 1.725 g (3 out of 18 fractions) as the “heavy” ^{13}C-DNA. Several fractions showed high 16S rRNA copy numbers in samples taken 22 and 60 days after the addition of ^{13}C-labelled biomass indicating cross-feeding (consumption of the primary consumers by secondary consumers resulting in less strongly labelled DNA), which can give a constant background signal (no assignment to primary or secondary utilizers of the biomass possible) of unspecific ^{13}C-DNA in many fractions (Lueders et al., 2004; Dumont et al., 2011).



Supplementary Figure S1: qPCR of the 16S rRNA gene after stable isotope probing of samples amended with either ^{13}C labeled biomass or non-labeled ^{12}C biomass. The fractions with a buoyant density of 1.68 g ml^{-1} were considered to contain mainly non-labeled DNA, whereas the fractions with a buoyant density of 1.72 g ml^{-1} was considered to contain mainly ^{13}C labeled DNA.

5.3.5 Phylogenetic analysis of the bacterial operational taxonomic units (OTUs) responding to the supply of biomass

After T-RFLP profiling of the "light" and the "heavy" fraction, the relative abundance of each OTU in the "light" ^{12}C fraction obtained after density gradient centrifugation was subtracted from the relative abundance of the matching OTU in the "heavy" ^{13}C fraction, to find OTUs that were predominately incorporating ^{13}C (positive values) or ^{12}C (negative values). These values were called 'subtraction values'. This is an adaptation of the concept proposed by Lankau (2010) using 'sensitive' and 'resistant' OTUs. As an example, when the relative abundance of the ^{12}C fraction of a certain OTU was 0.5% and the matching relative abundance of the ^{13}C fraction was 3%, then the subtraction value would be 2.5%. Therefore, this specific OTU was predominantly incorporating ^{13}C-labelled biomass. We only investigated the samples 4 and 8 days after addition of biomass as it was not possible to make a clear separation between the "heavy" and the "light" fractions 22 and 60 days after the

addition of biomass, because 16S rRNA copy numbers were increased in all fractions. Seventy-three OTUs showed a subtraction value either lower than -1 or higher than 1, which was designated as subtraction value of -1 / +1, 43 OTUs showed a subtraction value of -2 / +2, 30 OTUs a subtraction value of -3 / +3 and 22 OTUs a subtraction value of -4 / +4.

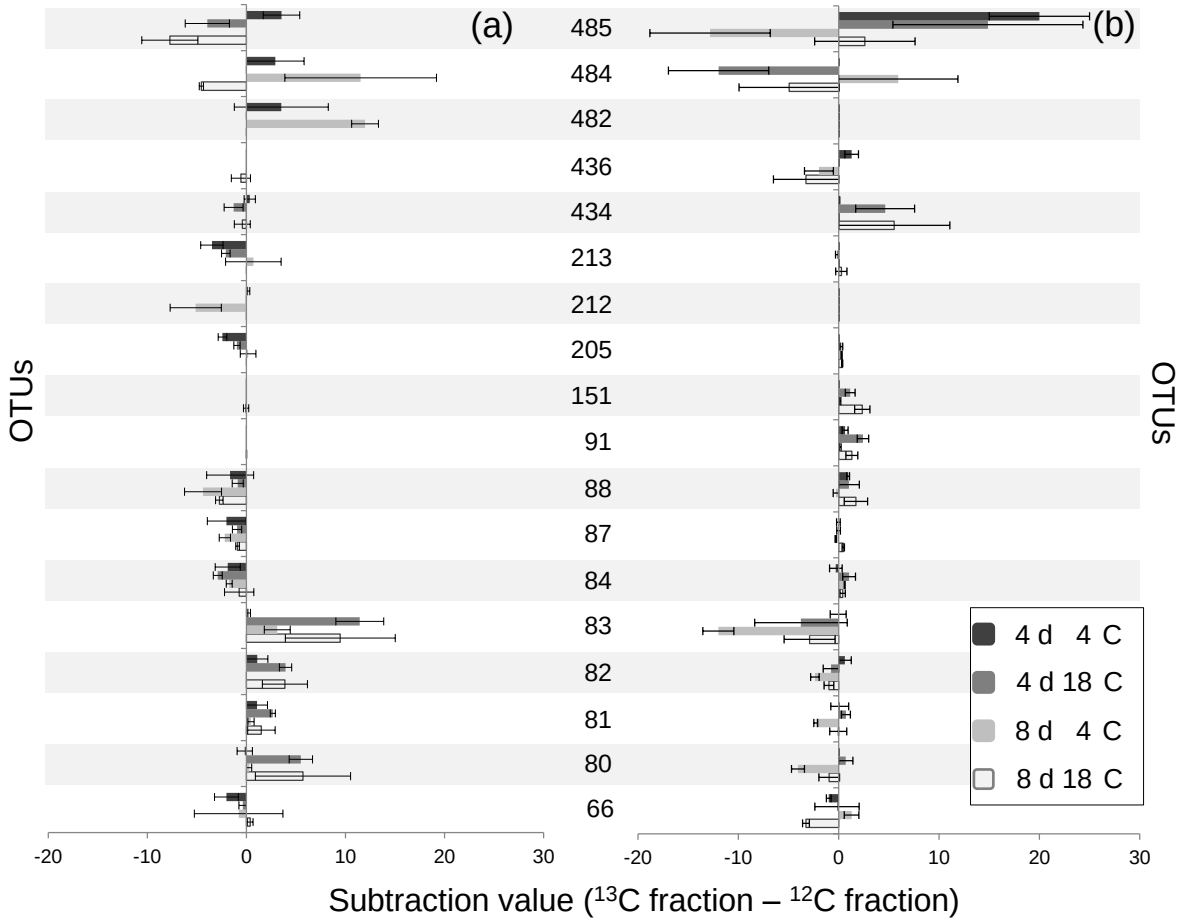


Figure 4: Bacterial OTUs (T-RFs in bp) incorporating ^{13}C from (a) fungal and (b) algal biomass after 4 and 8 days of incubation at either 4°C or 18°C. After T-RFLP profiling, the relative abundance of each OTU in the "light" ^{12}C fraction obtained after SIP was subtracted from the relative abundance of the matching OTU in the "heavy" ^{13}C fraction to find OTUs that were predominately incorporating ^{13}C or ^{12}C. Values were considered to be high if the subtraction value was below -3 or above 3 for at least one sample, as described in the main text. Positive values represent OTUs predominantly incorporating ^{13}C, negative values represent OTUs predominantly incorporating ^{12}C (mean ± SE, n = 3). The corresponding phylogenetic groups are given in Table 3.

Because of the limited number of clones investigated, we decided that a subtraction value of -3 / +3 (smaller than -3 and larger than +3) represented bacterial phylotypes principally incorporating either ^{13}C-labelled biomass (subtraction value +3) or other, non-labelled carbon substrates from the soil (subtraction value -3) (Figure 4). Out of these 80 sequenced OTUs, 18 showed a subtraction value of -3 / +3, and are listed in Table 2. Some of our OTUs (66, 84 and 87) showed a preferential allocation towards the heavy fraction in the ^{12}C amended samples. For all the other OTUs this was not the case. Therefore, the results for these three OTUs are tentative.

Table 2: Bacterial OTUs with the *MspI* terminal restriction fragment length in bp of the environmental samples and the clones, corresponding to bacterial OTUs incorporating ^{13}C labeled carbon derived from fungal or algal biomass, as shown in Figure 4.

Environmental sample OTU	Clone OTU	Clone name	NCBI accession no.	NCBI closest match		Phylogenetic Group	Habitat	Identity
				accession no.	clone or strain name			%
66	65	Bact_SIP_65	JX204372	EU132932	FFCH16325	*Actinobacteria*	prairie soil	96
80	80	Bact_SIP_80	JX204361	FR772064	*Flavobacterium* sp. R-38296	*Bacteroidetes*	Antarctic soil and water	97
81	81	Bact_SIP_81-2	JX204321	AB599368	TUDR-1_F09	*Chloroflexi*	rice roots	99
82	82	Bact_SIP_82	JX204343	AM177627	*Flavobacterium hercynium* WB 4.2-78	*Bacteroidetes*	hardwater creek	98
83	84	Bact_SIP_84	JX204311	AM177638	*Flavobacterium* sp. WB 4.4-73	*Bacteroidetes*	hardwater creek	98
84	84	Bact_SIP_84-2	JX204344	HM113619	*Flavobacterium* sp. 001xTSA12A_G06	*Bacteroidetes*	Alaskan soil	99
87	87	Bact_SIP_87	JX204353	AM177638	*Flavobacterium* sp. WB 4.4-73	*Bacteroidetes*	hardwater creek	98
88	89	Bact_SIP_89	JX204362	FJ694273	KL201E08	*Bacteroidetes*	Polar river	96
91	91	Bact_SIP_91	JX204375	EU861864	G08_bac_con	*Acidobacteria*	Alpine tundra soil	98
151	151	Bact_SIP_152	JX204323	EF516787	FCPT612	*alpha-Proteobacteria*	grassland soil	99
205	204	Bact_SIP_204	JX204335	AM945482	Malla4.140	*Bacteroidetes*	Arctic tundra soil	92
212	212	Bact_SIP_212	JX204336	EU298170	GASP-KB2S3_A06	*Firmicutes*	tallgrass prairie soil	98
213	214	Bact_SIP_214	JX204382	EF516615	FCPT647	*Chloroflexi*	grassland soil	94
434	434	Bact_SIP_434	JX204341	AM945469	Malla4.128	*Bacteroidetes*	Arctic tundra soil	97
436	437	Bact_SIP_437	JX204367	AB545620	Massilia sp. TSO8	*beta-Proteobacteria*	rice paddy soil	99
482	481	Bact_SIP_481	JX204330	EU978740	glb251b	*beta-Proteobacteria*	glacier ice	98
484	484	Bact_SIP_484	JX204331	AB186839	TSAF31	*gamma-Proteobacteria*	microcosm study	97
485	485	Bact_SIP_485	JX204352	EF018455	Amb_16S_1080	*beta-Proteobacteria*	soil under aspen	99

Supplementary Table S1: Physico-chemical parameters of the bare soil from the recently deglaciated forefield of the Damma glacier. Soil porosity was measured as pore volume in the corresponding soil volume ($m^3\ m^{-3}$). The mineral nutrient contents are given as µmol g^{-1} soil. Nitrogen (N) and carbon (C) are given in %.

pH	Coarse porosity	Fine-to-intermediate porosity	Water holding capacity	Sand	Silt	Clay	N	C	NH_4	Cl	NO_3	PO_4	SO_4	Ca	K	Mg	Na
	($m^3\ m^{-3}$)	($m^3\ m^{-3}$)	(%)	(%)	(%)	(%)	(%)	(%)									
5.3	0.169	0.294	64.5	77.0	20.5	2.7	0.007	0.10	0.05	0.11	0.01	0.01	0.04	0.61	0.29	0.13	0.25

Five bacterial phylotypes (OTUs 80, 81, 82, 83 and 482) predominantly incorporated ^{13}C from fungal biomass (Figure 4a). Most cloned sequences were phylogenetically affiliated to *Flavobacterium* sp. within *Bacteroidetes*, isolated from Antarctic soil and water or from a hardwater creek. Other clone sequences were closely related to the environmental clones obtained from glacier ice (Table 2).

Four bacterial phylotypes (OTUs 88, 91, 151 and 434) predominantly incorporated ^{13}C from algal biomass (Fig. 4b). The clone sequences were phylogenetically affiliated to environmental clones within *Bacteroidetes* obtained from a polar river and from an Arctic tundra soil. Other clone sequences were related to environmental clones within *Acidobacteria* obtained from Alpine tundra soil and within *alpha-Proteobacteria*, strain *Rhodopseudomonas* sp., obtained from a microbial mat in an Alpine lake.

Two bacterial phylotypes (OTUs 484 and 485) incorporated both fungal- and algal-derived biomass (Fig. 4). The clone sequences were closely related to environmental clones within *gamma-Proteobacteria* obtained from a microcosm study using polluted river sediment and within *beta-Proteobacteria* obtained from soil under aspen.

5.4 DISCUSSION

In bare glacier forefield soils, which are characterized by low C and N contents, moist and sandy batches are rapidly colonized by autotrophic, as well as heterotrophic microorganisms, leading eventually to higher organic C content and water availability. This significantly improves the living conditions for other organisms (Breen and Levesque, 2008). Thus, it can be assumed that algae and fungi are an important C source in recently deglaciated soils. Here we report the first application of DNA-SIP to trace C from dead green-algal and fungal biomass into the soil bacterial community.

CO_2 evolution in soils peaked 2 to 4 days after addition of ^{13}C-labelled biomass, indicating that the substrate was rapidly metabolized by indigenous soil microorganisms. The microorganisms known to increase their metabolic rates quickly following such a C input are mainly fast growing microorganisms (Kuzyakov et al., 2000; Stenstrom et al., 2001; Blagodatskaya et al., 2008). Polysaccharides such as cellulose or chitin, as well as glucose are generally utilized within days by microbes in soils (Manucharova, 2009; Stursová et al., 2012). But at such high microbial activity rates, the easily available C will be rapidly respired, leaving more recalcitrant material which is decomposed mainly by slow growing microorganisms. This can be seen in the decreased and near constant respiration rates from 13 days after addition of biomass onwards. These slow growing microorganisms are now more competitive for the remaining, but more complex C components from fungal and algal biomass, that have not been decomposed by the fast growing organisms before (Fontaine et al., 2004; Kindler et al., 2006).

It has been suggested that 50% of a carbon substrate or dying biomass applied to soil, is integrated into microbial biomass or contributes to soil organic matter, and 50% is respired as CO_2 (Kuzyakov et al., 2000; Kindler et al., 2009), if it is easily available. In our experiment around 20% of the supplied carbon was respired as CO_2 after 60 days at 18°C. Therefore a large part of the added C still remained in the soils after 60 days of incubation and could be decomposed by soil microorganisms. This is also

indicated by the higher CO_2 respiration in the soils supplied with fungal or algal biomass, than in control soils without the addition of a carbon source.

Generally, more CO_2 was respired at 18°C than at 4°C. This is in accordance with Andersson and Nilsson (2001) and Steinweg and colleagues (2008), who also observed that soil respiration increased at higher temperatures. However, the large amount of C respired at 4°C indicates that microbial communities had adapted to low temperatures and were active in the Damma glacier forefield (mean annual temperature is 1.8°C; (Zumsteg et al., 2012).

In samples supplied with algal biomass respiration and bacterial activity (leucine incorporation) were generally higher than in samples supplied with fungal biomass. One reason for this difference might be that the green algae contained more N (6.5%) than the fungi (1.9%). The combined addition of N as ammonium nitrate and C as glucose has been shown to result in higher bacterial growth rates than adding glucose alone (Demoling et al., 2007), which is evidence that N limitation prevents growth (Vitousek et al., 1991). The bacterial communities in bare soils of the Damma glacier forefield are also known to be C and N co-limited (Göransson et al., 2011). This implies that, by adding fungal biomass, N might become a limiting factor, leading to restricted respiration and activity. Another factor is that green algae and *Penicillium* sp. differ greatly in their cell wall composition. Green algal cell walls are mainly composed of cellulose, xylan and mannan (Tsekos, 1999), whereas the cell walls of *Penicillium* sp. are composed of chitin together with glucose and mannose (Andriyanova et al., 2011). Cellulose is the most abundant and rapidly utilizable biopolymer in soil, and is thought to be more easily degradable than chitin (Baldrian et al., 2011). This is mainly due to the hydrogen bonding in the chitin molecule (Minke et al., 1978), which makes it harder to break the bonds between the glucosamine units in N-acetylglucosamine than the glucose units in cellulose. The enzymes involved in the degradation of cellulose and chitin have been quantified in bare soil from the Mendenhall Glacier forefield in Alaska (Sattin et al., 2009) and in bare soil of the Damma glacier forefield (E. Bünemann, personal communication). In both locations the enzymes involved in cellulose

degradation were more abundant than the enzymes involved in chitin degradation, which implies that cellulose-degrading microbes, in general, are more abundant in glacier forefields.

The source of the supplied C (fungal or algal biomass) significantly influenced the bacterial community structures at 18°C. In contrast, the difference was not significant at 4°C, which could be due to the generally lower turnover rates at lower temperatures (Pettersson et al., 2003). The observed change in bacterial community structures with time could reflect a shift from a fast growing phenotype, feeding on easily available substrate, to a slow growing phenotype using the more recalcitrant C (Stenstrom et al., 2001), as indicated by the decreasing respiration rates at later time points.

Distinct bacterial phyla appear to metabolize different labile C sources (Padmanabhan et al., 2003; Singleton et al., 2007), but large proportions of cells are protein, RNA and DNA. Accordingly we found that some soil bacterial phyla could decompose both fungal and algal biomass. The main phylogenetic groups incorporating both fungal- and algal-derived C were *Bacteroidetes* and *beta-Proteobacteria*. The abundance of these two phyla was found to correlate with the C content in various soils across the USA (Fierer et al., 2007). Some active members of the *Bacteroidetes* and *beta-Proteobacteria* are initial metabolizers of labile C sources (not in association with soil particles) (Padmanabhan et al., 2003), which corresponds to the incorporation of ^{13}C-labelled biomass after already 4 or 8 days of incubation. *Bacteroidetes* are abundant in glacial habitats in general (Cheng et al., 2007; Simon et al., 2009), and have been reported to play an important role in the mineralization of microbial biomass in soil (Lueders et al., 2006). Similarly, *beta-Proteobacteria* are abundant in newly deglaciated granitic sediments (Zumsteg et al., 2012), and belong to the main decomposers of plant and microbial organic carbon in soil (Lueders et al., 2006; Bernard et al., 2007). Accordingly, the *Oxalobacteraceae*, which we found to incorporate fungal and algal biomass, are responsive to the addition of cellulose in soils (Eichorst and Kuske, 2012).

Chitin is one of the main components of the cell wall in the fungus *Penicillium* sp., and is degraded by fungi and bacteria in different habitats (Gooday, 1990). Of the bacterial phylotypes metabolizing and incorporating mainly the fungal-derived C in this experiment, OTUs 80, 82 and 83 were affiliated to *Flavobacterium* sp. (FR772064, AM177627 and AM177638) within *Bacteroidetes*, which are able to degrade chitin in soil (Manucharova, 2009). In this experiment, *Flavobacteria* sp. incorporated more ^{13}C at 18°C than at 4°C, even though they are known to be adapted to cold (Gangwar et al., 2011), and appear to dominate the bacterial community in shallow lakes in Antarctica together with *Pseudomonas* species (Michaud et al., 2012). Another bacterial OTU shown to primarily incorporate fungal- derived C (OTU 482) within *beta-Proteobacteria* was closely related to a clone (EU978740) obtained from glacier ice (Simon et al., 2009) and belongs to the genus *Polaromonas* sp., which are known to be active even in glacier ice (Barker et al., 2010). Interestingly, specific members of the *beta-Proteobacteria*, within the genus *Collimonas* sp., have been found to degrade the chitin of living fungal hyphae (de Boer et al., 2004). This genus has recently been isolated from the Damma glacier forefield (Lapanje et al., 2011) co-occurring with saprophytic fungi such as *Penicillium* sp., *Mucor* sp. and *Geomyces* sp., which were found to be abundant in the barren soils of the forefield (Brunner et al., 2011).

Algal cell walls are mainly composed of cellulose, which has been shown to be the main C source in soils (Stursová et al., 2012). Of the bacterial phylotypes metabolizing and incorporating mainly the algal-derived C in this experiment, one OTU (OTU 91) within *Acidobacteria* was closely related to a clone (EU861864) derived from an Alpine tundra soil fertilized with N for several years (Nemergut et al., 2008). It belongs to the Gp1 *Acidobacteria*, which has been shown to be actively involved in decomposition of cellulose in soil (Baldrian et al., 2012). Another bacterial OTU found to incorporate mainly algal-derived C (OTU 151) within *alpha-Proteobacteria* was closely related to *Rhodopseudomonas* sp. (AJ289108) obtained from an Alpine lake. The *Rhodopseudomonas* are characterized as photoheterotrophic bacteria containing bacteriochlorophylls (Lester et al., 2007). Therefore they use both organic carbon and light as nutrient and energy sources, which is an

advantage in colonizing a glacier forefield with low soil C contents. Besides incorporating fungal-derived C, we also found members of *Bacteroidetes* incorporating algal-derived biomass (OTUs 88 and 434), indicating the versatility of this phylogenetic group in using diverse C sources. OTU 434 was related to the genus *Mucilaginibacter* sp. which is abundant in soils after cellulose addition (Stursová et al., 2012), which is consistent with our findings.

In conclusion, we found that both ^{13}C-labelled substrates were used and respired by indigenous soil microorganisms at both 18°C and 4°C, with CO_2 respiration being higher at 18°C than at 4°C. Higher respiration and bacterial activity indicate that the nutrients are more easily available in algal biomass than fungal biomass. Additionally, the origin of the carbon source and incubation temperature had an effect on the bacterial activities and their community structures. We found that only 20% of C was respired as CO_2 after 60 days, and the rest, we presume, remained in the soil, increasing the soil organic matter content. We have therefore been able to show the important role of both fungi and algae in increasing the available carbon pool in recently deglaciated bare soils. We found that the two different substrates were incorporated by differing phylogenetic groups. Therefore, we could show that the kind of carbon substrate which is added to soil does influence the consumer communities. The main bacterial incorporators of fungal-derived C belonged to the *Bacteroidetes*, whereas the algal-derived C was incorporated mainly by *Acidobacteria* and *Proteobacteria*. When investigating microbial colonization of bare soils, one should therefore be aware of the carbon sources available in the soil when comparing different sites, besides considering the climatic conditions and diverse soil physicochemical parameters.

5.5 Experimental Procedures

5.5.1 Soil origin and physico-chemical parameters of the investigated soil

Bare soil used in this experiment was collected on the Damma glacier forefield, which is located in the Central Alps in Switzerland (N 46°38 E 08°27), within the Central Aar Massif at an altitude between 1950 and 2050 m a.s.l. The forefield has been subjected to extensive interdisciplinary studies (Bernasconi et al., 2011). The soil was collected close to the glacier terminus where the soil has been ice free for 5 to 7 years. Soil was taken from the first 5 cm in depth using a shovel and sieved through a 2 mm sieve to remove larger stones. The soil physico-chemical parameters of recently deglaciated bare soils were determined as previously described (Zumsteg et al., 2012), and are given in Table S1.

5.5.2 Carbon Sources

The fungus *Penicillium* sp. was isolated from the Damma glacier forefield (Brunner et al., 2011) and grown at 25°C for 3 weeks in a modified liquid MMN medium (Marx, 1969), with 9.3 mg ml^{-1} of ^{13}C-glucose (Sigma-Aldrich, St. Louis, MO, USA) as a substitute for malt. The green alga *Chlorella* sp. was isolated from the Damma glacier forefield (Frey et al., 2010) and grown in a modified liquid Bold's Basal Medium (BBM) (Bischoff et al., 1963), adding 10 mg L^{-1} sodium hydrogen carbonate ($NaH^{13}CO_3$) (Sigma-Aldrich), which is 100 times less than reported in Raoof et al. (2006) as a low amount also resulted in highly labelled algal biomass. Labelling with ^{13}C-sodium bicarbonate has been used to detect active primary producers like *Cyanobacteria* and chemoautotrophic bacteria and *Crenarchaeota* (Wuchter et al., 2003; van der Meer et al., 2007). The algae were grown at 25°C for 3 months and exposed to 10 h of light (2900 Lm) (Philips, Amsterdam, The Netherlands). Additional $NaH^{13}CO_3$ (10 mg L^{-1}) was added every 3rd week to the medium.

Sterile grown cultures were collected by centrifugation and the pellets were recovered and freeze-dried. The C and N contents were determined by elemental analysis (CN 1500, CE Instruments,

Wigan, UK) using 1 mg of freeze-dried material. Both fungal and algal biomass had a C content of 49%. The content of N was higher in *Chlorella* sp. (6.5%) than in *Penicillium* sp. (1.9%). As we provided the cells with one carbon source only, which was 99% ^{13}C-labelled, we assume that both fungal and algal biomass were highly (more than 90%) labelled with ^{13}C.

5.5.3 EXPERIMENTAL SET-UP

14 mg of freeze dried fungal and algal biomass were rewetted and homogeneously distributed over a glass-fibre filter-paper (d = 4.5 cm) (Gelman Sciences Inc., Ann Arbor, MI, USA). The filters with ^{13}C-labelled cells were then cut into small pieces (d < 2 mm) and thoroughly mixed with 10 g of soil. This mixture was wetted with 1 ml of supernatant of a soil slurry, prepared by shaking one litre of water with 200 g of bare soil for 1 h at 25°C on a Kuhner LS-X shaker (Kuhner AG, Birsfelden, Switzerland). The mixture of 10 g of soil with small pieces of filter papers was then put in a carbon-free experimental bag (5 cm x 10 cm) with a pore diameter of 25 µm (Ankom Technology, Macedon, NY, USA). Three bags were placed in a plastic specimen jar (100 ml; Greiner BioOne, Frickenhausen, Germany), which was then filled with 90 g of soil. A total of 42 mg of fungal or algal material was applied per microcosm, which is equivalent to 0.18 mg C g^{-1} soil, corresponding to a deposition of approximately 0.12 mg C m^{-2} a^{-1} at the Damma glacier forefield through snow (Brankatschk et al., 2011). During the experiment, the water content was gravimetrically monitored and any water loss compensated. Microcosms supplied with ^{12}C-labelled biomass or left untreated (sterile filters only without cells) were treated similarly and served as controls.

Microcosms were incubated at either 4°C or 18°C and exposed to 10 h of light (1200 Lm) (Philips). In addition to soil samples taken at the beginning of the experiment (just before biomass were added), samples were taken at four different sampling times after biomass were added (4, 8, 22 and 60 days). In total, 120 microcosms (each containing 3 bags) with one of the five C sources (^{12}C or ^{13}C -labelled *Penicillium* sp., ^{12}C or ^{13}C -labelled *Chlorella* sp. or no additional C source), two temperature regimes

(4°C and 18°C), four sampling times (4, 8, 22 and 60 days) and three replicates each were prepared. Preliminary tests showed that it was easier to homogenize the ^{13}C-labelled biomass in a small volume of soil than in a large volume. Furthermore, more DNA could be extracted from three smaller bags than from one larger soil sample. Consequently we decided to place three smaller experimental bags in each replicate microcosm. At each sampling time, the three replicate microcosms were removed from the incubation chambers (at either 4°C or 18°C). At each sampling time, 6 g fresh weight of soil per microcosm (2 g fresh weight of soil per experimental bag) was removed from the three replicates for each treatment and used for measurements of bacterial activity and the remaining soil was immediately frozen for subsequent molecular analyses.

5.5.4 CO_2 EVOLUTION AND ITS $\Delta^{13}C$ SIGNATURE

The CO_2 evolution from soil was measured separately in 250 ml bottles (Schott Inc., Elmsfort, NY, USA). The bottles were prepared in the same way as the microcosms. They contained the same amount of bare soil (120 g in total) and the same amount of ^{13}C-labelled biomass (0.18 mgC g^{-1} soil), which were distributed on filter papers, cut in small pieces, mixed with soil, wetted with a soil slurry and placed in bags. The water content was monitored and any loss compensated for. CO_2 samples were taken after closing the glass bottles for 24 h to allow the CO_2 accumulation to surpass the detection limit of 300 µmol CO_2. The bottles were closed with lids containing an airtight rubber septum for gas sampling. Gas samples were taken by retrieving 20 ml of air with a 25 ml syringe through the septum and by injecting the air into 12 ml pre-evacuated glass vials closed with an airtight rubber septum (Exetainer gas testing vials, Labco Limited, High Wycombe, UK). The glass vials were evacuated with a vacuum of 800 hPa immediately before sampling. The gas samples were analysed for both CO_2 concentration and $\delta^{13}C$ using a Gasbench II, coupled with an isotope ratio mass spectrometer (Delta Plus XL, Thermo Finnigan interfaced with a Delta-S Finnigan MAT, Bremen, Germany). The CO_2 was measured at 11 sampling times (2, 5, 9, 13, 16, 20, 23, 30, 38, 46 and 60 days) after the addition of biomass.

5.5.5 Bacterial activity

Bacterial activity was measured with the leucine incorporation technique according to Bååth (1998) in the samples taken at 0 (before adding the biomass), 4, 8, 22 and 60 days after the addition of ^{13}C-labelled biomass and additionally on the control samples without biomass addition. As the bags were frozen after sampling, the samples were thawed at room temperature for 24 h before the activity measurement, which was then conducted at room temperature (20°C). In contrast to the original protocol, 2 g of soil was used and the incubation period for the leucine incorporation was extended to 2.5 h instead of 2 h. As we measured the activities at room temperature, we were not able to compare the bacterial activities at the two different incubation temperatures (4°C and 18°C) but rather compared the carbon availability, which is suggested to decrease faster at higher temperatures (Rousk et al., 2012).

5.5.6 DNA Extraction and density gradient centrifugation

Genomic DNA was isolated from 600 mg of soil from one experimental bag. Thus three DNA extractions for every replicate microcosm were performed. DNA was extracted using a bead beating procedure according to Frey et al. (2011) with a Fast Prep-24 homogenizer (MP Biomedicals Europe, Illkirch Cedex, France). Extracted DNA was dissolved in AE buffer (1 g dry soil = 1ml of AE), quantified with Pico Green (Invitrogen, Carlsbad, CA, USA) and stored at -20°C. For further analysis the three extractions from each replicate microcosm were pooled to obtain sufficient DNA for stable isotope probing (SIP).

To investigate which bacterial phylotypes incorporated the C derived from ^{13}C-labelled biomass into their DNA, ^{13}C-DNA separation was performed. For all samples, 5 µg of extracted DNA were loaded into a gradient of CsCl (Sigma-Aldrich, St. Louis, MO, USA) of an average density of 1.723 g ml^{-1} dissolved in gradient buffer (0.1 M TrisHCl [pH 8], 0.1 M KCl and 1 mM EDTA [pH 8]). The optical

density of the solution was set to 1.4029 ± 0.0002, measured with a refractometer (Mettler Toledo Inc, Columbus, OH, USA), which corresponds to 1.723 g ml^{-1} CsCl. Density gradient centrifugation was performed in 4.9 ml Polyallomer quick-seal tubes in a VTi-65.1 vertical rotor using an Optima L-80 XP Ultracentrifuge (Beckman Coulter Inc, Brea, CA, USA). Centrifugations were at 45'000 rpm (177'000 g) at 20°C for 40 h. Centrifuged gradients were fractionated from bottom to top by perforating the centrifuge tubes with syringes (Lueders et al., 2004). Fractions of about 200 µl each were then collected and the density of each fraction was determined with a refractometer. Samples from unlabelled control experiments were always analysed in parallel. The DNA in each fraction was precipitated with 1.2 ml PEG (30% PEG 6000, 1.5 M NaCl, [Sigma-Aldrich]) and incubated at 37°C for 1 h, followed by centrifugation (30 min, 13'000 rpm, 4°C). The resulting DNA pellet was washed with 150 µl 70% EtOH (-20°C) and air dried. The DNA was dissolved in 30 µl of AE buffer, quantified in each individual gradient fraction using a Nanodrop 2000 (Thermo Fisher Scientific UK Ltd, Leicestershire, UK) and stored at -20°C.

5.5.7 Quantitative real-time PCR

The distribution of ^{13}C- and ^{12}C-DNA in the individual fractions after density gradient centrifugation was determined by targeting the bacterial 16S rRNA genes with quantitative real-time PCR (qPCR), using the universal bacterial primers 1369F and 1492R (Smith et al., 2006). qPCR was performed in MicroAmp optical 96-well plates using the automated ABI Prism 7500 Fast real-time PCR system (Applied Biosystems, Carlsbad CA, USA). Each 15 µl reaction contained 7.5 µl of SYBR green PCR master mix (including AmpliTaq Gold DNA polymerase, SYBR green PCR buffer, dNTP mix, SYBR green I dye and passive internal reference based on proprietary ROX (Applied Biosystems), 0.5 µM of each primer, 0.015 mg of BSA, 1.6 µl of water and 5 µl of diluted DNA. The PCR conditions were as follows: 15 min at 95°C for initial denaturation; then 40 cycles of 30 sec at 95°C for denaturation, 30 sec at 54°C for primer annealing and 30 sec at 72°C for elongation. The fluorescence was recorded during the annealing and elongation step in each cycle. After the DNA amplification cycles, melting curve

analysis on the PCR products was performed by increasing the temperature gradually from 60°C to 95°C to confirm primer specificity. The calibration curve, calculations and preparation of a plasmid standard containing the target region (16S rRNA gene) generated from sequenced clones was performed as described in Frey et al. (2008). Reaction efficiencies of qPCRs were between 91% and 97% with R2 values > 0.99 for all runs.

5.5.8 T-RFLP PROFILING AND CLONE LIBRARIES

The primers 27F and 1378r were used to amplify the bacterial 16S rRNA genes (Microsynth GmbH, Balgach, Switzerland). The PCR conditions were the same as previously described (Zumsteg et al., 2012). For T-RFLP analysis, PCR was performed on the three replicate samples taken 0, 4, 8, 22 and 60 days after the addition of ^{13}C-labelled biomass, before and after density gradient centrifugation. Unlabelled samples taken from microcosms containing ^{12}C-labelled microbial cells were included for comparison. The bacterial 16S rRNA amplicons were restricted with *MspI* and the T-RFLP analysis was performed as previously described (Zumsteg et al., 2012). The data were then normalized according to total peak height for further statistical analysis.

Four clone libraries were prepared with the same primers, but unlabelled. PCR was performed on the three replicate DNA samples (before density gradient centrifugation) extracted 8 and 60 days after the addition of either ^{13}C-labelled fungal or algal biomass. The PCR products of the corresponding time points were then pooled together for transformation (thus 3 samples were pooled for one clone library). The PCR products were ligated into the vector of the pGEM-T Easy Vector System and cloned into the competent cells JM109 (Promega Corporation, Fitchburg, WI, USA), according to the manufacturer's instructions. A PCR on the clones with M13 vector primers was then performed as described by Zumsteg et al. (2012), to ensure correct insertion of the 16S rRNA fragment. Subsequently, 72 clones of each of the four clone libraries were subjected to T-RFLP analysis (using the same 16S rRNA primers as above on the M13 PCR products) to compare the clone T-RFs with the

T-RFLP profiles of the samples before and after density gradient centrifugation. We prepared clone libraries for only two time points (8 and 60 days), but we assumed that the T-RFs found in these clone libraries could be assigned to any other T-RFLP profile before and after density gradient centrifugation. 140 clones with T-RFs corresponding to T-RFs found in the environmental samples were subsequently chosen to be sequenced, and the corresponding M13 PCR products were purified with a Millipore sequencing reaction clean up kit (EMD Millipore Corporation, Billerica, MA, USA) and bi-directionally sequenced with the primers 27f and 907rev using an ABI prism sequencer 3730XL (Applied Biosystems). The sequences were then sorted, aligned, chimera-checked and analysed as previously described (Zumsteg et al., 2012). The bacterial 16S rRNA gene sequences were deposited in GenBank under accession numbers (JX204305-JX204382).

5.5.9 Phylogenetic analysis of bacterial OTUs

All fractions obtained after density gradient centrifugation from soils supplied with ^{13}C-labelled biomass were subjected to T-RFLP profiling analysis. Each T-RF found through T-RFLP profiling was considered to be one OTU. To investigate which OTUs incorporated mostly ^{13}C-labelled biomass and which OTUs did not, we adapted the concept of 'resistant' and 'sensitive' OTUs according to Lankau (2010) by calculating the differences between the OTU relative abundance of the ^{13}C and the ^{12}C fractions. Positive values represented bacterial OTUs that mainly incorporated ^{13}C material into their DNA, and negative values represented the bacterial OTUs that mainly incorporated ^{12}C. These values were called 'subtraction values'. As an example, when the relative abundance of the ^{12}C fraction of a certain OTU was 0.5% and the matching relative abundance of the ^{13}C fraction was 3%, then the subtraction value would be 2.5%. Therefore, this specific OTU was predominantly incorporating ^{13}C-labelled biomass. A subtraction value of -3 / +3 was considered to represent bacterial groups incorporating mainly ^{13}C-labelled biomass or non-labelled carbon sources. We decided on this threshold of -3 / +3 as a lower value would lead to too many OTUs and a higher value to too few

OTUs that have incorporated the supplied biomass. The classified OTUs could then be assigned to phylogenetic groups, according to the T-RFLP profiles of the sequenced clones.

5.5.10 Statistical Analysis

Statistical analyses on the T-RFLP profiles were performed with the program Primer E (Primer E Ltd, Ivybridge, GB). The normalized T-RFLP profiles were subjected to a square root transformation before statistical analysis. The effect of C source, temperature and time on the bacterial community structures was determined on the T-RFLP profiles of samples supplied with fungal or algal biomass, before density gradient centrifugation with PERMANOVA, including all the sampling times. An effect was considered to be significant when $P < 0.05$. Principal coordinate analysis (PCO) was performed to compare the bacterial community structures of samples without the addition of a carbon source (control; mean of n = 3) and of those with the addition of fungal or algal biomass (n = 3). The bacterial activities between the samples supplied with fungal biomass and samples with algal biomass were compared with an independent t-test using SPSS Statistics (IBM, Armonk, NY, USA).

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6. General Discussion

6.1 Influence of Soil and Environmental Parameters on Microbial Communities in the Damma Glacier Forefield

There are multiple environmental parameters that were proposed to have a major influence on microbial community structures and activities, like temperature, pH, nutrient content of the soil, soil moisture and plant cover, to name a few examples. For instance, higher temperatures lead to higher bacterial and fungal activities in soil (Bekku et al., 2004; Barcenas-Moreno et al., 2009), and also the bacterial and fungal community structures change when soils are incubated at changing temperatures (Yergeau & Kowalchuk, 2008). Furthermore, it has been proposed that pH is one of the main factors influencing microbial communities in soil (Fierer & Jackson, 2006). Soil pH has not only a major influence on microbial community composition (Bååth & Anderson, 2003) but also on respiration and the activity level, as both decrease with lower pH (Rousk et al., 2010). Generally fungi are less sensitive to pH, as they have a wider range of pH which they are naturally adapted to (Rousk et al., 2010). Another main influencing factor for soil microbial communities is the soil organic matter content which is tightly connected to plant cover. There, fungi are more influenced by plant species composition than Prokaryotes (Zinger et al., 2011). The kind of plant species determines the quality and quantity of soil organic matter by exhibiting differing survival strategies and different architectures and therefore diverging in the composition of root exudates and litter decomposition products (Eviner & Chapin, 2003), this directly influences the often saprophytic or mycorrhizal fungal species in soil. Prokaryotes on the other hand are more influenced by soil properties like pH and C:N ratio (Nielsen et al., 2010; Zinger et al., 2011).

As there are so many factors influencing the soil microbial communities, it is not sufficient to focus on one to gain a thorough view on the topic. Therefore, I investigated several environmental factors and soil parameters that could influence microbial communities in glacier forefield soil during my PhD thesis. It has to be stated here, that often it is not clear if a factor is influencing the microbial

communities directly (cause and effect) or if it is simply correlating with an observed change (the factor is not the cause but correlates with the effect). To distinguish between the two, one would have to conduct laboratory experiments in order to control as many environmental factors as possible. However, my thesis was performed mainly in the field; therefore many environmental parameters could have an influence on the investigated microbial communities or correlate with the change. Therefore, the term "influence" will be used throughout, even though it is not always clear if it is an influence or a correlation.

Starting with changes in bacterial, archaeal and fungal community structure along the soil age chronosequence on the Damma glacier forefield, I found that the successional shift in the microbial communities correlates to the increasing C and N content as well as increasing plant cover (Zumsteg et al., 2012 a) (chapter 2). The soil age therefore, was a major driving factor of microbial succession along the Damma glacier forefield. Generally in the older, vegetated soil, decomposers of plant material (as diverse gram negative and positive bacteria as well as fungi), nitrate reducers (as some *Proteobacteria* species for instance) and plant associated microorganisms (as mycorrhizal fungi) were thriving, and outcompeted those that were successful in bare soil (as *Ascomycota* and *Cyanobacteria* for instance) (Deiglmayr et al., 2006; Schutte et al., 2009; Esperschütz et al., 2011 b; Zumsteg et al., 2012 a). For the initial colonization of recently deglaciated terrain no organic matter is needed in the soil, initial colonizers can live on ancient carbon from under the ice or deposited from the glacier ice (Bardgett et al., 2007). Later, plant colonization and soil organic matter content become important factors for soil microbial community structure (Edwards et al., 2006). Generally plant species composition determines the quality and quantity of soil organic matter, which in turn determines the soil microbial community composition (Zinger et al., 2011). Through the gradual colonization of the chronosequence by plants and therefore the succession of differing plant species along the soil age gradient, the soil organic matter content and composition changes along the glacier forefield. Therefore, the microbial communities have to adapt to these changes, leading to a microbial community succession (Allison et al., 2007). This is in accordance to my results, showing a

relationship of the soil C and N content with microbial succession (Zumsteg et al., 2012 a) (chapter 2). Of course it is not only crucial that organic C is present in soil, but also if it is available for microorganisms. For instance it was shown that at higher soil temperatures the C sources are metabolized faster and therefore become less available with time (Rousk et al., 2012). This means that generally at more moderate temperatures the organic carbon is more available for microorganisms, which means for a topographically very heterogenic forefield, that the substrate availability can differ in sites close to each other, which was reflected in my soil transfer experiment (Zumsteg et al., 2011, Zumsteg et al., 2013) (chapters 3 and 4).

While the older soil communities correlated to increasing C and N content and plant cover, especially the archaeal and fungal communities in the younger soils correlated with a higher pH (Zumsteg et al., 2012 a) (chapter 2). That the pH is a main influencing factor for *Crenarchaeota* has already been proposed by Lehtovirta et al. (2009), showing a decline of *Crenarchaeota* with increasing pH. This is in accordance to our findings along the Damma glacier forefield, where the *Crenarchaeota* abundance increased with decreasing pH. But it was also shown that fungi are less influenced by pH than bacteria (Rousk et al., 2010) which is not in accordance to our results where the fungal community structures correlated to soil pH (Zumsteg et al., 2012 a) (chapter 2). That, on the other hand, the abundance of *Basidiomycota* degreases with increasing pH (Rousk et al., 2010) is in accordance to our study as we found mainly *Ascomycota* living in the young soil with a higher pH. It has to be added that the extent of the pH change along the forefield (from approximately 5.5 to 4) cannot be expected to inflict a large influence on the microbial communities. It can therefore be expected that the influence of the change in C and N content and availability as well as plant cover are much larger in comparison.

In addition to the soil parameters, I also focused on environmental and microclimatic parameters possibly influencing microbial communities. I showed that temperature has a large influence on microbial community structures and activities in general (Zumsteg et al., 2011; Zumsteg et al., 2012 b; Zumsteg et al., 2013) (chapters 3 – 5). When investigating the difference in bacterial activities after

biomass addition in a laboratory experiment, the activities were higher at 18°C when compared to 4°C. This is in accordance to other studies that showed a microbial activity increase at higher temperatures (Loffler et al., 2008; Barcenas-Moreno et al., 2009). However, in contrast to these findings in a soil transfer experiment in the field, an increase in temperature did not increase the microbial activities (Zumsteg et al., 2011; Zumsteg et al., 2013) (chapters 3 and 4). On the contrary, the activities rather increased after soil transfer from a warmer site to a colder site, therefore opposing my earlier findings and the literature. The optimal growth temperature in soil is between 25°C and 35°C (Pietikåinen et al., 2006) and temperature fluctuations are known to reduce bacterial productivity (Harris & Tibbles, 1997). Therefore, the activities increased after transfer to a colder site, as there the temperature maxima (20°C) and daily fluctuations (up to 10°C) were more favorable. Additionally, the soil moisture in the samples originally from the colder sites was generally higher, even after transfer, than the samples from the warmer site. I therefore concluded that, in addition to temperature, the soil moisture was also a main influencing factor for the microbial activities, as a higher soil moisture increases microbial activities (Poll et al., 2008). This is in accordance to findings that soil moisture enhances the microbial access to nutrients and also enhances their motility in soil (Long & Or, 2009), therefore higher soil moisture can be advantageous to maintain a certain activity level, in order to survive at extreme locations like at the warmer and drier site (Zumsteg et al., 2013) (chapter 4). Another reason for the low microbial activities at the warmer and drier site could be the reduced nutrient and C availability at higher temperatures (Rousk et al., 2012) and the therefore better nutrient and C availability at the colder and moister site after soil transfer.

However, not only the microbial activity and community structure is influenced by environmental parameters, as it was found in the Morteratsch glacier forefield that the weathering rate between the north-facing sites differed significantly from the south-facing sites. The north-facing sites showed distinctively higher weathering rates and the soil moisture was found to play an important role in mineral weathering there (Egli et al., 2012). The microclimatic changes in sites close to each other (but with a differing exposition to the sun) are an important topic during this thesis, and this finding

emphasizes the high importance of exposition for soil developmental aspects, not only from a microbial, but also from a geological and mineralogical point of view.

A recently emerging topic in soil microbiology is the self-organization of soil microbes. There it is proposed that the structure of a soil determines the environmental conditions in which the soil microbes live in, but in turn the intrinsic soil microbes also affect soil structure through their activity (Young & Crawford, 2004; Crawford et al., 2012). It is clear that the parameters I measured like enzymatic and microbial activities do depend on the soil structure, but the enzymes and metabolites do contribute to the formation and stabilization of aggregates in soil, which in turn changes the soil structure. Therefore, microbes can cause a soil structure change but it is not yet clear if this change has an effect on microbial activity (Crawford et al., 2012). As a consequence for my experiments, it might be that the changes I observed in microbial activity might not only be due to a change in the soil properties, but also due to a change in the microbe-soil interactions after the observed community structure change.

I can conclude that, as expected, multiple factors like C and N content, plant cover, pH, temperature and soil moisture influence the microbial community structures and activities on a glacier forefield. Therefore the utmost care has to be taken when selecting experimental sites to investigate microbial communities, especially in glacier forefields, where the exposition and the dynamic nature of glacier advancing and retreating can result in a large heterogeneity. This heterogeneity can result in a differing microclimate and differing soil properties in sites close to each other. The soil properties and environmental parameters should therefore always be included in the interpretation of microbial community structure and activity data. One way to diminish the impact of heterogeneity between sites in the field is to sample at as many different sites as possible, therefore creating a large dataset which represents the whole sampling area (Andrén et al., 2008; Fuhrmann, 2009; Brankatschk, 2012). Furthermore, the sampling procedure and the experimental design should be fitted to the research questions, to ensure a thorough statistical analysis (Andrén et al., 2008; Brankatschk, 2012).

6.2 Carbon Balance in Recently Deglaciated Soil

By supplying bare soil with ^{13}C-labeled fungal (*Penicillium* sp.) or algal (*Chlorella* sp.) biomass, I was able to show, that carbon derived from microbial biomass can be metabolized by heterotrophic organisms in bare glacier forefield soil (Zumsteg et al., 2012 b) (Chapter 5). Therefore, autotrophic or heterotrophic organisms living in bare soil can become a carbon source for other microbes after their death. Freeman et al. (2009) quantified the contribution of autotrophic organisms to the C input in bare soil (23 gC m^{-2} y^{-1}), but the heterotrophic contribution has not been quantified so far. Looking at the C quantification made in the Damma glacier forefield, the soil respiration (8 – 10 gC m^{-2} y^{-1}) (Gülland et al., 2012 b) in bare soil already exceeds the annual C deposition (0.5 – 4 gC m^{-2} y^{-1}) (Pogodina, 2003; Brankatschk et al., 2011). Gülland et al. (2012 a; 2012 b) explained this discrepancy by the fact that a large part of the soil respiration originated from the decomposition of old carbon. However, to actually reach the proposed C accumulation rate of 100 gC m^{-2} y^{-1} (Smittenberg et al., 2012) (Figure 9), approximately 105 gC has to be additionally brought into the bare glacier forefield soil. I did for instance find that only 20% of the added C was respired as CO_2 after 60 days, the rest supposedly remained in the soil, increasing the soil organic matter content.

Therefore, I could show the important role of algae as well as fungi in increasing the available carbon pool in recently deglaciated bare soil. A large part could be deposited on the forefield by avalanches or by animal detritus. However, in a study on avalanches going down on the forefield it was shown that most of the young soil sites do not receive many avalanches (Tobias Jonas, WSL, personal communication) (Figure 10). Thus the carbon accumulation has to come mostly from primary production, as already tentatively suggested by Smittenberg et al. (2012). Thus exceeding the values calculated by Freeman et al. (2009) four fold. All these calculations show that algal and fungal biomass can both indeed serve as carbon sources in a glacier chronosequence, either reaching the forefield by deposition, wind, avalanches or through primary production.

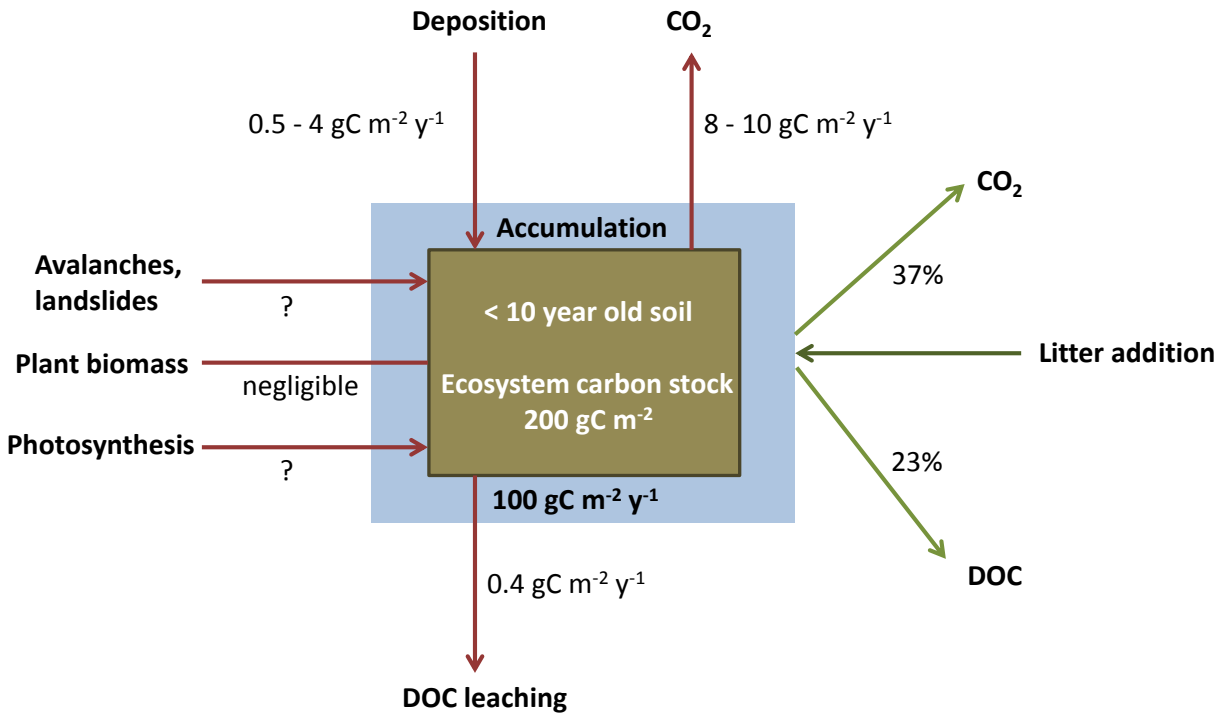

Figure 9: Carbon pools and fluxes in the bare soil of the Damma glacier forefield, according Pogodina (2003), Brankatschk et al. (2011), Smittenberg et al. (2012) and Gülland et al. (2012 a; 2012 b) as already shown in Figure 7 (a) in the introduction.

Interestingly, the bacterial community structure after fungal biomass addition was different from the bacterial community structure after algal biomass addition. Therefore, in addition to soil parameters like plant cover, C and N content and pH, the origin of the carbon source also affected the microbial community structures.

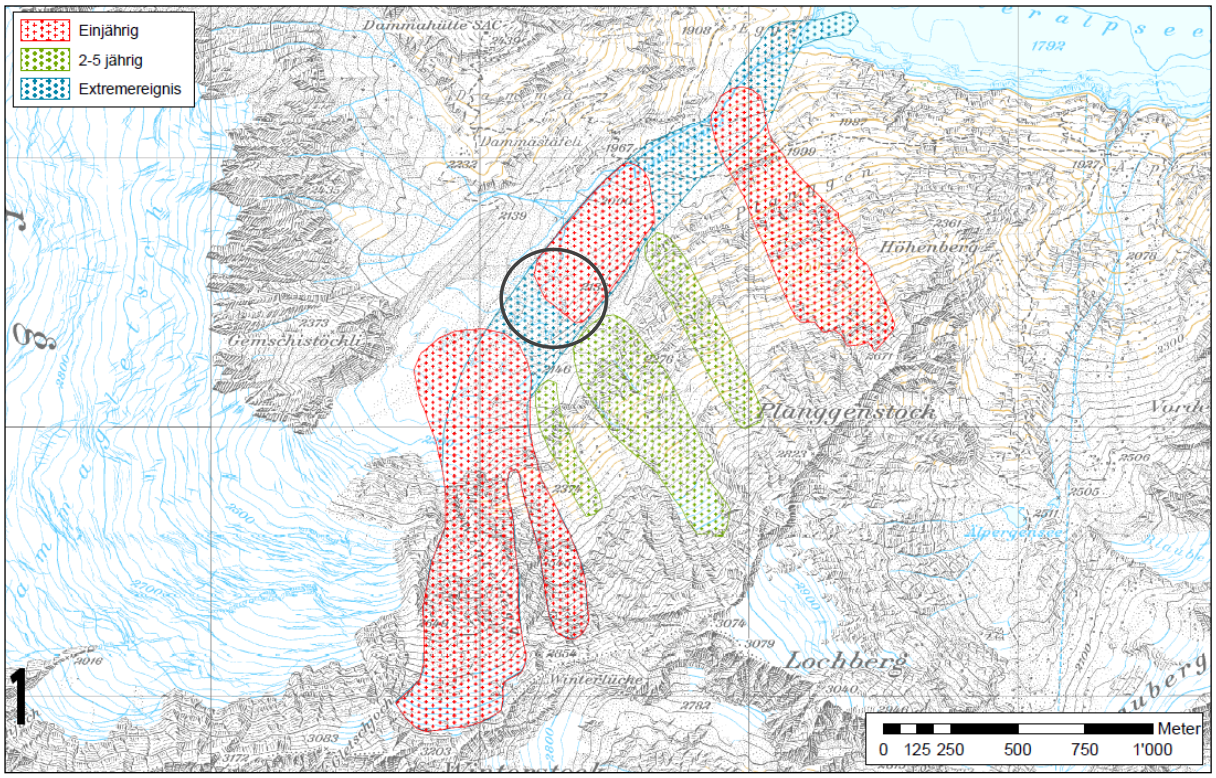

Figure 10: Possibility for avalanches in the Damma glacier forefield and surroundings (Tobias Jonas, personal communication). The circle marks the area of the young soil sites where most of my thesis experimental plots were located. The avalanche regions marked in red are yearly occurrences, green are 2 – 5 year occurrences and in blue are the very rare avalanche occurrences.

The rate that SOM decomposes in soil is dependent on soil temperature, soil moisture, nutrient availability, pH and on the chemical composition of the SOM (Eskelinen et al., 2009). It is generally accepted that with increasing temperature the decomposition rate of SOM increases, which is consistent with my findings. However, as shown in the transfer experiment (Zumsteg et al., 2011; Zumsteg et al., 2013) (Chapters 3 and 4), the substrate availability decreases in the long run at a warmer site, as the easily degradable carbon is decomposed and the more recalcitrant carbon remains. Therefore, substrate availability is not only dependent on the temperature at a given moment, but also on the temperature history at a specific site. Soil moisture is of course always linked with temperature and as microbes use extracellular enzymes to mediate the degradation, transformation and mineralization of soil organic matter (Sinsabaugh, 2010), a high soil moisture can be advantageous for the distribution of such enzymes in the soil. Therefore, for a complete

decomposition of SOM, a warm site with a high soil moisture is needed, like for instance the north-facing soil transferred to the south-facing site in my transfer experiment (Zumsteg et al. 2011; Zumsteg et al. 2013) (Chapters 3 and 4).

Surprisingly it was found that SOM mineralization is not dependent of microbial biomass size, community structure or specific activity (Kemmitta et al., 2008). Therefore, my observed difference in the bacterial community structure between the samples amended with differing carbon sources did not have an influence on the decomposition rate, only the SOM itself, meaning the availability and therefore its quality, is influencing the decomposition rate. This is consistent with my findings, where the more easily degradable algal biomass induced a higher respiration rate than the fungal biomass (Zumsteg et al., 2012b) (Chapter 5). In soil it is generally thought that the mass of dead bacterial cells is 40 times higher than the living biomass and that up to 80% of SOM is originally derived from microbial biomass (Liang & Balser, 2011). This microbial biomass is then distributed between mineralization, incorporation into other microorganisms and stabilization in SOM (Miltner et al., 2012). That such a large part of the SOM is from microbial origin supports my view that microbial cells can indeed be an important C source, also in a glacier forefield ecosystem, where up to 100% of the SOM might be of microbial origin.

6.3 Microbial Community Composition

Not only it is important to know the influencing factors for the microbial community structures, but also to know the microbial community composition and how certain phylogenetic groups react to a change in soil properties or the microclimate. To visualize the results obtained through the three studies performed, the reactions of specific phylogenetic groups to the changes in soil properties or the microclimate are combined below.

The colonization of bare soil by microorganisms at glacier forefields has previously been described (Sigler et al., 2002 a; Jumpponen, 2003; Nemergut et al., 2007). And also on the Damma glacier forefield, the bacterial, archaeal and fungal communities showed a succession along the chronosequence (Figure 11).

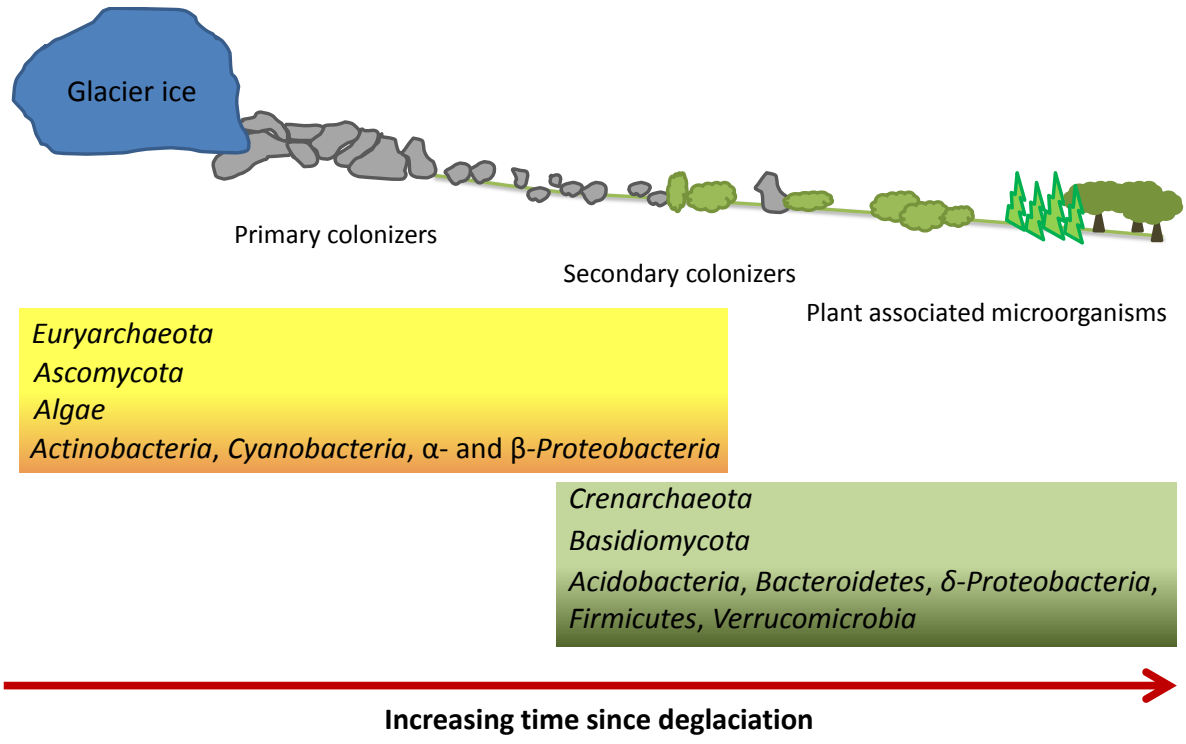

Figure 11: Microbial succession along the glacier forefield. *Cyanobacteria*, *Proteobacteria*, algae, *Euryarchaeota* and *Ascomycota* are colonizing the younger soil ages whereas *Crenarchaeota*, *Basidiomycota*, *Acidobacteria*, *Bacteroidetes* and *Firmicutes* are colonizing the older soil ages.

The most frequently found clone sequences I detected to be dominant in the soil samples from the Damma glacier forefield during all my studies belonged to the bacterial class *Proteobacteria*. *Proteobacteria* are phenotypically very versatile and consist of numerous phototrophs, photoheterotrophs and chemolithotrophs (Kersters et al., 2006). *Proteobacteria* are divided into four subclasses, the *alpha-*, *beta-*, *gamma-* and *delta-Proteobacteria*, all of which were found in the Damma glacier forefield. *Alpha-* and *beta-Proteobacteria* abundance decreased with rising soil age, they were resistant to soil transfer along a microclimatic gradient and were able to metabolize algal and fungal derived biomass (Zumsteg et al., 2011; Zumsteg et al., 2012 a; Zumsteg et al., 2013) (Chapters 2, 4 and 5). *Alpha-* as well as *beta-Proteobacteria* are known to be abundant in bare glacier forefield soil (Sattin et al., 2009; Schloss et al., 2010; Zumsteg et al., 2012 a). However, mainly the *beta-Proteobacteria* were shown to belong to the main decomposers of organic material in soil (Bernard et al., 2007), to be active in granite weathering (Frey et al., 2010; Lapanje et al., 2011) and to utilize differing C sources (Simon et al., 2009) which is in accordance to my findings. The two other *Proteobacteria* subclasses *gamma-* and *delta-Proteobacteria* were less abundant along the chronosequence. The g*amma-Proteobacteria* belong to the main decomposers of plant residues and soil organic matter (Bernard et al., 2007; Lee, C. G. et al., 2011), consistent to my findings that they did decompose fungal- and algal- derived biomass.

The bacterial class *Bacteroidetes* was the main metabolizers of fungal and algal derived biomass added to young soil (Zumsteg et al., 2013) (Chapter 5). Indeed *Bacteroidetes* were found to be one of the main organisms for the sequestration and mineralization of biomass in soil (Lueders et al., 2006) mainly of labile C sources (Padmanabhan et al., 2003) , and their abundance was found to correlate to soil C (Fierer et al., 2007). I especially found the genus *Flavobacterium* to be involved in the degradation of fungal derived C (Zumsteg et al., 2012 b) (Chapter 5). *Flavobacteria* sp. are known to be able to degrade chitin in soil (Manucharova, 2009), which is the main component of fungal cell walls, especially of the fungal genus *Penicillium*, which I used here as a C source (Andriyanova et al., 2011). Furthermore, they are adapted to cold environments like the Himalayas (Gangwar et al.,

2011). In addition, it was observed that *Flavobacteria* sp. are more important in carbon transfer in general than one would guess by their numbers, as even a small number of *Flavobacteria* sp. can show a high activity (Zeder et al., 2009). *Flavobacteria* sp. are also known to increase in their abundance with increasing substrate availability in marine systems (Alonso & Pernthaler, 2006) and it was shown that they are particularly successful if an excess of substrate is present. The excess substrate which I added to the glacier forefield soil could therefore, have triggered an enhanced growth of *Flavobacteria* sp. compared to other phylogenetic groups.

I found the photoautotrophic *Cyanobacteria* mainly in the young soils of the Damma glacier forefield (Zumsteg et al., 2012 a) (chapter 2). This is in accordance to other studies showing that *Cyanobacteria* can survive on bare rocks as first colonizers (Brinkmann et al., 2007; Fermani et al., 2007), either as single cells or as lichens in a symbiotic relationship with fungi. Their ability to survive in bare soil at high altitudes is partly due to the production of protective compounds against UV light (Vincent et al., 2004). Therefore, they are protected against the higher UV radiation in the recently deglaciated terrain near the Damma glacier terminus, where no vegetation dampens the radiation.

Actinobacteria was another bacterial class found to be mainly colonizing the young soil in the Damma glacier forefield (Zumsteg et al., 2012 a) (chapter 2). As heterotrophic organisms, they could be active in the decomposition of organic materials like pollen, spores or dead microbes in the bare soil. I could show is that they are sensitive to changes in the microclimate (Zumsteg et al., 2013) (chapter 4), which is in accordance to an observed sensitivity to seasonal changes (Lazzaro et al., 2012).

Sequence analysis of archaeal clone libraries showed a shift from a *Euryarchaeota*-dominated archaeal community in the young soil to a *Crenarchaeota*-dominated archaeal community in the older soil (Zumsteg et al., 2012 a) (chapter 2). Archaea in general are known to be influenced mainly by soil properties and not by plant cover (Zinger et al., 2011). In the Damma glacier forefield it seems that the *Euryarchaeota* might outcompete the *Crenarchaeota* in the younger soil ages with low nutrient content, whereas in the older soil ages the *Crenarchaeota* outcompete the *Euryarchaeota*.

In permafrost regions, the active layer is primarily colonized by *Crenarchaeota*, whereas the permafrost soil is colonized by *Euryarchaeota* (Steven et al., 2008), which seems to reflect my findings, where the *Euryarchaeota* colonize the harsh environment (low nutrient content, high temperature fluctuation, low soil moisture content) and the *Crenarchaeota* the more moderate (plant covered soil with higher nutrient content, lower temperature fluctuations and higher soil moisture contents). The diversity of archaea was decreasing along the chronosequence, which is consistent with studies showing that archaea diversity is generally low compared to bacterial and fungal diversity in soil and that archaea diversity results from a large number of closely related phylotypes rather than from diverse lineages (Auguet et al., 2010).

Interestingly, it was found that the *Euryarchaeota* living in permafrost soil or in glacier ice produce methane (Tung et al., 2005; Wagner et al., 2007), and major groups of methane oxidizers also belong to the *Euryarchaeota* (Schleper et al., 2005). Methane concentrations in glacier forefield soils in Switzerland indeed proved to be in a detectable range (Nauer et al., 2012) which supports the finding of the *Euryarchaeota* colonizing the younger soil. With increasing plant cover, these *Euryarchaeota* could then be outcompeted by *Crenarchaeota*, as they are often found in plant rhizosphere soils (Timonen & Bomberg, 2009), where they were proposed to play a role in the nitrogen cycle, as they are important ammonia oxidizers in soil (Schleper & Nicol, 2010). Ammonia oxidation is an important part of the nitrogen cycle, it determines the balance between reduced and oxidized forms of nitrogen and it can result in leaching of nitrogen from soil (Wessen et al., 2010). It was postulated that non-thermophilic *Crenarchaeota* use ammonia as their primary energy source and that at least some groups could be chemolithoautotrophic ammonia oxidizers (nitrifiers) (Schleper et al., 2005). Recently the mesophilic archaea, to which also the ammonia oxidizers belong, have been placed in a separate archaea group, the *Thaumarchaeota,* which is phylogenetically as well as genetically diverse from both *Crenarchaeota* and *Euryarchaeota* (Gupta & Shami, 2011). The Crenarchaeal groups I found in the Damma glacier forefield were the *Crenarchaeota* group 1.1a, 1.1b and 1.1c, of which 1.1a and 1.1b are typical soil archaea found around the globe and are thought to be important

nitrifiers (Auguet et al., 2010), whereas the *Crenarchaeota* group I is more generally thought of being ammonia oxidizers (Stahl & de la Torre, 2012). This would make the *Crenarchaeota* important contributors in the nitrogen cycle in general and in the case of the Damma glacier forefield to important colonizers in the more developed soil. Therefore, besides a shift in the archaeal community composition, a functional shift in the importance of archaea in these early ecosystems' nutrient cycles might also take place along the chronosequence. There the methanogenic *Euryarchaeota* community, which might play an important role in the carbon cycle, is replaced by the ammonia oxidizing *Crenarchaeota*, which are important players in the nitrogen cycle.

An interesting question is from where these archaea originate. Bacteria and archaea were both found to survive bellow glaciers and in glacier ice (Priscu et al., 1999; Stibal et al., 2012). Archaea were also found to be present in alpine lakes and streams and permafrost soil (Pernthaler et al., 1998; Wagner et al., 2007). The ice itself and the glacial stream are therefore probable sources of the archaea I found in the Damma glacier forefield, in addition to Aeolian deposition and the carry-in by animals and humans. As seen above, their functions in these ecosystems are under investigation. However, it is estimated that 25% of the methane in permafrost soils is originating from archaea. It has also become clear that the cold adapted archaea contribute to the global organic and inorganic carbon and nitrogen cycles (Cavicchioli, 2006), but it is not known to what extent.

Most of the fungal phylotypes I found to react to soil transfer along a microclimatic gradient in the Damma glacier forefield were affiliated to *Ascomycota* (Zumsteg et al., 2013) (Chapter 4). Members of this group were found to be active in weathering in the Damma glacier forefield (Brunner et al., 2011) and outnumber the *Basidiomycota* in recently deglaciated soil (Zumsteg et al., 2012 a) (chapter 2). The reason for this is the absence of plants. It was for instance shown that ectomycorrhizal propagules were scarce in bare soil close to the glacier terminus but increased with time since deglaciation occurred as soon as the first host plants were present (Jumpponen et al., 2002). In the transfer experiment (Chapters 3 and 4) the fungal clones closely related to *Hymenoscyphus ericae* reacted sensitively to transfer. The genus of *Hymenoscyphus* belongs to the mycorrhiza forming fungi

(Vralstad et al., 2002), therefore the transfer could have affected the fungus' access to his host plants and therefore we observed a decrease of this genus' abundance after transfer in general. Two clones related to the *Pezizomycotina* (*Helicoon fuscosporum* and *Lasiobolidium orbiculoides*) reacted sensitively to a transfer from a hot and dry environment to a colder and more humid soil. Both are involved in litter decomposition of wood and plant litter (Moustafa & Sharkas, 1982; Goos, 1987), which is difficult to explain as both sites should contain a similar amount of degradable litter. The clones closely related to *Nidula niveotomentosa* reacted sensitively to the opposite direction (cold and humid to hot and dry) but with resistance to the transfer from hot and dry to cold and humid. This is surprising as *Nidula* sp. often contain UV-A light-induced stress-related proteins, including catalases and heat-shock proteins (Taupp et al., 2008) which should enable them to withstand the higher radiation at the hot and dry site. However, it was also found that growth-related enzymes were up-regulated as a response to irradiation with UV-A, which could have inhibited their growth after the transfer (Taupp et al., 2008).

Generally, the fungal community changed from *Ascomycota* which are able to live on rocks (Gleeson et al., 2005) or as free living dark septate endophytes like some *Dothidiomycetes*, to the *Basidiomycota*, which are often associated with plants, along the forefield chronosequence (Zumsteg et al., 2012 a) (chapter 2). These findings corroborate those of Jumpponen (2003) on the Lyman glacier forefield. The fungal community structure in bare soil was furthermore negatively correlated to the C and N content in the soil and to plant cover, whereas the fungal community structures in old soils correlated positively with these parameters (Zumsteg et al., 2012 a) (chapter 2) which supports this hypothesis of fungal succession from *Ascomycota* to *Basidiomycota*. When there is no plant litter available and no mycorrhizal plants are present like at many sites in the bare glacier forefield soil, the fungi can survive by weathering of granite (Brunner et al., 2011) or by degrading other organic compounds, like for instance chitin, which is a major component of the fungal cell walls and in the outer shell of arthropods (Setälä & McLean, 2004).

Interestingly, the fungal sequences found in the transfer experiment (Zumsteg, et al. 2013) (Chapter 4) were close relatives of the clones obtained during the successional study performed before (Zumsteg, et al. 2012 a) (Chapter 2). This indicates that the most dominant fungal phylotypes found are homogeneously distributed over the recently deglaciated terrain and therefore found specific niches where they are adapted to.

To identify the diversity and function of the microbial population one could also identify active and functional genes and perform T-RFLP analysis and clone library construction on these specific genes. This molecular analysis combined with for instance stable isotope probing could then identify the function of specific species (Wellington et al., 2003). Additionally in the future, with the new pyrosequencing tools, one will be able to sequence basically every DNA present in the soil, leading to a coverage close to 100% and eliminating the bias introduced by cloning individual DNA molecules (DeLong, 2009). But generally, one major problem with investigating environmental DNA remains, as normally also non-active microbial DNA is extracted and therefore analyzed. The subsequent diversity therefore includes non-active species. A novel approach to overcome this problem is to first extract rRNA (phylogenetic marker) and mRNA (is needed for functional genes) and then perform a reverse transcriptase reaction to obtain cDNA, which can then be used for pyrosequencing (DeLong, 2009). This can give the environmental phyla actually active in a given environment, which in turn can also give information about the function of the species found (mRNA), which was not possible before.

6.4 Evaluation of the Methods used

In the ETH project BigLink within CESS, a continuous sampling approach of 24 sites was chosen to cover the whole forefield chronosequence from recently deglaciated to developed soils, which I also used in chapter 2 to assess microbial succession (Bernasconi & BigLink, 2008). This approach was certainly useful to assess the overall carbon budget along the forefield (Smittenberg et al., 2012) together with other soil parameters that were investigated (Bernasconi et al., 2011). A large drawback of this approach is however, that spatial heterogeneity between sites is neglected, as no replicas are taken. In retrospect it would have therefore been better to sample three parallel transects along the forefield, to circumvent the later observed large heterogeneity between sites close to each other. As was seen later (chapters 3 – 5), microbial communities are largely affected by the microclimate, thus to sample parallel transect would have allowed to draw more accurate conclusions about microbial community succession along the chronosequence. Furthermore, the samples I took for the clone libraries were not replicate samples in the common term (Prosser, 2010), as I performed several different PCR reactions on the same samples, which came from several separate DNA extraction of the same soil. However, it can be expected that the community composition in the forefield is heterogeneous varying from one site to another, which was therefore not accounted for. One possibility to overcome this problem could have been to extract DNA from many different sites within similar soil ages (for instance from all the young sites) and then perform the PCR reaction on this DNA extract. This would have taken the heterogeneity into account.

Soil transfer, which was used in chapters 3 and 4, is a powerful tool to investigate the reactions of microbial communities to changing environmental parameters (Bottomley et al., 2006; Boyle et al., 2006; Lazzaro et al., 2011). Microclimatic differences between sites can especially be investigated and monitored in the same soil environment. Overall I could gain satisfying results about the microbial adaptation and reaction to a changing microclimate in non-vegetated soil. The only restriction was the time span that could be investigated during my PhD, which might have been not long enough to let the system react adequately to the change. Therefore, I now observed short- to

intermediate- term effects (few days to 1.6 years). It is of course not known if the long term observations of more than 3 years would have rendered differing results.

Generally, the methods used to investigate the microbial community structure and composition (T-RFLP, cloning and sequencing) proved to be useful tools but they also had limitations. While T-RFLP is certainly well suited to assess differences in community composition between different samples, T-RFLP data often provides wrong estimates of diversity in microbial communities (Blackwood et al., 2007). This is due to the fact that several taxa generate the same terminal restriction fragment (T-RF) and that rare T-RFs are often excluded, due to their low abundance (Blackwood et al., 2007). Another drawback of the T-RFLP methods is that through DNA extraction (which is never 100% efficient) and the fact that one primer pair combination does miss up to 50% of all sequences present in a DNA pool (Hong et al., 2009), one does automatically underestimate the diversity in a given sample. The opposite problem (overestimating of diversity) arises when so called pseudo-T-RFs are formed. These are peaks that appear in the T-RFLP analysis but are not actually a restriction site when compared to the sequence (Egert & Friedrich, 2003). It was suggested that T-RFLP derived diversity indices should be interpreted as a mere reflection of community composition, and not as true community diversity (Blackwood et al., 2007).

A drawback of methods using PCR in general is that rare phyla are often under-represented (Pedros-Alio, 2006). Additionally, the construction of clone libraries is cost and time intensive, which inevitably leads to insufficient sequencing of clones, leading to a low coverage (coverage $C = 1 - (n N^{-1}) \times 100$; where n is the number of unique clones and N is the total number of clones examined (Ravenschlag et al., 1999)). For instance it was found that several hundreds of sequences have to be analyzed to obtain a reasonable coverage in soil (Prosser, 2002). However, in rather low diversity ecosystems like Antarctica, already 200 sequenced bacterial clones can lead to a coverage between 50% and 85% (Aislabie et al., 2008).

The construction of clone libraries is time consuming and cost intensive, therefore only around 500 clones were sequenced in total which led to a rather low coverage of the clone libraries and therefore to a limited overview of the species involved in the three studies. The number of different genomes in one gram of soil can range from 2'000 to 18'000 genes (Dunbar et al., 2002), illustrating that in a clone library comprising 300 clones most of the species will be represented by only one or two clones, therefore indicating a high probability for a sampling error (Dunbar et al., 2002). One way to avoid such a large error can be to use microarrays, which reveal greater diversity in environmental samples, allow to analyze the samples rapidly and allow replications (DeSantis et al., 2007). Another emerging way to describe microbial diversity in general today is pyrosequencing. There, large amounts (billions) of genes are sequenced, creating huge datasets of the soil genome. Of course this technique is cost intensive and relies largely on bioinformatics tools as well as sufficient pure DNA samples. However, it can give information about the microbial community structure and compositions as well as on functions of diverse microbial communities in a certain environment (Kakirde et al., 2010). The pyrosequencing analysis would be even more efficient when extracting mRNA from soil, performing a reverse transcriptase reaction to produce cDNA and then do the pyrosequencing reactions (DeLong, 2009). Thus one would know all the microbial species present in the soil which are actually alive and active, as mRNA is quickly degraded after the death of a cell or is only produced in low quantities if a cell is not active.

Especially in the case of the fungal 18S rRNA clone libraries, it has to be taken into account, that there is a potential bias involved, as the primers are not always specific for fungi (Anderson et al., 2003). This was reflected in my analysis, where I found several sequences belonging to the *Eimeriidae* (less than 5% of the sequences), which belong to the phylum *Apicomplexa* and are often parasites of diverse animals (Duszynski & Upton, 2001). Additionally, I found that the NCBI database does contain much more sequences retrieved from fungal ITS sequences than of 18S rRNA sequences, which often resulted in a bad similarity index of my sequences. This is consistent with findings that performing T-RFLP and constructing clone libraries on the fungal ITS region does lead to a better assessment of

species diversity and identity than the fungal 18S region (Lord et al., 2002). Therefore, one can assume that there are limited numbers of 18S sequences generated in general and therefore there is a limited amount of sequences available for 18S. Furthermore, it has to be taken into account that it is still not possible to assign species names to specific sequences, as the clone sequences are never 100% identical to other sequences when blasted in NCBI. Therefore, only the class names can be taken as a certain assignment to a specific phylogenetic group, but not the species names. Therefore, one can only discuss the results on the class level, where, especially for microbes, it is difficult to assign specific functions.

SIP is recently becoming very popular in microbial ecology, as it allow to link community structure to function (Dumont & Murrell, 2005; Whitby et al., 2005; Kreuzer-Martin, 2007). Nevertheless there are several issues to be considered when using the SIP technique. One important limitation of DNA-SIP is the need for DNA synthesis and cell division at the conditions investigated to obtain incorporation of sufficient label into DNA (20% incorporation of a ^{13}C labeled substrate into DNA) for gradient separation (Whitby et al., 2005; Neufeld et al., 2007 b). Therefore, it is important that the substrate is mainly used for growth (incorporated into DNA), otherwise the label would become too diluted to detect (Wellington et al., 2003). In addition to produce detectable levels of material, it may be necessary to add excess labeled substrate. This can bias the results for example by promoting growth of certain particular organisms, therefore falsifying the results on the abundances of certain phylogenetic groups.

Besides this, I observed that especially after longer incubation with a labeled substrate, it was not possible anymore to clearly separate the “heavy” ^{13}C-DNA from the “light” ^{12}C-DNA (Zumsteg et al., 2012 b). This is due to cross-feeding of ^{13}C labeled material, thus secondary utilizers feeding on metabolites of the primary incorporators of the ^{13}C labeled biomass or on the primary utilizers themselves (Dumont et al., 2011). As those metabolites or cells are not as strongly labeled as the added substrate (which was up to 100% labeled), the secondary consumers will therefore be less labeled than the primary consumers. Therefore cross-feeding leads to high DNA copy numbers in all

the fractions after density gradient centrifugation, as the DNA of the secondary utilizers is heavier than ^{12}C DNA but lighter than ^{13}C DNA. I had to therefore focus on the organisms incorporating the added substrate quickly (4 to 8 days), and could not investigate the slow growing communities incorporating the added substrate later. Additionally, cross-feeding can falsify results when such secondary utilizers are wrongly classified as primary utilizers of a substrate. On the other hand cross-feeding can be used to investigate food-webs (Lueders et al., 2006; Ruf et al., 2006; Glaubitz et al., 2009; Esperschütz et al., 2011 a). Additionally, we could only observe the taxa directly incorporating the added substrate into their DNA, but might miss out the species which incorporate the substrate into lipids, and not into the DNA (Nelson & Carlson, 2012). This problem could be overcome by performing a ^{13}C isotopic analysis of the extracted PLFA in parallel to DNA-SIP.

Another concern involving the SIP technique is usually the need of higher substrate concentration than it would be the case in the environment, in order to gain a sufficiently high labeling of the DNA. This elevated substrate amounts could lead to different uptake dynamics from the natural environment (Nelson & Carlson, 2012).

6.5 Conclusions

In this thesis I investigated the microbial community structure and composition as well as microbial activities along a successional gradient in the Damma glacier forefield and their reaction to changes in several soil and environmental parameters.

The succession of microorganisms along a glacier chronosequence is driven by the physical and chemical environment, but also by the availability of organic and anorganic nutrient sources and the interactions with other species. In contrast, to what extent microbial communities can adapt to a new environment is largely determined by intrinsic soil parameters like soil moisture. In my thesis I could show that the soil moisture and the nutrient availability (C and N) seemed to be the main limiting factors for the microbial activities. The bacterial and fungal community structures, on the other hand, were strongly influenced by temperature and soil moisture content, but also by the extent of the microclimatic change, e.g. large temperature fluctuations. However, this can also be seen from another perspective: whilst microclimate and physical soil structure determine the environmental conditions for the soil microorganisms, microbes themselves act as ecosystem engineers, changing soil structure and chemistry, which is altering their habitat. This will then enhance survival and extinction of certain phylogenetic groups, which again greatly influence their surroundings. One can therefore not simply say the environmental parameters influenced the soil microbial community, but that the soil-microbe and plant-microbe interactions lead to the observed changes in microbial community structures and activities.

The bacteria found along the forefield did not differ from other soil environments; thus one can assume that there are specialist bacteria species for almost any soil type in any of the classes I found. Archaeal phylogenetic classes on the other hand, reacted more sensitively to changes in soil parameters, leading to a different species composition along the chronosequence. This implies that for diversity and species composition in general, one could be able to predict a certain archaeal community composition. In contrast, the most abundant bacterial groups are omnipresent, making

predictions more difficult. However, so far very little is known about archean diversity and function in soil and further investigations are needed before any clear predictions can be made.

The fungal phylogenetic groups changed along the Damma glacier chronosequence, mainly due to an increase in plant cover and the resulting increase of mycorrhizal plants. But the non-vegetated soils harbored similar fungal species, regardless of differences in environmental parameters. This is an indication that the initial fungal propagules are evenly distributed in the non-vegetated soils.

Conducting my studies in the non-vegetated soil enabled me to investigate the influence of few environmental parameters on the microbial community composition without the confounding effects of plants. Such studies can eventually lead towards a better understanding of the function and the response to a changing environment of the microbial populations in soil.

From an ecological perspective, my work shows that even non-vegetated sandy and stony soil harbors a vast diversity of microorganisms, which are able to adapt and survive in harsh environments. As in any ecosystem every organism is linked to other organisms in one way or the other, ensuring the function of the whole ecosystem. This fact is even more important in a glacier forefield, where the organisms strongly rely on the availability of nutrient and carbon sources, forming a complex network of interactions (together with mineral weathering, plant colonization and other factors), eventually leading to soil formation. This again highlights the importance of microbe-soil and microbe-environment interactions I investigated during this thesis, without which no soil formation would take place.

6.6 OUTLOOK

Thomas Rime is a new PhD student at WSL who will continue my work at the Damma glacier forefield. The main goals during his thesis will be the determination of the active microbial community, its utilization of carbon and its reaction to nutrient addition not only along the chronosequence but also in differing soil depths. In parallel, an investigation of the main sources of C input and their amounts will be done, by monitoring the C deposition in the forefield.

Some of the first main questions to be answered are if C utilization varies in soils of different development stages and soil horizons, how the different microbial communities at the different soil ages influence C use and if microbial evolution depends on the initial community structure, or if the C source is determining the direction of evolution. These questions will be determined using molecular approaches as T-RFLP profiling, DNA-SIP, quantitative PCR, PLFA analysis and pyrosequencing, together with microbial activity and enzyme measurements.

Furthermore a carbon budget will be done along the forefield by measuring the wet and dry deposition, the soil respiration, stream C content and estimating carbon turnover in three different soil ages in the Damma glacier forefield.

It would be very interesting to further investigate the archaeal communities along the glacier forefield, as archaea were mostly investigated in extreme and marine environment so far. Their reaction to changing environmental parameters and possible carbon sources could give insight into the function of soil archaea in recently deglaciated terrain.

Furthermore a comparison of the bare soil microbial communities between different glaciers around the world would be interesting. Manly to determine if the primary succession is similar around the world and if the main source for primary colonizers is the Aeolian deposition or the glacier melt-water, a question which is still not answered in general.

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9. CURRICULUM VITAE

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2012 – 2012	Research assistant at the institute for biogeochemistry and pollution dynamics, ETH Zürich, Switzerland
2008 – 2012	First research assistant then PhD student at the federal research institute for forest, snow and landscape research, WSL, Birmensdorf, Switzerland
2007 – 2008	Young graduate trainee at the European space agency ESA, Noordwijk, The Netherlands
2007	Trainee at the Baltic Sea research institute, Warnemünde, Germany
2001 – 2007	Master of science in biology, ETH Zürich, Switzerland
1998 - 2001	Gymnasium Thun Schadau, Thun, Switzerland
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9.2 PUBLICATIONS

2013

Anita Zumsteg, Erland Bååth, Beat Stierli, Josef Zeyer, Beat Frey; Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield, "*Soil Biology and Biochemistry*", 61 (2013) 121 - 132.

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2010

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Christina E. Reynolds-Henne, Anita Langenegger, Jan Mani, Nicole Schenk, **Anita Zumsteg**, Urs Feller; Interactions between temperature, drought and stomatal opening in legumes, *"Environmental and Experimental Botany"*, 68 (2010) 37–43

9.3 Conference Presentations

2012

Anita Zumsteg, Ivano Brunner, Josef Zeyer, Beat Frey. “Fungal community and activity shifts along soil age, temperature and soil moisture gradients in a glacier forefield”. (Oral presentation at the Zürich Mycology Symposium, Zürich, Switzerland)

Anita Zumsteg, Gerhard Furrer, Erland Bååth, Josef Zeyer, Beat Frey. „Reactions of microbial communities to soil transfer along a climatic gradient in the Damma glacier forefield (Switzerland)”. (Oral presentation at the Eurosoil conference, Bari, Italy)

2011

Anita Zumsteg, Josef Zeyer, Beat Frey. “Microbial succession along the Damma glacier forefield”. (Oral presentation at the Swiss Society of Microbial Ecology Meeting, Engelberg, Switzerland)

Anita Zumsteg, Stefano M. Bernasconi, Josef Zeyer, Beat Frey. “Microbial activity and community shifts after soil transplantation in a glacier forefield”. (Oral and poster presentation at the International Symposium on the Geochemistry of the Earth Surface, Boulder CO, USA)

Anita Zumsteg, Josef Zeyer, Beat Frey. “Reactions of microbial communities after the transplantation of soils along a climatic gradient in a glacier forefield”. (Oral presentation at the Polar and Alpine Microbiology Conference, Ljubljana, Slovenia)

Anita Zumsteg. “Measurements of bacterial and fungal activity in a glacier forefield soil”. (COST Short Term Scientific Mission to Prof. Dr. Erland Bååth at the University of Lund, Sweden, 5.-18. November 2011)

2010

Anita Zumsteg, Ivano Brunner, Jörg Luster, Hans Göransson, Rienk Smittenberg, Josef Zeyer, Beat Frey. “Bacterial, Archaeal and Fungal Community Succession along the Damma Glacier Forefield”. (Poster presentation at the Swiss Society for Microbiology Meeting, Zürich, Switzerland)

Anita Zumsteg, Jörg Luster, Hans Göransson, Rienk H. Smittenberg, Ivano Brunner, Stefano M. Bernasconi, Michael Plötze, Gerhard Furrer, Josef Zeyer and Beat Frey. “Bacterial, archaeal and fungal succession in the forefield of a receding glacier”. (Poster presentation at the CCES Latsis Symposium, Zürich, Switzerland)

Anita Zumsteg, Josef Zeyer, Beat Frey. “Microbial succession and adaptation along the Damma glacier forefield”. (Oral Presentation at the Meeting of Young Researchers in Earth Sciences, Cottbus, Germany)

2009

Anita Zumsteg, Ivano Brunner, Gerhard Furrer, Michael Plötze, Josef Zeyer, Beat Frey. “Fungal and bacterial community succession along the Damma glacier forefield“. (Poster presentation at the Goldschmidt Conference, Davos, Switzerland)