UNIVERSITY OF TURIN

DOCTORAL SCHOOL OF
SCIENCES AND INNOVATIVE
TECHNOLOGIES

PHD PROGRAM IN
AGRICULTURAL, FOREST AND FOOD SCIENCES

CYCLE: XXX

ECOLOGY AND FUNCTION OF MICROBIAL COMMUNITIES IN DIFFERENT ALPINE, PERIGLACIAL ECOSYSTEMS

Ilaria Mania

Advisor:
Roberta Gorra, Ph.D

Coordinator:
Prof. Aldo Ferrero

YEARS
2015, 2016, 2017

Table of contents

1. Introduction

1.1 The periglacial environment

1.1.1 General characteristics

The term periglacial was introduced for the first time by Lozinski at the beginning of the 20th century to describe the climatic and geomorphic conditions of areas peripheral to Pleistocene ice sheets and glaciers (Lozinski 1912). More recently (Washburn 1979, Harris et al. 1988) the definition has been extended to include all the conditions, processes and landforms associated with cold, non-glacial environments where geomorphic processes are dominated by frost action. Based on this wider definition, it is estimated that the so-called 'periglacial domain' represents approximately 25% of Earth's land surfaces, and it comprises both high-latitude and high elevation, mid- and low-latitude areas. Therefore, periglacial environments basically associate with five different climatic conditions (French 2007):

1. High Arctic climates - large seasonal but small daily temperature fluctuations; in polar latitudes (e.g. Canadian Arctic). The ice-free areas of the Antarctic continent show similar features, but are characterized by more intense cold, windiness and aridity.
2. Continental climates - extreme seasonal but small daily temperature fluctuations; in sub-arctic latitudes (e.g. Central Siberia, Interior Alaska).
3. Alpine climates - large seasonal and diurnal temperature fluctuations; in middle latitudes, mountain environments (e.g. European Alps, North American Rocky Mountains).
4. Quinghai-Xizang (Tibet) plateau - large seasonal and diurnal temperature fluctuations, above-normal insolation due to elevations of 4200-4800 m a.s.l.; low-latitude, high-elevation mountain environment.
5. Other climates - small seasonal and diurnal temperature fluctuations (e.g. islands in sub-arctic latitudes, high-latitude mountain environments).

Besides the occurrence of freeze-thaw oscillations (Tricart 1968), another central, but not defining element characterising the periglacial environment is the presence of permafrost (Péwé 1969). Müller et al. (1943) defined permafrost as "rock or soil material, with or without included moisture or organic matter, that has remained below 0° C continuously for two or more years". However, also in perennially frozen grounds, an active layer is normally present. This is a surface layer overlying the permafrost table that undergoes seasonal freeze-thaw events, whose thickness can vary from year to year and from place to place, spanning from a few centimetres up to several metres.

Permafrost distribution is controlled by several factors, from the climatic conditions to site-specific controls, such as parent material, topography, aspect, vegetation, snow cover and presence of water bodies (French 2007). In general, terrestrial permafrost can be found in high-latitude and high-altitude areas. The first is called latitudinal, or polar permafrost; the second, mountain permafrost. A particular case is represented by relict permafrost, that occurs in locations where current ground temperatures would not allow today permafrost formation, and thus reflects past climatic conditions. Another type of classification is based on the proportion of land surface that remains perennially frozen in a permafrost-affected area. In these terms permafrost is commonly classified into continuous (90-100%), discontinuous (50-90%), sporadic (10-50%) or isolated (0-10%).

The presence of permafrost leads to the formation of typical landforms that, due to the wide diffusion of permafrost in periglacial environments, often constitutes the periglacial landscape itself. Some of these landforms are mainly linked to permafrost aggradation processes, such as thermal-contraction-crack polygons, rock glaciers, frost mounds (e.g. palsas and pingos) and patterned ground; others, like ice wedges, thermokarst-derived relieves and depressions and taw lakes result from the thaw and erosion of ice-rich permafrost. In this dissertation, two typical periglacial features will be investigated: patterned grounds and rock glaciers.

1.1.2 High-altitude environments

High elevation areas represent an important fraction of periglacial environments worldwide: for instance, it has been estimated that about 72% of the permafrost in the boreal hemisphere occurs in mountains, uplands and plateaus (Brown et al. 1997). The expression *mountain permafrost* is used to indicate permafrost found in mountain areas, regardless their geographical position (Cheng and Dramis 1992). Indeed, despite alpine and polar mountain regions are characterised by different climatic conditions, the influence of mountain topography on permafrost properties in these areas is analogous. In particular, the main unifying element is the extreme spatial variability with respect to several surface and near-surface characteristics, such as elevation, slope, exposition, subsurface material thickness and composition. The heterogeneity of these properties is then connected to heterogeneity in surface micro-climatology, drainage, snow cover and vegetation type and density (French 2007).

The systematic study of permafrost in high mountain areas started in the late 1970s, although most of the scientific literature focusing on the topic has been concentrated in the last three decades, as a consequence of the growing attention to climate change issues and their effects on cold environments (Haeberli et al. 2010). Indeed, since permafrost sensitivity to changes in atmospheric temperature has been widely documented (Romanovsky et al. 2010, IPCC 2013), and clear evidences of air temperature rising have been reported in different high-altitude areas of the Northern Hemisphere (Harris et al. 2003, Li et al. 2008), a better understanding of climate change consequences on these environments has become necessary. Among the most concerning aspects potentially linked to alpine permafrost warming there are the increase of slope instability and natural hazard in mountain areas (Haeberli 1992, Harris et al. 2001a, Harris et al. 2009), the possible variations in water resources availability and quality (Viviroli et al. 2011, Liljedahl et al. 2016, Yang et al. 2017) and the effects on biogeochemistry and ecology of associated ecosystems. The next chapters will specifically focus on this last point.

1.2 Microbial ecology in permafrost and seasonally frozen soils

1.2.1 Frozen ground: a hostile environment?

Periglacial environments represent a potentially challenging habitat for microbial life under different aspects: the low temperatures that reduce enzymatic reaction rates and, especially at subzero conditions, limit water and nutrients availability; the freeze-thaw succession occurring in permafrost active layer and in seasonally frozen soils that can damage microbial cells and the levels of background radiation, particularly high in high-altitude areas. For these reasons, permafrost and frozen grounds have been considered for a long time simply as a reservoir of ancient forms of life surviving in a dormant or dead state. This assumption has firstly been overtaken by some pioneer studies demonstrating the possibility to isolate viable microorganisms from permafrost (James and Sutherland 1942; Boyd and Boyd 1964; Horowitz et al. 1972; Cameron and Morelli 1974), and by the development of new sampling techniques and methods to monitor exogenous microbiological contamination, that allowed to confirm the actual origin of the isolates (Gilichinsky et al. 1989, Christner et al. 2005, Juck et al. 2005). Further evidence of microbial growth and activity in cold ecosystems, even at subzero temperatures, has then been provided by several studies involving the application of RNA-based approaches as well as measures of soil carbon respiration, enzyme activity and isotopically labelled substrates (Nikrad et al. 2016).

Therefore, permafrost and frozen grounds are currently recognized as habitats colonised by abundant, diverse and, at least in part, active populations of microorganisms well adapted to live and thrive in such harsh conditions. Actually, microbial life at low temperatures required the evolution of a series of structural and functional adaptations that are common in many organisms living in cold environments. Among these:

- the ability to regulate the membrane fluidity (Russell 2008; Shivaji and Prakash 2010);

- the production of cold-adapted proteins/cold-active enzymes (Feller et al. 2003, Siddiqui et al. 2006);
- the presence of a cold-shock response, involving the production of cold-shock proteins (Phadtare 2004, Phadtare and Severinov 2010);
- the production and accumulation/secretion of osmoprotectants and antifreeze proteins (Sleator and Hill 2002, Christner 2010);
- the production of exopolymeric substances involved in cell protection, adhesion and biofilm formation, nutrients immobilization and mediation of biochemical interactions (Mancuso Nichols et al. 2005, Krembs and Deming 2008);
- the development of strategies to control oxidative processes, such as the production of antioxidant enzymes (e.g. catalase, superoxide dismutase) or the suppression of ROS-producing pathways (Médigue et al. 2005; Methé et al. 2005).

1.2.2 Abundance and diversity of permafrost microorganisms

Microbial cell counts have been assessed in different permafrost environments, by using different techniques (Steven et al. 2009). Culture-dependent techniques (e.g. plate count, MPN) show variable results, with cell counts spanning from 0 up to 10^9 cells g-1dw. Conversely, direct microscopy or flow cytometry counts tend to give more stable values, ranging from 10^7 to 10^9 cells g^{-1} dw, even in samples where no microorganisms have been detected with culturing techniques. Indeed, the proportion of recovered viable cells on total cells count is estimated to be around 0.1-10% in Arctic permafrost and 0.001-0.1% in Antarctic permafrost (Vorobyova et al. 1997), similar to the values reported for several other ecosystems (Rappé and Giovannoni 2003). Such a low recovery rate may be partially linked to the limits of standard culturing techniques in satisfying the growth requirements of a part of the microbial population, but also to the presence of large amounts of cells in viable but non-culturable state (described in detail in Oliver, 2005).

In terms of microbial community composition, cold soils have proved to contain a great variety of different microorganisms, including bacteria, archaea, green algae, fungi and protozoa (Margesin and Miteva 2011), although bacteria generally show the highest diversity (Jansson and Taş 2014). Data from 16S rRNA genes sequencing usually report the presence in perennial and seasonally frozen grounds of Proteobacteria (with a particularly relevant proportion of Gammaproteobacteria), Firmicutes, Chloroflexi, Acidobacteria, Actinobacteria and Bacteroidetes, as well as several uncharacterized and novel bacterial phyla (Jansson and Taş 2014, Frey et al. 2016). Archaeal sequences affiliated with Euryarchaeota, Thaumarchaeota and Crenarchaeota have also been detected (Jansson and Taş 2014), suggesting higher complexity in archaeal community than described in the first culture-dependent studies, mainly reporting methanogenic and halophilic isolates (Steven et al. 2009).

Focusing on eukaryotes, filamentous fungi and yeasts have been commonly found in cold ecosystems, with a total amount varying from less than 10 to almost 10^4 CFUs g^{-1} material (Ozerskaya et al. 2009). Despite the initial evidences of an absolute dominance of yeasts over mycelial organisms, only present as spores in permafrost samples (Vorobyova et al. 1997, Vorobyova et al. 2001), more recent studies confirmed the ability of both dimorphic yeasts (genera *Leucosporidium*) and filamentous fungi (genera *Geomyces*) to grow on solid culture media at below 0°C temperatures (Panikov and Sizova 2007). In terms of diversity, all the major fungal phyla have been detected in Arctic, Antarctic and alpine permafrost and tundra soils, including Zygomycota, Ascomycota, Basidiomycota and Chytridiomycota (Margesin and Miteva 2011, Frey et al. 2016), with *Geomyces*, *Cladosporium*, *Aspergillus* and *Penicillium* among the most common genera for mycelial fungi (Ozerskaya et al. 2009), *Cryptococcus* and *Rhodotorula* for yeasts (Buzzini and Margesin 2014).

Finally, viable green algae have been isolated from both Arctic deep sediments (Vorobyova et al. 1997) and Siberian permafrost samples (Vishnivetskaya 2009), supporting the hypothesis that these photoautotrophs not only can survive in a dormant state in permafrost, but are also readily reversible

to an active photosynthetic state when favourable conditions occur (Vishnivetskaya et al. 2003).

1.2.3 Importance of microorganisms in periglacial environments

As anticipated in section 1.2.1, a number of evidences confirm the presence of metabolically active microorganisms in permafrost and seasonally frozen soils. Recent studies based on the application of meta-omic techniques pointed out the presence of a wide metabolic potential in these ecosystems, including not only genes linked to cold adaptation and survival, but also genes involved in fermentation and respiration of several organic compounds, anaerobic respiration processes, nitrogen, sulphur and methane cycling pathways (Yergeau et al. 2010, Mackelprang et al. 2011, Fierer et al. 2012, Hultman et al. 2015, Mackelprang et al. 2016). Thus, microorganisms represent a key element to be investigated in order to better understand the functioning and the potential future evolution of permafrost and associated environments.

As for the present, recently deglaciated environments, often including soils at different degree of evolution, offer ideal locations to study the actual role of microbial communities in processes linked to soil formation and stabilisation, such as mineral weathering, establishment of initial C and N pools and early stages of plant colonisation (Schultz et al. 2013, Bradley et al. 2014). They give also the possibility to investigate the relationships existing between microbial populations and abiotic factors in a simplified context, if compared to more evoluted soils in temperate and tropical climates, intensively colonized by plants and macroorganisms, and exposed to stronger anthropogenic pressures (i.e. agriculture, industrial and residential land use). Moreover, due to its permanently frozen state, permafrost is also considered a suitable terrestrial analogue for the study of microbial life on cryogenic planets, including Mars (Cavicchioli 2002, Jakosky et al. 2003, Gilichinsky et al. 2007, Martins et al. 2017), besides being an important source of microorganisms and cold-adapted enzymes with enormous biotechnological potential (Margesin and Feller 2010, Cavicchioli et al. 2011).

Moving to the future, one of the main concerns linked to global warming is its effect on permafrost and the associated microbial communities. It has been estimated that the increase in global temperatures will lead to widespread thawing of permafrost and thickening of the seasonally active layer, resulting in an increase of both microbial metabolic activity and nutrients mobilisation; this would promote the microbial turnover of labile organic carbon, resulting in a positive feedback on greenhouse gases production (Schuur et al. 2009; Koven et al. 2011). Therefore, the inclusion of microbiological parameters into the models evaluating future scenarios of carbon turnover in high-latitude environments is one of the main challenges of climate change-focused research today (Hollesen et al. 2011; Graham et al. 2012, Ebrahimi et al. 2017). In addition, in mountain regions at lower latitudes, that are more densely populated than hig-latitude regions and represent important water reservoirs for large areas (Huss 2011), further hazards could derive from an increase in permafrost thawing from a biological point of view. Among these, a possible increase in the release of rock weathering products in headwaters (Ilyashuk et al. 2017), or the dispersal of biologic material previously stored in permafrost (Petrova et al. 2008, D'Costa et al. 2011, Legendre et al. 2014).

1.3 Aim of the work

As discussed above, one of the main characteristics of the alpine ecosystem is the extreme spatial variability directly linked to mountain topography. If, on the one hand, due to the different combinations of environmental drivers acting at different sites, this aspect considerably complicates the study of periglacial ecosystems functioning and evolution, on the other hand it offers a unique opportunity to test the combined effect of multiple abiotic factors on microbial community characteristics. Starting from such considerations, the aim of this work was to increase the current knowledge on microbial communities inhabiting periglacial alpine ecosystems and to offer new insights in the relationships existing between microbial diversity and distribution and environmental drivers. Three main research questions are addressed in this thesis:

1. Does the effect of repeated freeze and thaw cycles directly affect microbial community composition, diversity and abundance?
2. Which are the main environmental drivers involved in shaping microbial community in these ecosystems?
3. What is the hierarchical order of these environmental drivers? Does the same driver have the same importance in different ecosystems?

In order to answer these questions, our investigation targeted two different periglacial landforms: patterned ground (PG) and rock glaciers (RG). Chapter 2 focuses on the influence of the parent material lithology, as well as different levels of freeze-thaw intensity, on microbial community in different PG features. In chapter 3 the investigation is restricted to a single system with a higher level of complexity. The prokaryotic community associated to different habitats within a RG-pond system is compared in terms of abundance and diversity, and several geochemical properties potentially related to microbial distribution are observed.

For both the studies, a culture-independent approach based on total DNA directly extracted from the environmental matrixes has been applied in order to assess microbial community structure, diversity and microbial abundance. Since

DNA pools in soils and sediments are expected to be relatively stable (Agnelli et al. 2004, Borin et al. 2008, Pietramellara et al. 2009, Torti et al. 2015), the choice of DNA as molecular marker allows to give an overview of the microbial community potentially characterising the different sampling points and resulting from the long-term exposition to environmental pressures. This is coherent with the explorative nature of the studies collected in this thesis, representing preliminary investigations on the organization of microbial communities in poorly characterized systems.

2. Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)

Adapted from:

[1]Mania I, [1]D'Amico M, [1]Freppaz M, [1]Gorra R

Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)

Biology and Fertility of Soils (2016) 52:1135–1148.
doi: 10.1007/s00374-016-1147-z

[1] DISAFA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO) Italy

2.1 Abstract

Patterned ground (PG) is one of the most evident expressions of cryogenic processes affecting periglacial soils, where macroscopic, repeated variations in soil morphology seem to be associated with small-scale edaphic and vegetation gradients, potentially influencing also microbial communities. While for high latitude environments only few studies on PG microbiology are available, the alpine context, where PG features are rarer, is almost unexplored under this point of view.

We followed a double approach, based on Denaturing Gradient Gel Electrophoresis (DGGE) and quantitative PCR (qPCR), in order to investigate microbial community composition and abundance of phylogenetic markers and functional genes (bacterial and archaeal *amo*A) within single PG features and among different sites from four areas in the Western Italian Alps, characterized by different lithotypes.

Bacterial, archaeal and fungal community composition was quite homogeneous within single features, with more differences among samples collected from different lithologies. The abundance of phylogenetic and functional markers was uniform at different sites, except for the highest altitude one showing the lowest bacterial, archaeal and ammonia-oxidizing archaea abundance. Nevertheless, at a small-scale level, a concentric distribution of microbial markers was described within single features, paralleling soil chemical properties trends. These first results support the hypothesis that microbial ecology in alpine, periglacial ecosystems is driven by a complex series of environmental factors, such as lithology, altitude and cryogenic activity, acting simultaneously on community shaping both in terms of diversity and abundance.

2.2 Introduction

Patterned ground (PG) derives from cryogenic processes and represents one of the most spectacular expression in periglacial landscapes. Cyclic soil freezing and thawing, accompanied by ice lens formation, leads to severe ground modifications, resulting in surface geometric patterns including circles, polygons, networks or stripes. The presence of textural sorting, with stony areas alternated to soil and fine debris characterizes *sorted* patterned ground, while in *nonsorted* patterned ground single features are defined by differences in ground relief or vegetation cover. In both cases, two domains are often recognizable: central parts of finer material or bare ground, more strongly affected by cryogenic processes; and peripheral areas, richer in stones and/or vegetation (Ballantyne et al. 2013; Walker et al. 2008). These macroscopic, repeated variations in ground morphology produce also small-scale gradients in physical and chemical soil properties, changing between centres and rims (Barrett et al. 2004; D'Amico et al. 2015; Michaelson et al. 2012; Wagner et al. 2005). In this sense, single patterned ground features can be seen as small, ubiquitous model unities useful to investigate the effect of cryoturbation processes on soil evolution, plant colonization, and organic C accumulation and storage in geographically, climatically and topographically diverse environments. In fact, patterned ground formation is widespread in high-latitude environments, such as polar or sub-polar regions, but occurs also in alpine areas, in presence of permafrost or seasonal ground freezing conditions and favourable topographic conditions (Ballantyne et al. 2013).

Until now, several works recognized the presence of a strong interaction between soil processes and vegetation in patterned ground formation and functioning (D'Amico et al. 2015; Michaelson et al. 2012; Walker et al. 2008), but the effects on soil microbial properties are poorly known. Microorganisms are able to survive, grow and be metabolically active in very harsh conditions, such as subzero temperatures, presence of ice and freeze-thaw cycles (Margesin and Miteva 2011; Steven et al. 2006). Moreover, they play key roles in

weathering processes, pedogenesis, biogeochemical cycling and plant colonization of permafrost soils and recently deglaciated areas, like glacier forefields (Bajerski and Wagner 2013; Jansson and Taş 2014; Nemergut et al. 2006). Considering the impact of microbial communities on soil ecosystem properties, it is necessary to increase our comprehension of the role of microbial communities in a complex and dynamic pedo-environment like patterned ground.

To date, only a limited number of studies considered patterned grounds from a microbial point of view, all referring to Arctic or Antarctic areas. In particular, several studies focused on polygonal soils, due to their large diffusion in tundra ecosystems and to their potential role in global methane production linked to the waterlogged, anoxic conditions affecting these soils. Differences in terms of community composition were described along depth gradients (Frank-Fahle et al. 2014; Wagner et al. 2005), comparing central and marginal areas of single features (Wagner et al. 2005), and considering different polygonal soils (Frank-Fahle et al. 2014; Lawley et al. 2004). Another line of investigation concerned patterned grounds along the North American Arctic Transect. Timling et al. (2014) and González et al. (2014) compared microbial communities in terms of biomass and fungal community composition in patterned ground features (PGF) and adjacent vegetated soils (AVS) along a topographic and climatic gradient, detecting significant differences between PGF and AVS both in terms of microbial biomass and diversity, coherently with differential distribution of plant cover and soil properties already described (Walker et al. 2008, 2011). On a broader scale, they noticed that the hierarchy of environmental factors potentially involved in community shaping changes within the bioclimatic gradient. For instance, in more extreme environments, such as the higher latitude polar deserts, they found that disturbances linked to cryoturbation have smaller impacts on microbial biomass and community composition than at lower latitudes, resulting in limited differences between PGF and AVS. Conversely, topographic position, analysed by González et al. (2014) by comparing dry, wet and mesic zones, becomes a more important driver in warmer subzones.

While patterned ground landscapes are extensively distributed in Arctic and subarctic regions, in mid-latitude mountain ranges their diffusion is limited to few areas characterized by flat surfaces, rapid snow removal by wind and high water availability (Bockheim and Munroe 2014). For this reason, the identification of sites suitable for the study of the combined action of climatic conditions and cryoturbation disturbances on soil microbial communities is rather complex. On the other hand, the presence of the same phenomenon replicated in sites geographically close to each other gives the opportunity to investigate the influence of other environmental drivers potentially involved in communities and ecosystem modelling, like altitude, parent material lithology and soil properties. The parent material lithology, in particular, has a strong impact not only on the morphology of patterned ground features, due to the different resistance to ice-driven weathering, but also on soil chemical properties and plant colonization (Michaelson et al. 2008). This suggests that also the composition and distribution of microbial communities might be influenced by these properties, as already reported for other cold ecosystems (Boyd et al. 2007; Larouche et al. 2012; Nyyssönen et al. 2014; Reith et al. 2015).

With this study, we performed a preliminary investigation -the first, on our best knowledge- on microbial communities inhabiting patterned ground features in an alpine context, in terms of composition, overall diversity and abundance. We chose four active patterned ground sites in the North-Western Italian Alps, developed on different lithotypes creating large gradients in chemical soil properties such as available nutrients and heavy metal contents. Our hypothesis was that, as for chemical soil properties and plant distribution, cryoturbation should have an impact also on microbial population, both in terms of biomass distribution and community composition. The intensity of this influence should be modulated by site-specific edaphic properties linked to parent material lithology. This work had thus three main objectives: 1) to give a first insight in the microbial ecology of a fascinating and previously almost unexplored ecosystem; 2) to describe and compare microbial diversity and distribution both at a small-scale, within single PG features, and among different

sites; 3) to define soil properties potentially involved in shaping microbial communities composition.

2.3 Materials and methods

2.3.1 Samples collection

For this study, four active patterned ground areas, located in the Western Italian Alps and dominated by stripes, sorted and nonsorted circles, were chosen. All areas were located in protected areas (Mont Avic Natural Park and Gran Paradiso National Park). The different sites were characterized by different parent material (Table 2.1): calcschists (CS site), serpentinite and metamorphic gabbros respectively in SP and GB sites, and frost shattered gneiss at GN site. In CS and GB, the parent materials were enriched in small quantities of serpentinite derived from upslope areas. For each site, one typical PG feature was examined in order to minimize the sampling impact on these ecosystems. Five surface samples (0-10 cm) were collected equally spaced along a north-south transect drawn across the circle/stripe. Hence, we obtained two external samples, taken from the stony/vegetated rims (N and S), one central sample (C) and two intermediate samples (NC and SC), as shown in Fig. 2.1. A total of 20 samples was obtained.

Table 2.1 Localization and environmental properties of the study sites.

	Site localization	Elevation (m a.s.l.)	Parent material	PG type	Dimensions (m)
1 - (CS)	Fenêtre de Champorcher (Champorcher, AO)	2705	Calcschists (serpentinite in traces)	Nonsorted circles, hummocks	0.8/1.5
2 - (SP)	Colle di Raye Chevrère (Champdepraz, AO)	2710	Serpentinite	Sorted stripes	0.8/1.5-3/8
3 - (GB)	Lac des Heures (Champdepraz, AO)	2780	Gabbro (serpentinite in traces)	Sorted elongated circles	1.2/2.5
4 - (GN)	Piata Lazin (Ronco Canavese, TO)	3054	Gneiss	Sorted circles	0.8/2

Sampling took place in late September 2012. In that period, nighttime air temperatures were expected to drop below freezing point, enhancing freeze-thaw

cycling; below the sampled depth, in fact, the soils were completely frozen. All the samples were collected in the early afternoon, stored at 4 °C in the field and during the transport and at – 20 °C in the laboratory prior to further analysis.

Climatic conditions of the study areas and morphological, mineralogical and textural characteristics of PG soils, as well as vegetation type and distribution, are described in detail in D'Amico et al. 2015. Soil chemical properties and percentage of vegetation cover, measured at each sampling point and reported in the same study, are summarized in Table 2.2.

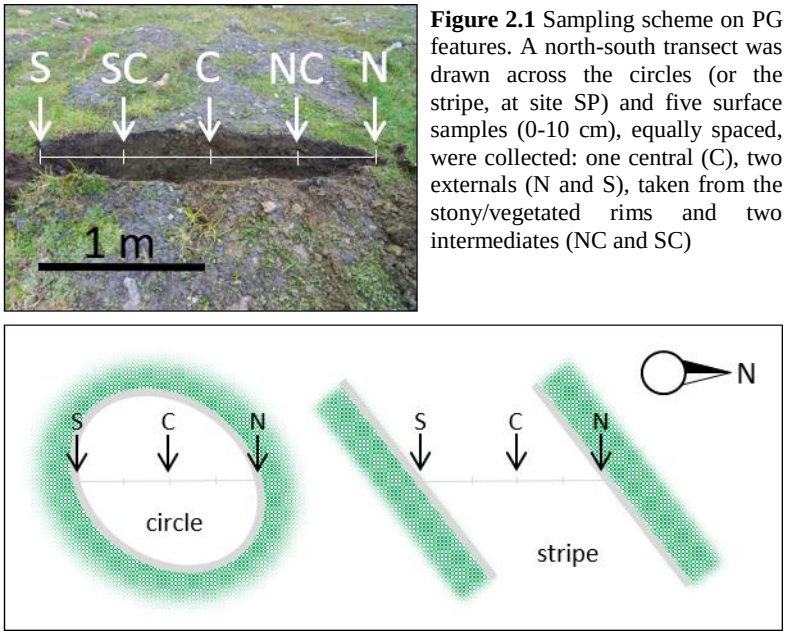


Figure 2.1 Sampling scheme on PG features. A north-south transect was drawn across the circles (or the stripe, at site SP) and five surface samples (0-10 cm), equally spaced, were collected: one central (C), two externals (N and S), taken from the stony/vegetated rims and two intermediates (NC and SC)

2.3.2 Soil DNA extraction

Total soil DNA was extracted from 0.5 g of soil samples using the FastDNATM SPIN Kit for Soil and the FastPrep® Instruments (MP Biomedicals) in accordance with the manufacturer's instructions. Quantity, quality and integrity of extracted DNA were evaluated using a NanoDrop ND-1000 spectrophotometer (Thermo Fischer Scientific) and agarose gel electrophoresis.

Table 2.2 Main chemical soil properties and plant cover distribution in the sampling sites (data from D'Amico et al. 2015)

Site	Sample	pH	TOC (%)	C/N	Exchangeable Ca cmol kg-1	Exchangeable Mg cmol kg-1	Exchangeable Ni mg kg-1	Available P mg kg-1	Vascular plant cover (%)
CS	1N	5.2	2.65	14.7	6,32	2.05	11.77	8.09	98
	1NC	5.7	2.05	14.6	3,27	1.25	5.86	2.26	30
	1C	6	1.12	18.7	1,15	0.31	6.94	1.35	5
	1SC	5.3	3.01	14.3	3,76	1.23	3.50	2.66	20
	1S	5.5	3.12	14.2	6,57	1.18	1.94	7.18	100
SP	2N	5.7	1.59	13.3	1,30	1.64	24.74	2.41	50
	2NC	6	1.26	14.0	0,91	1.09	20.34	1.38	10
	2C	6.1	1.21	13.4	0,88	0.99	16.36	1.25	5
	2SC	5.9	3.33	15.1	1,31	1.97	20.12	2.26	20
	2S	5.4	11.78	13.4	5,02	4.57	30.24	10.77	50
GB	3N	5.4	4.21	16.8	2,92	1.52	0.03	12.02	40
	3NC	5.6	0.95	13.6	0,97	1.44	0.18	2.04	5
	3C	6.4	0.42	10.5	1,02	1.05	1.57	0.68	1
	3SC	5.3	2.43	12.2	1,15	0.53	0.00	6.08	10
	3S	5.2	6.16	15.0	2,58	1.99	1.34	12.71	30
GN	4N	5.3	0.60	12.0	0,24	0.07	0.00	14.3	5
	4NC	5.4	0.52	8.7	0,22	0.08	0.00	16.46	1
	4C	5.6	0.30	7.5	0,20	0.11	0.00	26.43	0
	4SC	5.4	0.42	8.4	0,26	0.08	0.00	14	1
	4S	5.3	0.27	6.8	2,06	0.06	0.00	9.77	5

2.3.3 PCR-DGGE

Polymerase chain reaction (PCR) products for denaturing gradient gel electrophoresis (DGGE) were obtained by amplifying total bacterial and archaeal 16S rRNA genes and fungal 26S rRNA genes. While bacterial and fungal genes were amplified directly from the extracted DNA, a nested approach was followed for Archaea. Primer pairs were 357F-GC and 518R for bacteria (Muyzer et al. 1993), NL1 and LS2 for fungi (O'Donnell 1993; Cocolin et al. 2000), A2F and 1492R and SaF-GC and PARCH519R for the first and second step of archaeal PCR respectively (Reysenbach et al. 1995; Lane 1991; O'Donnell 1993; Cocolin et al. 2000). Primer sequences and reaction conditions are reported in Annex I.

All PCR reactions were carried out in a DNAEngine® Peltier Thermal Cycler (Bio-Rad Laboratories) in a 25-µl reaction volume containing 1x reaction buffer (Bioline), 3 mM $MgCl_2$, 0.02 mg/ml bovine serum albumin (BSA), 0.2 mM of each dNTP, 0.4 µM of each primer, 1.25 U of BIOTAQ™ DNA polymerase (Bioline) and 2 µl of soil DNA diluted 1:10 in sterile DNase-treated water (Sigma). Second steps of nested PCR were performed without BSA, using 1 µl of the first step product as template.

DGGE was carried out as previously described by Webster et al. (2006) using a DCodeTM Universal Mutation Detection System (Bio-Rad Laboratories), with a gradient from 30 to 60%. Electrophoresis was run at 200 V for 5 h (with an initial 10 min at 80 V) at 60°C in 1x TAE buffer. Gels were stained for 30 min with SYBR® Gold nucleic acid gel stain (Invitrogen) and visualized under UV with an UVIpro Platinum Gel Documentation System (UVItec). Reproducibility of DGGE profiles was tested by comparing PCR products obtained by using DNA extracted in triplicate from the same sample as template. Considering that good reproducibility was achieved, DGGE gels were organized in order to compare single samples within PG features and among different sites (Annex II, III). DGGE bands recurrent at site level, or shared among different sites were excised, incubated one night at -20°C, washed and crushed in 10-20 µl of molecular-grade water. Supernatant (1 µl) was used as template and PCR was performed as above except for the elimination of BSA and the employment of modified linker-PCR

archaeal and bacterial primers described in O'Sullivan (2008). PCR products were sequenced and searched for sequence similarities in the National Center for Biotechnology Information database using nucleotide Basic Local Alignment Search Tool (BLASTn) analysis (Altschul 1990).

Obtained 16S bacterial rRNA gene sequences were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession numbers LT613607-LT613635.

2.3.4 Quantitative PCR

The abundance of different phylogenetic markers and functional genes was estimated by real-time quantitative PCR (qPCR).

For standard curves construction, the reference genes were amplified from genomic DNA extracted from pure cultures of standard organisms: *Lactococcus lactis* subsp. *cremoris* for bacterial 16S, *Methanococcoides methylutens* for archaeal 16S, *Saccharomyces cerevisiae* for eukaryotic 26S and *Nitrosomonas europaea* for bacterial *amo*A. PCR products were than purified with the PCRExtract Mini Kit (5 Prime), in accordance with the manufacturer's instructions, quantified by NanoDrop and used to prepare serial dilutions in molecular-grade water. Primer pairs used for standard preparation were 27F and 1492R for bacteria (Lane 1991), S-D-Arch-0025-a-S-17 and 1517R for archaea (Vetriani et al. 1999), NL1 and LS2 for fungi (O'Donnell 1993; Cocolin et al. 2000) and amoA-1F and amoA-2R for bacterial *amo*A genes (Mc Tavish et al. 1993). Primer sequences and references for PCR conditions are reported in Annex IV, while master mix composition was as described above (excluding BSA). Only for archaeal *amo*A gene, PCR products obtained by amplifying total DNA extracted from PG sample 2N with primer pair Arch-amoAF and Arch-amoAR (Francis et al. 2005) were pooled, purified, quantified by NanoDrop, serially diluted and used for standard curve construction.

qPCR reactions were performed using a Chromo4^{TM} Real Time PCR Detection System (Bio-Rad Laboratories), and data were analysed with the MJ Opticon Monitor software (version 3.1). Primer pairs were the same as for

standard preparation, except for Bacteria and Archaea. The first were substituted by the pair 519F and 907R (Lane 1991; Muyzer et al. 1995), the second by the pair S-D-Arch-0025-a-S-17 and S-D-Arch-0344-a-S-20 (Vetriani et al. 1999). The PCR mixture contained 0.3 µM of each primer, 10 µl of SsoAdvanced™ SYBR® Green Supermix and 2 µl of soil DNA diluted 1:10 - 1:100, in a total volume of 20 µl. Optimal DNA dilution was chosen in order to minimize inhibition problems linked to low A260/A230 ratio of extracted DNA. All the samples and the standards were analysed in triplicate on PCR strip tubes (Bio-Rad Laboratories) with the following thermal cycling conditions: 95° for 5 min, followed by 40 cycles of 95°C for 30 sec, annealing for 30 sec and 72°C for 1 min. Different annealing temperatures are reported in Annex 2.4. PCR specificity was verified by melting curves analysis. Standard curves R2 value was always higher than 0.996, and all the reactions showed efficiencies higher than 70%.

2.3.5 Statistical analysis

Statistical analysis was performed using R 3.0.1 software (R Foundation for Statistical Software, Institute for Statistics and Mathematics, Vienna, Austria).

Significant differences in gene abundance among different lithologies were checked by Brown-Forsythe one-way ANOVA combined with post hoc Games-Howell test and displayed as boxplots, using the userfriendlyscience package.

Microbial communities were grouped using Cluster Analysis (CA), average linkage agglomeration criteria, Bray-Curtis dissimilarity algorithm. The best dissimilarity algorithm (Bray-Curtis) was selected according to the function rank index in the Vegan package (Oksanen et al. 2013), which correlates many dissimilarity algorithms with a given gradient (in this case, soil-environmental properties). As the clusters were usually very well separated, their statistical significance was not checked.

Gradients in microbial community composition within the different patterned ground sites were observed using unconstrained ordination methods (NMDS, Kruskal, 1964, distance Bray-Curtis). The analysis was carried out with

metaMDS within R vegan, using a Wisconsin double standardization and a maximum number of 100 runs to reach the best solution (two axis). To visualize relationships between microbial community composition and environmental parameters, the resulting NMDS biplot was interpreted using a post-hoc correlation with significant soil and environmental parameters (function envfit).

Pearson's linear correlation coefficients were calculated for assessing significant relations between microbial abundance and environmental parameters.

2.4 Results

2.4.1 Community structure (PCR-DGGE)

Bacterial DGGE profiles showed a quite homogeneous community composition within single PG features, with more differences among the four sites (Annex III). A pool of ubiquitous, brightly stained bands was clearly recognizable beside several site-specific, often weaker bands. Sequence analysis of excised bands highlighted the presence of at least 7 different phyla (Proteobacteria, Actinobacteria, Verrucomicrobia, Acidobacteria, Bacteroidetes, Cyanobacteria, and Chloroflexi), with all the ubiquitous phylotypes belonging to Acidobacteria or Alphaproteobacteria (Table 2.3). Only two of the detected bacterial phylotypes were closely related to described bacterial species (>97% sequence similarity), while the majority was related to uncultured bacteria previously found in soils or periglacial ecosystems. Cluster analysis confirmed the presence of distinct bacterial community composition at all the sites (Fig. 2.2).

Archaeal DGGE profiles appeared more heterogeneous compared to Bacteria at a small-scale level, both in terms of bands number and intensity, and few dominant bands recurred in samples collected from different sites (Annex III). Archaeal community composition in PG sites separated in different groups, as indicated by cluster analysis. Only at SP site a separation between centre and rims was detected (Fig. 2.2). All the sequences obtained from excised bands belonged to Thaumarchaeota and showed 93-96% of sequence similarity with Candidatus *Nitrososphaera gargensis* or Candidatus *Nitrososphaera*

evergladensis. Nearly all the phylotypes were closely related to uncultured Archaea previously detected in high-altitude soils (as reported in Table 2.4) but also with DNA sequences retrieved from temperate agricultural and forest soils.

Our PCR-DGGE approach was able to detect only low fungal diversity. As for Bacteria, the main differences among profiles seemed to be linked to the site, rather than the position within PG features. Cluster analysis supported this interpretation, even if for GB and GN samples a clear separation has not been found. Sample 3N was excluded from the analysis due to the impossibility to obtain a clear DGGE profile (Fig. 2.2). Only few sequences were obtained from fungal excised bands, including bands recurrent at site level, or shared among different sites, all corresponding to Ascomycota or Basidiomycota (Table 2.5); interestingly, four of them were strictly related (97-98% similarity) to uncultured Ascomycota detected in PGs from North American Arctic by Timling et al. (2014).

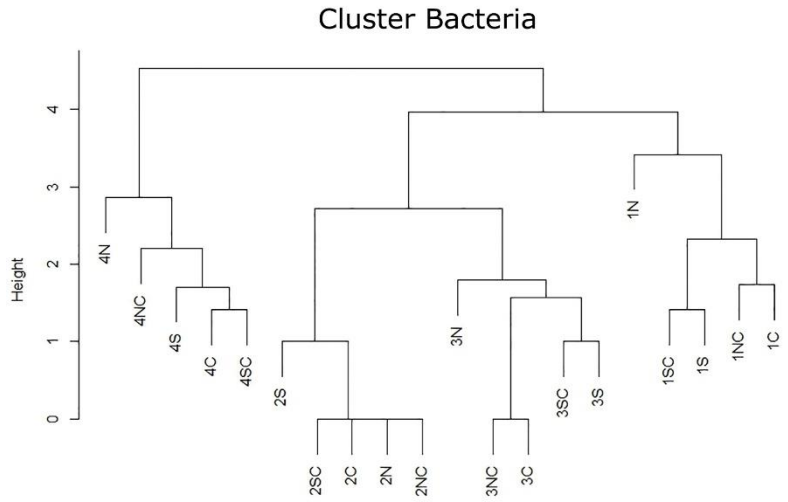
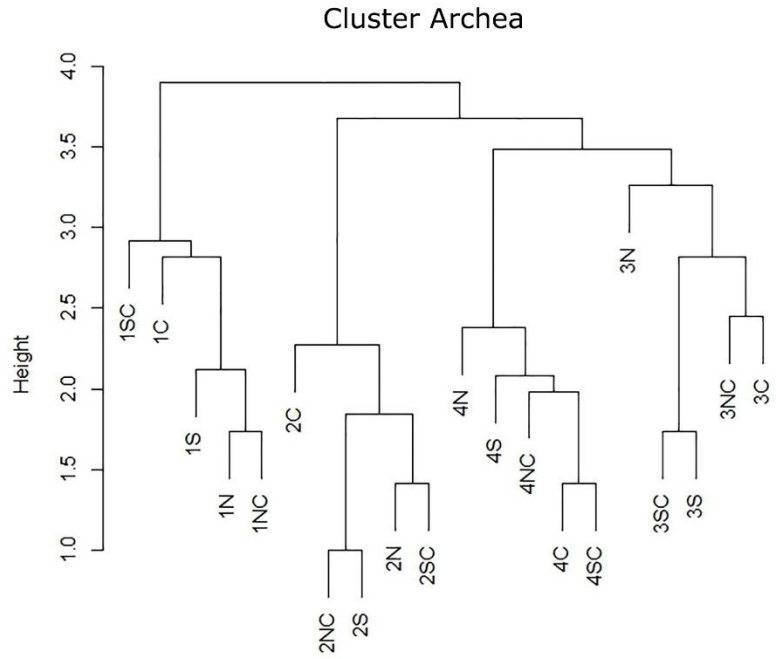


Figure 2.2 (Continues in the next page) Cluster analysis of DGGE profiles obtained for bacterial, archaeal and fungal PG communities (site 1=CS; 2=SP; 3=GB; 4=GN), based on Bray-Curtis dissimilarity algorithm.

Figure 2.2 (Continues from the previous page)



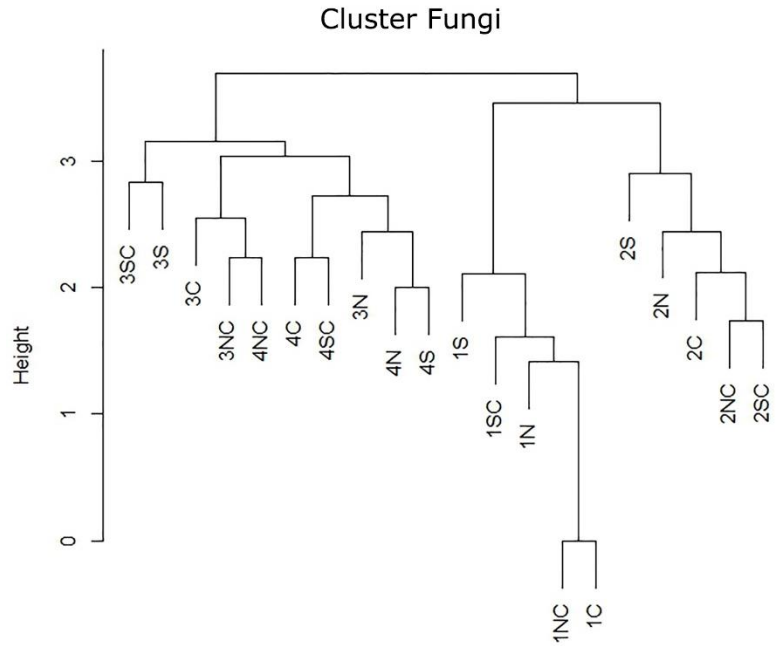


Table 2.3 Closest 16S rRNA gene sequence matches to excised bacterial DGGE bands using the NCBI BLASTN search tool.

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs			
							CS	SP	GB	GN
B3	*Edaphobacter aggregans* (NR_043898)	forest soil	95	*Acidobacteria*	*Edaphobacter aggregans* (NR_043898)	95	x	x	x	x
B1	*Nitrobacter winogradskyi* (NR_074324)	-	100	*Alpharoteobacteria*	*Nitrobacter winogradskyi* (NR_074324)	100	x	x	x	x
B17	Uncultured bacterium clone KA13 (JQ973360)	forest soil	96	*Alpharoteobacteria*	*Microvirga guangxiensis* (NR_044563)	95	x	x	x	x
B5	Uncultured bacterium isolate DGGE gel band 02_F2 clone 01 (JX986102)	soil	99	*Alpharoteobacteria*	Phaeospirillum fulvum (NR_025836)	94	x	x	x	x
B2	Uncultured bacterium clone B8-80 (KF494605)	permafrost	99	*Alpharoteobacteria*	*Sinorhizobium fredii* (NR_102919)	97	x	x		x
B7	Uncultured SOIL BACTERIUM clone GC0AA4ZA03PP1 (JQ919779)	soil	96	*Bacteroidetes*	*Pedobacter borealis* (NR_044381)	88		x	x	
B16	Chloroflexi bacterium Ellin7237 (AY673403)	soil	86	*Chloroflexi*	Chloroflexi bacterium Ellin7237 (AY673403)	82				x
B4	*Arthrobacter ramosus* (KF387693)	Himalaya	90	*Actinobacteria*	*Arthrobacter ramosus* (KF387693)	90		x		
B15a	Bacterium PE03-55G21 (AB127830)	eutrophic lake	97	*Alphaproteobacteria*	*Filomicrobium insigne* (NR_044095)	95	x		x	
B24	Uncultured bacterium clone HF127 (KF037272)	soil	97	*Betaproteobacteria*	*Nitrosospira multiformis* (NR_074736)	93		x		
B15	Chroococcales cyanobacterium PE5G6 (HE805950)	lake water	86	*Cyanobacteria*	*Cyanobium gracile* (NR_102447)	85				x
B8, B9	*Pseudomonas antarctica* (NR_025586)	McMurdo Valley, Antarctica	100	*Gammaproteobacteria*	*Pseudomonas antarctica* (NR_025586)	100				x
B12, B13	Uncultured bacterium clone P1s-43 (GQ287601)	periglacial soil Himalaya	100	*Verrucomicrobia*	*Prosthecobacterfluviatilis* (NR_041608)	86	x			
B14	Uncultured bacterium isolate 1112869339975 (HQ11871)	forest soil	95	*Verrucomicrobia*	Bacterium Ellin507 (AY960770)	90				x

Table 2.4 Closest 16S rRNA gene sequence matches to excised archaeal DGGE bands using the NCBI BLASTN search tool.

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs			
							CS	SP	GB	GN
1	Uncultured archaeon clone DT-1T-A-10 (KJ834100)	permafrost sediments (Qilian moutain)	98	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	94	x	x	x	x
A	Uncultured archaeon clone QRS_2 (KF445503)	newly deglaciated soil (Western Himalayas)	100	*Thaumarchaeota*	Candidatus *Nitrososphaera evergladensis* SR1 (CP007174)	94	x	x	x	x
B	Uncultured archaeon clone AS.A17 (GU298233)	glacial cryconite (Signy Island, Antarctica)	100	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	96	x	x	x	x
C	Uncultured clone DT-14Q-2T-17 (KR066470)	permafrost soil (Qinghai, Tibetan Plateau)	100	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	95		x	x	x
D	Uncultured archaeon clone DZ2-14Q-3T-1 (KR066484)	permafrost soil (Qinghai, Tibetan Plateau)	100	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	95		x	x	x
E	Uncultured archaeon clone ARCdr-50 (GQ126886)	soil (Mount Mila, Tibetan Plateau)	97	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	93		x	x	x
68	Uncultured archaeon clone ARCu-227 (GQ127350)	soil (Mount Mila, Tibetan Plateau)	96	*Thaumarchaeota*	Candidatus *Nitrososphaera gargensis* Ga9.2 (NR_102916.1)	94	x			

Table 2.5 Closest 26S rRNA gene sequence matches to excised fungal DGGE bands using the NCBI BLASTN search tool.

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs			
							CS	SP	GB	GN
F1	Uncultured fungus clone 126_NA10_P32_C19 (KC966197)	Patterned Ground (North American Arctic)	98	*Ascomycota*	*Coniochaetaceae* sp (AB752287)	97	x	x	x	
F2	*Pleosporales*sp (JX244063)	*Populus deltoides* roots	95	*Ascomycota*	*Pleosporales* sp (JX244063)	95	x	x	x	
F3, F4, F5	Uncultured fungus clone 112_NA3_P31_P17 (KC966078)	Patterned Ground (North American Arctic)	97	*Ascomycota*	*Geomyces sp.* (AB752279)	*97*	x	x	x	x
F6	*Cortinarius cf. saniosus* (FN687648)	alpine soil (root apex of *Salix herbacea*)	97	*Basidiomycota*	*Cortinarius cf. saniosus* (FN687648)	97	x	x		
F7	*Clavulina cf. cristata* (JN228225)	Guiana Massif	89	*Basidiomycota*	*Clavulina cf. cristata* (JN228225)	89			x	x
F1	Uncultured fungus clone 126_NA10_P32_C19 (KC966197)	Patterned Grounds (North American Arctic)	98	*Ascomycota*	*Coniochaetaceae* sp (AB752287)	97	x	x	x	
F2	*Pleosporales*sp (JX244063)	*Populus deltoides* roots	95	*Ascomycota*	*Pleosporales* sp (JX244063)	95	x	x	x	

2.4.2 Microbial abundance (qPCR)

Microbial abundances were assessed by quantifying bacterial and archaeal 16S rRNA genes and fungal 26S genes with a qPCR-based method. Abundance of ammonia oxidizers was estimated by quantifying bacterial and archaeal ammonia monooxygenase subunit A (*amo*A) genes. Total DNA concentration was considered as a proxy for biotic presence.

Comparing samples from different lithologies, significant differences ($P<0.05$) were only found for DNA, bacterial and archaeal markers and for archaeal *amo*A genes (Fig. 2.3). In general, lower abundances were reported for GN samples (average of 9.10, 6.31 and 6.67 Log copies per g of dry soil for Bacteria, Archaea and AOA respectively), while CS, SP and GB showed similar values (average of 9.92-10.12, 6.69-7.57 and 7.21-7.87 Log copies per g of dry soil for Bacteria, Archaea and AOA respectively). Fungal marker abundance ranged from an average of 8.57 to 9.27 Log copies per g of dry soil, without significant differences among sites. Neither for bacterial *amo*A genes abundance, ranging from an average of 4.34 to 5.59 Log copies per g of dry soil, significant differences were detected. Comparing only functional genes, a predominance of archaeal over bacterial *amo*A genes, with AOA/AOB Log copies ratio ranging from 1.4 to 1.9, was detected in all the four sites.

At a small-scale level, a slightly concentric variation was reported for all the phylogenetic markers: the abundance of bacterial and archaeal genes decreasing from the rims toward the centre of single features in CS, GB and GN, and showing an opposite trend in SP; fungal markers reaching the highest values in intermediate positions (Fig. 2.4). DNA concentration followed a similar trend, decreasing from the rims to the centre in CS, GB and GN and from the centre to the rims in SP. No clear repeated patterns were described observing the distribution of AOA and AOB markers within single PG features. The only recognizable trends were a concentric decreasing of AOB in GB and AOA in GN or the north-to-south decreasing of AOB in GN and AOA in SP.

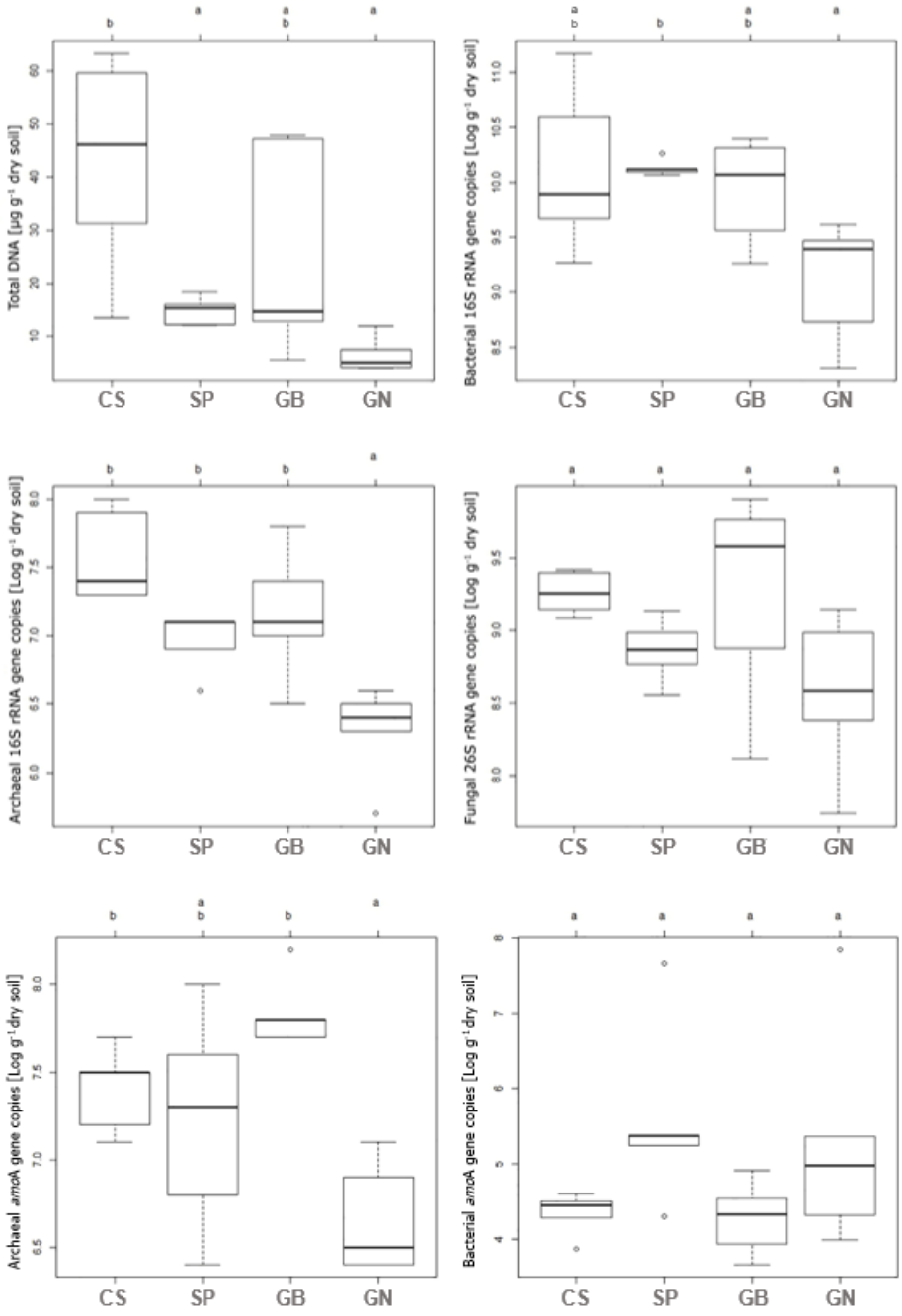


Figure 2.3 DNA concentration and abundance of bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, archaeal and bacterial *amo*A genes in the four sites. Different letters indicate sig-nificant differences ($P < 0.05$) among sites according to Games Howell *post hoc* test.

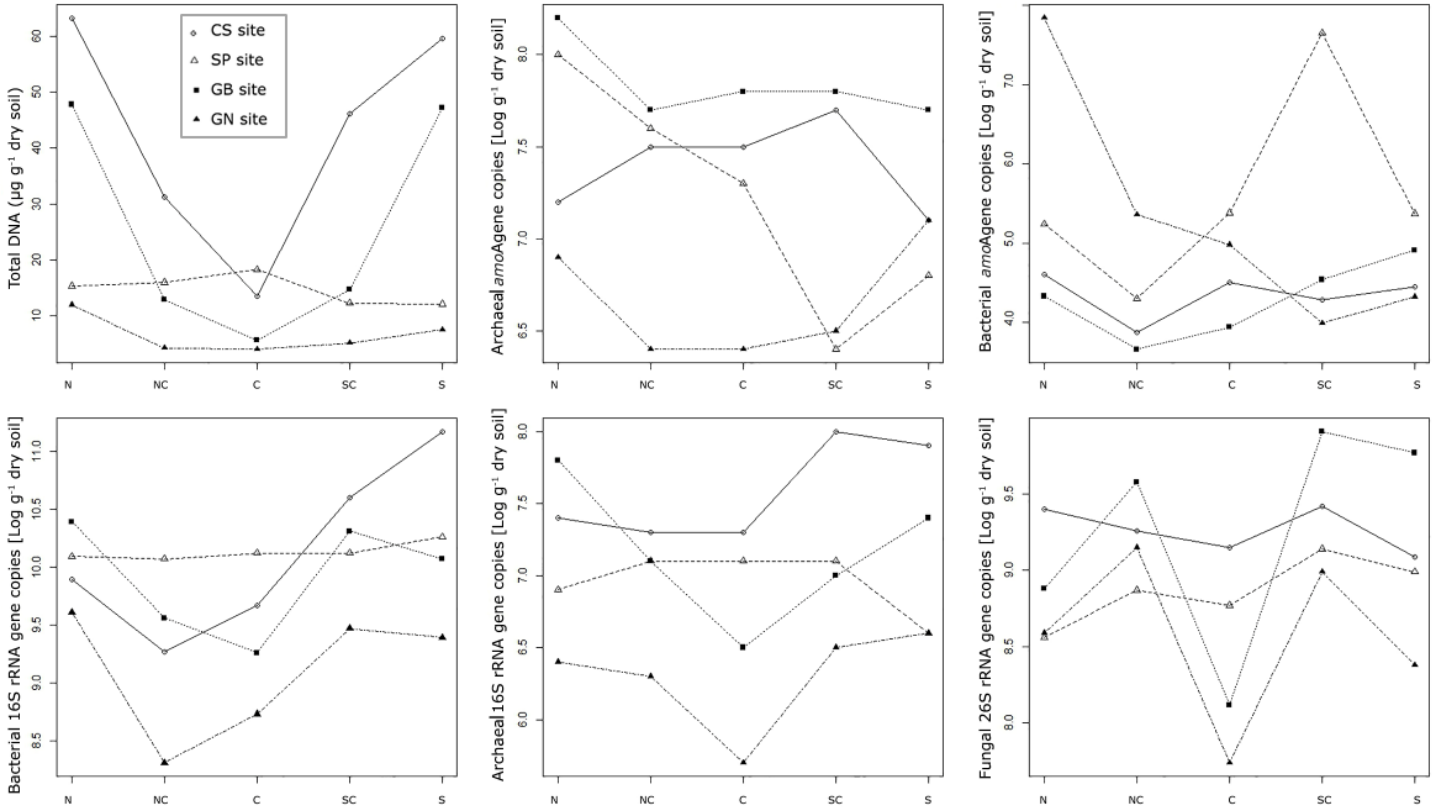


Figure 2.4 Distribution of different biological markers within single PG features in the four sites: DNA concentration, bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, archaeal and bacterial *amo*A genes.

2.5 Discussion

We performed a preliminary survey, in order to explore the composition and abundance of microbial communities inhabiting patterned ground features developed on lithologically distinct sites in a mid-latitude alpine environment. A molecular approach combining PCR-DGGE (community structure analysis) and qPCR (quantitative analysis) was applied, targeting bacterial, archaeal, and fungal phylogenetic markers. Moreover, in order to focus on microbial driven processes affecting these ecosystems, the functional gene ammonia-monooxigenase was included in the quantitative analysis. Both Bacteria (AOB) and Archaea (AOA) ammonia-oxidisers drive the first and rate-limiting step of nitrification. Their use as process indicators provides important information due both to their function and to differential response to environmental factors influenced by their diverse ecological niches (Prosser and Nicol 2012).

DNA-targeting techniques do not discriminate the active from the total population and the presence of highly resilient extracellular DNA and DNA deriving from dead cells may lead to a biased view of the ecosystem ecology (Pietramellara et al. 2009). Moreover, PCR-DGGE approach allows to perform a comparison among samples at "low resolution", compared to metagenomic sequencing. However, the objective of this work was to investigate the long-term influence of cryoturbation and edaphic properties on soil microbial community shaping. In this sense, the data obtained provided exhaustive information about the overall microbial complexity, the more represented groups and evidenced the ecosystem properties possibly involved in shaping and influencing the community.

Overall, the composition of bacterial communities found on these patterned ground features results quite similar to those described in more detail on alpine soils (Nemergut et al. 2005), or in other periglacial landscapes like polygonal soils (Frank-Fahle et al. 2014), ice wedges (Wilhelm et al. 2012), or glacier forefields (Bajerski and Wagner 2013), in accordance with Delmont et al.

(2014) that showed how similar habitats may lead to the development of communities with similar composition.

The presence of phylotypes belonging to at least seven different phyla indicates quite complex bacterial communities. Among primary producers, phototrophic Cyanobacteria and Alphaproteobacteria families including chemolithotrophic and chemoorganotrophic organisms (Bradyrhizobiaceae, Rhodospirillaceae, Hyphomicrobiaceae) were detected. Moreover, in all the sites several Acidobacteria-related phylotypes were found. The ability to grow at low nutrient conditions and tolerate variations in soil humidity often characterize Acidobacteria (Ward et al. 2009), giving a potential explanation for their ubiquity in the examined patterned ground ecosystems. Finally, the presence of at least one representative of Bacteroidetes in all the sites suggests that these communities can host also a group of degraders of complex substrates (Nemergut et al. 2005).

Archaea showed lower differentiation, with all the investigated phylotypes belonging to Thaumarchaeota division. Considering that Thaumarchaeota includes all known archaeal ammonia oxidizers, this result is also consistent with the high abundance of *amo*A gene copies, which exceed their bacterial analogues in all the samples. Similar situations are quite common in different ecosystems, particularly in acidic soils (Prosser and Nicol 2012; Qin et al. 2013; Tian et al 2014; Xu et al. 2012) and have been reported by Frank-Fahle et al. (2014) for polygonal tundra. Nevertheless, the same study pointed out a clear predominance of methanogens, not detected in this study. Previous studies highlighted the importance of waterlogging, common phenomenon affecting polygonal soils, in driving permafrost microbial community composition (Ollivier et al. 2014). However, the study sites present quite different characteristics, in terms of water content, 12-28% (unpublished data), from those reported for high latitude patterned ground ecosystems. Therefore, the predominance of aerobic phylotypes over anaerobic is not surprising and could indicate a relevant role of Thaumarchaeota in influencing N availability in mid-latitude PGs.

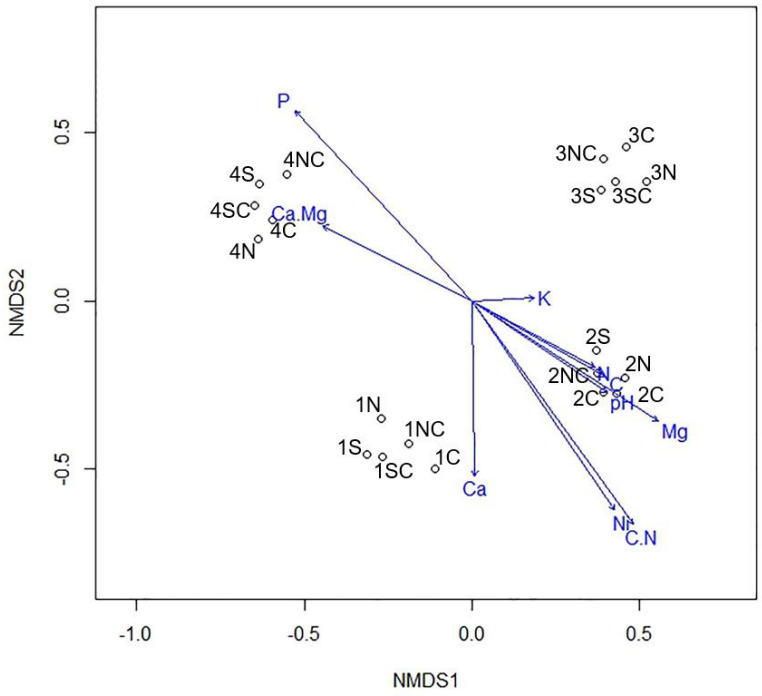


Figure 2.5 NMDS ordination of the four sites (1=CS; 2=SP; 3=GB; 4=GN), based on DGGE profiles (for each sampling point information obtained from archaeal, bacterial and fungal profiles were combined). Vectors show the direction and strength of environmental variables.

	NMDS1	NMDS2	r^2	p-Value
pH	0.83	-0.56	0.21	0.118
Ca	0.02	-1.00	0.25	0.089
Mg	0.84	-0.54	0.40	0.008
K	1.00	0.04	0.03	0.756
Ni	0.56	-0.83	0.52	0.008
Ca/Mg	-0.89	0.45	0.22	0.003
N	0.88	-0.48	0.16	0.222
C	0.87	-0.50	0.19	0.140
C/N	0.59	-0.81	0.61	0.001
P	-0.68	0.73	0.54	0.001
Altitude	-0.65	0.76	0.91	0.001

Table 2.6 Correlation values and significance between the soil chemical properties and the NMDS factors shown in Fig. 2.5.

In terms of archaeal sequences, the presence of identical sequences (100% similarity) in our samples and in both cold or rock-associated ecosystems and temperate agricultural and forest soils might suggest the cosmopolitan nature of at least a part of the community.

In general, analysis of bacterial, archaeal and fungal phylogenetic markers revealed a quite homogeneous community composition within single PGFs, without a clear separation between samples collected from the vegetated rims and the central, nearly bare soil portion. This is also coherent with the results that Timling et al. (2014) obtained for fungal phylogenetic markers investigating patterned grounds in the northernmost bioclimatic subzone of the North American Arctic Transect. Differences in community composition were related more consistently to the sampling site than to the position across the PGFs. The NMDS analysis (Fig. 2.5), performed on the average band distribution of all the phylogenetic markers, supported this separation among sites, pointing out the main parameters (data from D'Amico et al. 2015) involved in site differentiation and, potentially, in shaping the composition of microbial community (Table 2.6). For instance, the GN site was located at the highest altitude, and was characterized by highest P content and exchangeable Ca/Mg ratio. Conversely, high levels of exchangeable Mg and Ni fitted with the SP community; CS sites were mainly characterized by high levels of Ca, while GB communities were correlated with intermediate levels of most soil parameters. This seems to suggest that in this mid-latitude, alpine context parent material lithology can be a strong driver for microbial community differentiation in terms of community composition, overcoming the effect of strong, small-scale gradients in edaphic properties produced by cryoturbation and patterned ground development. The importance of parent material lithology on composition of microbial communities has been described in a variety of different ecosystems, like soils (Reith et al. 2015), continental crystalline crust (Nyyssönen et al. 2014), pristine aquifers (Boyd et al. 2007) and arctic streams (Larouche et al. 2012). D'Amico et al. (2015), analysing the same study sites, reported a similar vegetation diversity pattern, with a lack of differentiation between rims and centres, and a strong

separation of plant communities developed on different matrixes. Therefore, parent material lithology and the associated soil chemical properties, plant colonization and microbial community composition seem to be strictly linked.

Quantitative analysis, performed by qPCR, presents a different picture. In fact, microbial abundance resulted quite homogeneous among different sites. The only one showing significantly lower abundances (in terms of Bacteria, Archaea, AOA markers and DNA) was GN site. This is probably linked to the lower temperatures, associated to highest altitude, which affects also total vegetation cover (D'Amico et al. 2015).

Conversely, some interesting patterns appear by comparing samples within single features. In particular, concentric trends reported for bacterial, archaeal and fungal markers, as well as for total DNA concentration, on sites CS, GB and GN are coherent not only with vegetation cover, but also with the small-scale variation of chemical properties, organic C and nutrient con-tent (D'Amico et al. 2015). For instance, TOC, N and exchangeable bases decreased from the rims to the centres, in parallel with microbial abundances, while pH, possibly also affecting microbial activity, showed an opposite trend. This distribution has already been reported for Arctic patterned grounds (González et al. 2014; Timling et al. 2014), where higher levels of microbial biomass were found in vegetated rims if compared with patterned ground features. However, one of the four sites did not follow this scheme. In fact, archaeal, bacterial and total DNA abundances showed a different trend in SP site, increasing or remaining nearly constant, for bacteria from the rims toward the centre of the sorted stripes, despite an opposite trend of plant cover and contents of organic C and nutrients. SP site strongly differs from the others for the high ex-changeable Ni concentration, which increases from the centre toward the rims. Therefore, in this case, it is possible that Ni toxicity becomes the prevalent driving factor in microbial distribution across the features, overwhelming the effect of other chemical properties. An inverse correlation between microbial biomass and respiration and Ni content has been observed in subalpine forest soils in the same area by D'Amico et al. (2009). Unfortunately, the impossibility to collect samples from replicate transects within

single PG features prevents a statistical verification of the micro-topographic trends discussed above. However, the presence of common trends in all the investigated features, and the coherence between trends reported for different parameters suggest intra-feature variations as a promising target for further, more specific studies.

Finally, exploring more in detail the relationships existing between microbial abundances and soil chemical parameters (Table 2.7), Bacteria showed the highest number of significant correlations with different chemical properties (vegetation cover and contents of Ca, Mg, K, TN and TOC and C/N). On the other hand, Archaea abundances only correlated with the soil C/N but correlated to all the microbial markers except for bacterial amoA. Similar situations have been previously reported for alpine forest soils (Siles and Margesin 2016) and for tundra soils (Blaud et al. 2015), with bacterial abundance following chemical soil properties trend, and archaeal abundance independent from them. However, in those cases, all the microbial markers resulted positively correlated to each other, suggesting an indirect action of environmental parameters on the whole microbial population. In our case we can hypothesize that, despite the presence of a bacterial population apparently more sensitive to variations in substrate composition, Archaea seems to represent the link among the different microbial domains, and so the real keystone of the ecosystem. Moreover, considering that Thaumarchaeota seem to represent an important portion of archaeal community in this ecosystem, the low level of correlation with any environmental parameter but C/N, reported for both archaeal and AOA markers, could be linked to the wide ecophysiological potential of this group, including not only autotrophy but also mixotrophy and heterotrophy lifestyles (Pester et al. 2011, Prosser and Nicol 2012). Concerning fungal abundances, the only correlation with soil properties was found with C/N. Nevertheless, the presence of a unique intra-feature distribution in gene abundance repeated in all the sites suggests the presence of other factors, not considered here but suitable for further investigations, driving fungal distribution, such as organic matter composition and quality.

Table 2.7 Correlation analysis among chemical soil properties and DNA content or microbial abundances estimated by qPCR (reported Pearson's coefficient and significance level of the correlation).

Variables	Bact	Arch	Fun	AOB	AOA	DNA
Plant coverage %	0.698**	0.666**	0.321	0.094	0.281	0.802**
pH	-0.167	-0.129	-0.396	-0.089	0.047	-0.187
Ca	0.588**	0.717**	0.368	-0.164	0.257	0.710**
Mg	0.478*	0.534*	0.362	0.060	0.275	0.594**
K	0.606**	0.349	0.343	0.224	-0.108	0.414
Ni	0.412	0.352	0.009	0.163	0.123	0.430
Ca/Mg	-0.089	0.075	0.071	-0.144	-0.230	0.024
P	-0.162	-0.347	-0.620	0.326	-0.439	-0.264
N	0.789**	0.643**	0.526*	0.186	0.216	0.695**
C	0.785**	0.654**	0.492*	0.205	0.229	0.712**
C/N	0.510*	0.815**	0.526*	-0.033	0.287	0.747**
Bact		0.626**	0.284	0.185	0.329	0.672**
Arch			0.556*	-0.323	0.480*	0.916**
Fun				-0.195	0.197	0.454*
AOB					-0.450*	-0.125
AOA						0.495*

*P < 0.05, **P < 0.01,

2.6 Conclusions

With this work, we obtained information about the overall complexity of the community and the more represented microbial groups, giving a preliminary insight in a previously unexplored ecosystem like alpine PG. Our results seem to indicate that Archaea and, in particular, Thaumarchaeota seem to play a key role in ecosystem coordination end functioning, suggesting this domain as a target for further, more detailed investigations.

In terms of ecological drivers, if micro-topographic heterogeneity produced by cryogenic processes seems to influence microbial distribution within PG features in terms of abundance, it has no clear effects on community composition. Conversely, lithology might strongly influence community composition but has not evident effect on overall microbial abundance, which is probably more linked to other variables, like altitude and temperature conditions. Only in the serpentinite sampling site it is possible to hypothesize an indirect influence of lithology on small-scale microbial abundance distribution: in fact, the presence of a gradient in heavy metals, produced by cryogenic processes, affects microbial distribution determining opposite trends with respect to all the other parent materials.

In conclusion, our results offer a picture quite in accordance with previous studies focused on Arctic PGFs, adding lithology to the complex hierarchy of controls modulating the effect of cryoturbation on soil microbial communities.

Further studies are needed in order to assess how the investigated drivers impact on community diversity and its potential metabolic activity. Moreover, an RNA-based analysis would allow to compare not only spatial, but also seasonal or daily community variations, giving more insights on the real ecosystem functioning in relation to temperatures variation and exposition to freeze-thaw activity.

2.7 References

References of this chapter are integrated into the overall reference section (Chapter 6).

2.8 Annexes

Annex I

Primer sets and amplification details used for PCR-DGGE analysis.

Primer pair	Sequence (5'-3')[a]	Target gene	Fragment lenght	Reference	Amplification details	Protocol reference
357F-GC[b]	CCTACGGGAGGCAGCAG	*Bacteria* 16S rRNA	193	Muyzer et al. 1993	95 °C 5 min; 10 cycles: 94 °C 30 s, 55 °C 30 s, 72 °C 1 min; 25 cycles: 92 °C 30 s, 52 °C 30 s, 72 °C 1 min; 72 °C 10 min	Webster et al. 2006
518R	ATTACCGCGGCTGCTGG					
A2F	TTCCGGTTGATCCYGCCGGA	*Archaea* 16S rRNA (PCR step I)	c. 1500	Reysenbach and Pace 1995	94 °C 5 min; 36 cycles: 95 °C 1 min, 50 °C 1 min, 72 °C 1 min; 72 °C 5 min	-
1492R	GGTTACCTTGTTACGACTT			Lane 1991		
SaF-GC[b,c]	Sa1f-CCTAYGGGGCGCAGCAGG Sa2f-CCTACGGGGCGCAGAGGG	*Archaea* 16S rRNA (PCR step II)	192	Nicol et al. 2003	95 °C 5 min; 5 cycles: 94 °C 30 s, 53.5 °C 30 s, 72 °C 1 min; 30 cycles: 92 °C 30 s, 53.5 °C 30 s, 72 °C 1 min; 72 °C 10 min	Nicol et al. 2003
PARCH519R	TTACCGCGGCKGCTG			Øvreås et al. 1997		
NL1-GC[b]	GCCATATCAATAAGCGGAGGAAAAG	Fungal 26S rRNA	c. 250	O'Donnell 1993	95 °C 5 min; 36 cycles: 95 °C 1 min, 52 °C 45 s, 72°C 1 min; 72 °C 7 min	Cocolin et al. 2000
LS2	ATTCCCAAACAACTCGACTC			Cocolin et al. 2000		

[a]D=G, A or T; H=A, T or C; K=G or T; M=A or C; R=A or G; S=G or C; W=A or T; Y=C or T

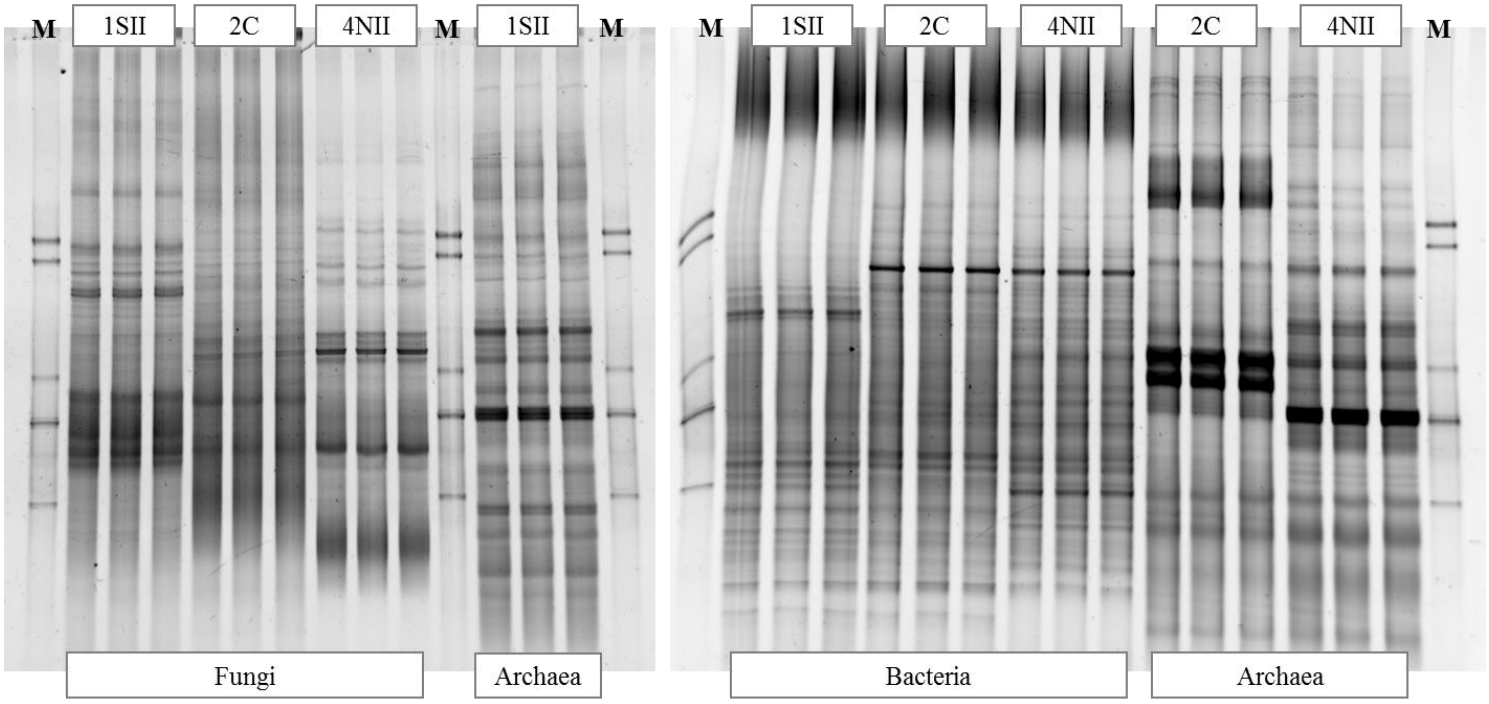
[b]GC-clamp at the 5' end, CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG (Muyzer et al. 1993)

[c]Primer SaF is a mixture of primers Sa1F and Sa2F at a molar ratio of 2:1

Annex II

DGGE reproducibility test.

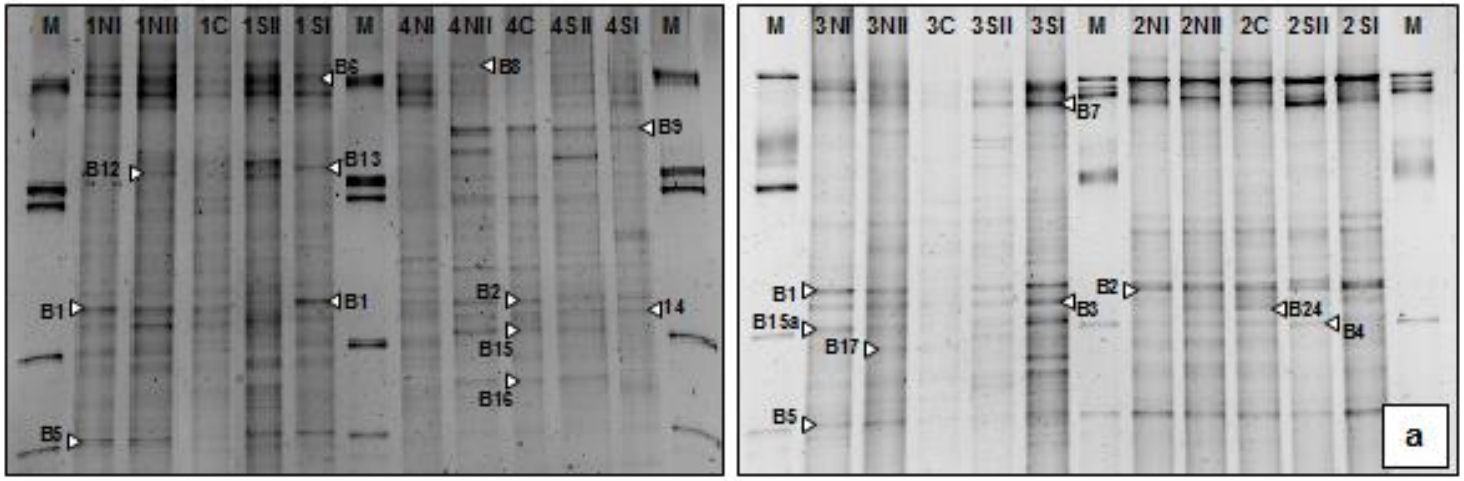
DGGE profiles of PCR products obtained from DNA extracted in triplicate from the same sample. DGGE were run in parallel for bacterial, archaeal and fungal phylogenetic markers, in order to check the reproducibility of profiles. Here, DGGE profiles from three randomly chosen samples, representative of different sites and sampling points (1SII, 2C and 4NII) were reported as an example. In general, all the samples gave good reproducibility for all the analysed markers.



Annex III

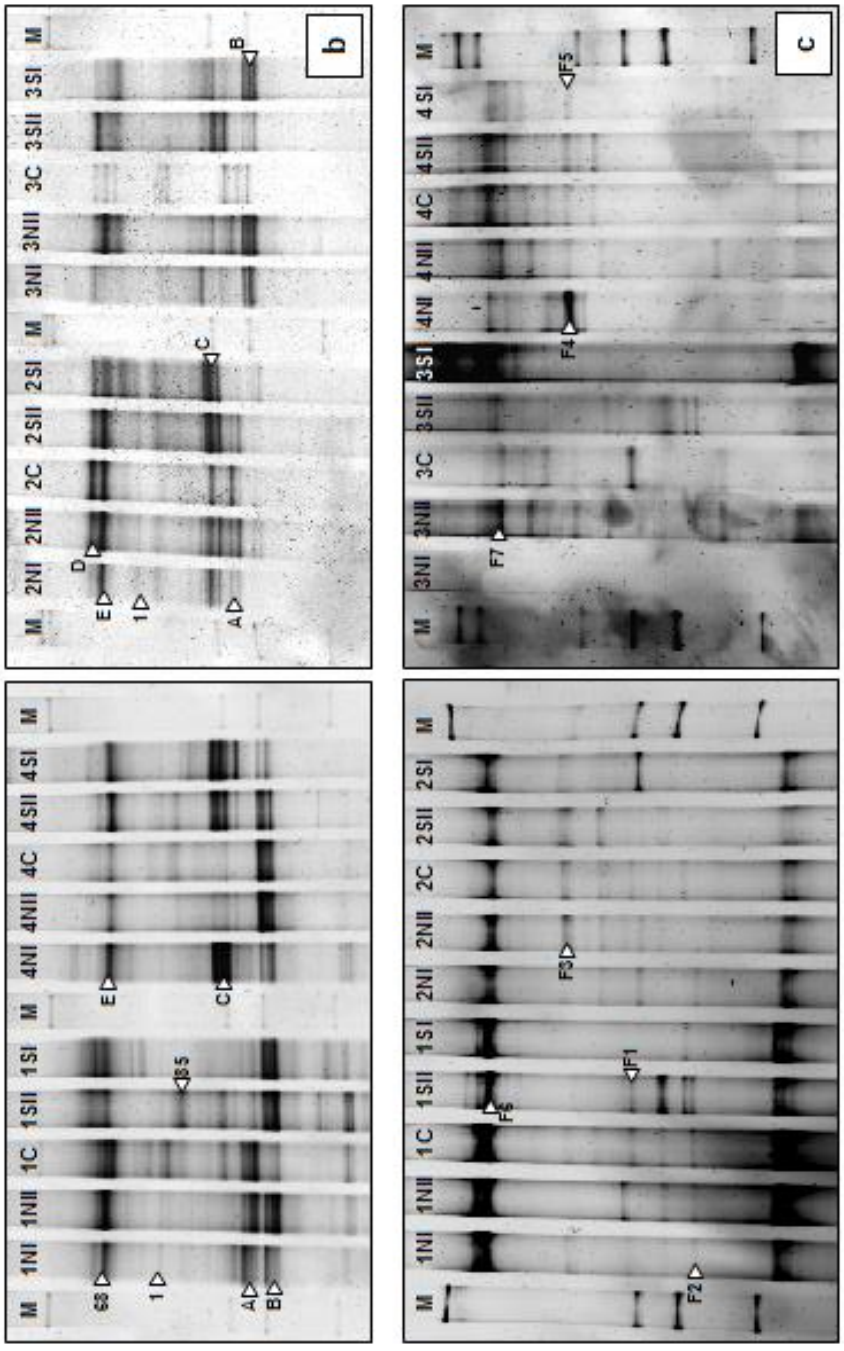
Comparison among sites and sampling points.

DGGE profiles of bacterial 16S rRNA genes (a), archaeal 16S rRNA genes (b) and fungal 26S rRNA genes (c) in the four sites.



(Continues in the next page)

(Continues from the previous page)



Annex IV

Primer sets and amplification details used for qPCR analysis.

Primer pair	Sequence (5'-3')[a]	Target gene	Fragment length	Reference	Amplification details	Application
27F	AGAGTTTGATCMTGGCTCAG	*Bacteria* 16S rRNA	c. 1500	Lane 1991		qPCR standard
1492R	GGTTACCTTGTTACGACTT					
S-D-Arch-0025-a-S-17	CTGGTTGATCCTGCCAG	*Archaea* 16S rRNA	c. 1500	Vetriani et al. 1999		qPCR standard
1517R						
NL1	GCCATATCAATAAGCGGAGGAAAAG	Fungal 26S rRNA	c. 250	O'Donnell 1993	95 °C 30 s; 44 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
LS2	ATTCCCAAACAACTCGACTC			Cocolin et al. 2000		
amoA-1F	GGG GTT TCT ACT GGT GGT	Bacterial *amo*A gene	491	Mc Tavish et al. 1993	94 °C 3 min; 40 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 45 s	qPCR standard, qPCR
amoA-2R	CCCCTCKGSAAAGCCTTCTTC					
Arch-amoAF	STAATGGTCTGGCTTAGACG	Archaeal *amo*A gene	635	Francis et al. 2005	95 °C 30s; 40 cycles: 95 °C 30 s, 53 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
Arch-amoAR	GCGGCCATCCATCTGTATGT					
519F	CCAGCAGCCGCGGTAATAC	*Bacteria* 16S rRNA	c. 390	Lane 1991	95 °C 30 s; 40 cycles: 95 °C 30 s, 50 °C 30 s, 72 °C 1 min	qPCR
907R	CCGTCAATTCMTTTRAGTTT			Muyzer et al. 1995		
S-D-Arch-0025-a-S-17	CTGGTTGATCCTGCCAG	*Archaea* 16S rRNA	c. 320	Vetriani et al. 1999	95 °C 30 s; 40 cycles: 95 °C 30 s, 48 °C 30 s, 72 °C 1 min	qPCR
S-D-Arch-0344-a-S-20	TCGCGCCTGCTGCGCCCCGT					

[a] D=G, A or T; H=A, T or C; K=G or T; M=A or C; R=A or G; S=G or C; W=A or T; Y=C or T

3. Prokaryotic diversity and distribution in different habitats of an alpine rock glacier-pond system

Adapted from:

Mania I[1,4], Gorra R[1], Colombo N[2,3], Freppaz M[1], Martin M[1], Anesio AM[4]

Prokaryotic diversity and distribution in different habitats of an alpine rock glacier-pond system

Microbial Ecology *[under revision]*

[1] DISAFA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO) Italy

[2] Department of Earth Sciences, University of Turin, Via Valperga Caluso 35, 10125 Torino – Italy

[3] Department of Geography and Environmental Studies, Carleton University, 1125 Colonel By Drive, Ottawa, ON, K1S 5B6 – Canada

[4] Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, University Road, Bristol BS8 1SS - United Kingdom

3.1 Abstract

Rock glaciers (RG) are assumed to influence the biogeochemistry of downstream ecosystems because of the high ratio of rock:water in those systems, but no studies have considered the effects of a RG inflow on the microbial ecology of sediments in a downstream pond. An alpine RG-pond system, located in the NW Italian Alps has been chosen as a model, and Bacteria and Archaea 16S rRNA genes abundance, distribution and diversity have been assessed by qPCR and Illumina sequencing, coupled with geochemical analyses on sediments collected along a distance gradient from the RG inflow. RG surface material and neighbouring soil have been included in the analysis to better elucidate relationships among different habitats.

Our results showed that different habitats harboured different, well separated microbial assemblages. Across the pond, the main differences in bacterial community composition (e.g. Cyanobacteria relative abundance) and porewater geochemistry (pH, DOC, TDN and NH_4^+) were not directly linked to RG proximity, but to differences in water depth. However, despite the microbial ecology of the pond system seems not to be influenced by RG, anomalies in community diversity and composition and sediments geochemistry reported after a phase of high RG hydrological contribution suggest that perturbations from such equilibrium are possible and, in particular circumstances, the RG influence may become relevant.

3.2 Introduction

Active rock glaciers (RGs) are slow-flowing mixtures of rocks and ice, representing one of the most common geomorphological features in high mountain environments (Barsch 1996), and are considered indicators of permafrost presence (Haeberli et al. 2006, Berthling 2011). Permafrost degradation has been reported to impact chemical characteristics of surface freshwater across the globe (Frey and McClelland 2009, Colombo et al. 2018) and RGs have shown the capability to influence waters passing through and originating from them, releasing cold meltwaters enriched in solutes (Giardino et al. 1992, Williams et al. 2006, Fegel et al. 2016). Given the widespread distribution of RGs worldwide (Barsch 1996), the high concentrations of heavy metals (Thies et al. 2013) and nutrients (Williams et al. 2007, Fegel et al. 2016) in their meltwaters, and their lower ice-melting rate in comparison to glaciers (Millar and Westfall 2008, Woo 2012), their importance in shaping headwater biogeochemistry is probably going to increase in the future, in a climate change scenario.

Several studies (reviewed in Slemmons et al. 2013) have investigated how the presence of glacial-meltwater inputs can affect downstream freshwater ecosystems in terms of physical and chemical properties, with subsequent effects on their biological structure and function. Far less information is available on RG meltwaters, and particularly on their influence on the ecology of associated water bodies. For instance, Thies et al. (2013) compared the diatom population of RG-influenced and reference streams in the NE Italian Alps, highlighting that both diatom species composition and diversity can be affected by the increase in water acidity determined in some cases by RG activity. Always in the Italian Alps, Ilyashuk et al. (2014, 2017) reported high concentrations of metals in the waters of RG lakes, which seem to explain the high rate of morphological abnormalities in chironomid population inhabiting lake sediments. Focusing on microbial community, Fegel et al. (2016) described the bacterial community composition in stream sediments collected close to the terminus of several glaciers and RGs,

distributed across three North America mountain ranges. They found that RG stream sediments were generally characterised by higher alpha diversity, and harboured a more cosmopolitan bacterial population, including genera more commonly found in soil. However, to our best knowledge, the potential impact of RG meltwaters on the total prokaryotic community of a complex freshwater ecosystem has not been investigated yet.

In this study, we focused on a RG-pond system located in North-western Italian Alps, whose structural settings and hydrological dynamics have been previously described by Colombo et al. (2017). This pond represents for many reasons a suitable model system to investigate the influence of RG on downstream aquatic ecosystems. The oligotrophic conditions, as well as the lack of algal or macrophyte cover on the bottom and the absence of anthropogenic disturbance reduce the number of factors influencing the microbial community inhabiting sediments. Moreover, the limited dimensions potentially increase its susceptibility to external environmental pressures (Buraschi et al. 2005, Hamerlik et al. 2014), and the presence of a localised inflow from the RG (Colombo et al. 2017) allows the definition of a hypothetical horizontal gradient in RG influence. It is important to specify that previous observations described this meltwater inputs as intermittent, concentrated only in the snow-free season and mainly linked to precipitation events. However, beside RG inputs can be limited in frequency and magnitude (Colombo et al. 2017), microbial communities can quickly react to environmental pressures acting directly or indirectly on them (Classen et al. 2015), becoming a powerful instrument for the detection of ecosystem perturbations.

Our hypothesis was that, despite the RG meltwater inputs being intermittent and limited in magnitude, their characteristic lower temperatures and higher levels of solutes in comparison to the pond waters, and their potential to carry microorganisms of subglacial origin, may influence the sediments geochemistry and microbial abundance and diversity. In order to test this hypothesis, we assessed archaeal and bacterial assemblages present in three main habitats (RG, soil and lacustrine sediments) of the RG-pond system. We

investigated whether the sediment microbial community and geochemistry varied across the pond, reflecting possible relationships with neighbouring compartments. Based on this information, we proposed a number of microbial markers that can be used to discern the influence of RG on proximal sediments.

3.3 Materials and methods

3.3.1 Study site

The Col d'Olen Rock Glacier ("Corno Rosso 2 Rock Glacier" in the Aosta Valley rock glacier cadastre, http://geonavsct.partout.it/pub/GeoNavSCT/index.html?metadato=MTD010N0001) is a talus-tongue shaped RG (Haeberli et al. 2006), covering an area of about 37,500 m^2. It is located in the NW Italian Alps, at the boundary between the Aosta Valley and the Piedmont regions (45°52'8.22''N, 7°51'46.98''E, WGS84; Fig. 3.1a). The surface is covered by clasts (calcschists and serpentinites) varying from pluri-decimetric to metric size, with outcrops of fine-grained, serpentinitic material at the terminus and along the lateral scarps. The Col d'Olen Rock Glacier Pond is situated at the RG terminus, on the right side of the tongue (Fig. 3.1b), at an elevation of 2722 m a.s.l.. The pond has an area of 1,600 m^2, with maximum length and width of approximately 60 x 40 m, and reaches a maximum depth of about 3 m. More details on the catchment structural settings and hydrological dynamics of the pond are described in Colombo et al. (2017). The research site is a node of the Long-Term Ecological Research (LTER) network in Italy (http://www.lteritalia.it).

3.3.2 Sampling procedures

For this study, different typologies of sample were considered in order to assess the microbial assemblages present in the different habitats of the system (Fig. 3.1b-c, Table 3.1). Sediment samples were collected at three points (S1, S2, S3) along a distance gradient starting from the RG front and crossing the lake in NE-SW direction. Points S1 and S3 were at approximately 1 m of water depth, while point S2 was positioned in the deepest point of the pond, at 3 m of water depth. The surface sediments (top 10 cm) were collected with a core sampler, transferred

in sterile plastic jars without preserving sediments stratification, and kept covered with water during the transportation. Fine-grained serpentinitic material (top 10 cm) was sampled on the RG front and soil was collected from the North vegetated slope. Only the top 10 cm, after removal of vegetation and roots, were considered. At every sampling point, three replicate samples of approximately 300 g located at a distance of about 50 cm from each other were collected. All the samples were kept refrigerated and in the dark during the transport. In the laboratory, material for chemical and scanning electron microscopic analysis was stored at 4 °C, while aliquots for molecular analysis were preserved at – 20 °C prior to further processing.

The complete sampling procedure was repeated for two consecutive years, always during the advanced snow-free season (September 9th 2015, July 30th 2016), in order to avoid the additional effect of snowmelt processes on the system.

Figure 3.1 Location of the study area (a) and positioning of the sampling points (b, c) in the Col d'Olen Rock Glacier Pond and neighbouring compartments. In S1, S2 and S3 sediments and porewater were sampled and analysed. In RG and G fine material (0-10 cm depth) was sampled

Table 3.1 Description of the sampling sites.

Code	Environmental matrix	Dominant lithology	Depth (m)	n. of replicates
S1	Lacustrine sediment	Serpentinite	1	3
S2	Lacustrine sediment	Serpentinite	3	3
S3	Lacustrine sediment	Serpentinite	1	3
RG	RG fine grained surface material	Serpentinite	-	3
G	Soil	Calcschist	-	3

3.3.3 Samples physicochemical characterization

Lake sediments, RG material and soil

Solid samples were homogenized and sieved to 2 mm. A fresh aliquot of 20 g was extracted with 100 ml KCl 1M. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were determined on 0.45 µm filtered extracts with a total organic carbon analyser (Vario TOC, Elementar, Hanau, Germany). Ammonium and nitrate concentration in KCl extracts were measured spectrophotometrically (U-2000, Hitachi, Tokyo, Japan). For extractable ammonia determination a Berthelot method based on reaction with salycilate in presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson 1971) was used while, for extractable nitrate, the Gneiss reaction (Miranda et al. 2001) was applied. Total carbon (TOC) and nitrogen (TN) in the solids were determined on samples dried at 105°C by elemental analysis (NA2100, CE Instruments, Milan, Italy).

Porewater

Porewater was extracted from aliquots of fresh sediment by centrifugation and 0.20 µm filtered. pH was measured using a Basic 20 pH meter (Crison, Modena, Italy). Anions (NO_2^-, NO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-}) were analysed by ionic chromatography with a Dionex 500 (Thermo Scientific). Major cation (Ca^{2+}, Mg^{2+}, Na^+, K^+) concentrations were measured by Atomic Absorption Spectrophotometry (Perkin Elmer AAnalyst 1400), while Si was determined spectrophotometrically according to standard methods (Jones and Dreher, 1996).

3.3.4 Scanning electron microscopic (SEM)

Sampling point S2 was selected for scanning electron microscopic analysis as the most distinctive area of the pond, theoretically less influenced by the input of microorganisms from the neighbouring compartments. Fresh aliquots of sediment samples collected in 2015 were prepared for scanning electron microscopic analysis by spreading them onto a 0.2 µm sterile filter. Filters were partially dried at room temperature for 30 minutes and cut. Samples were fixed at 4°C for 12 h

in glutaraldehyde and dehydrated by a combination of ethanol baths at increasing concentration and hexamethyldisilazane (HMDS), as described by Araujo et al. (2003). Dry samples were coated with graphite and observed under a Cambridge S-360 SEM.

3.3.5 DNA extraction

Genomic DNA from sediments, soil and RG fine-grained material was extracted from 0.5 g of sample using the FastDNA™ SPIN Kit for Soil and the FastPrep® Instruments (MP Biomedicals) in accordance with the manufacturer's instructions. Extracted DNA was quantified using Qubit® (Life Technologies), quality was assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fischer Scientific) and DNA integrity was evaluated by agarose gel electrophoresis.

3.3.6 Quantitative PCR (qPCR)

Quantification of 16S rRNA genes was performed by quantitative PCR (qPCR) using primer pairs 340F-1000R for Archaea (Gantner et al. 2011) and 338F – 518R (Muyzer et al. 1993) for Bacteria. The reaction mixture contained 10 µl of SsoAdvanced™ SYBR® Green Supermix, 0.3 µM of each primer, and 2 µl of DNA diluted 1:10 (sediment, RG samples) or 1:100 (soil samples), in a total volume of 20 µl. Plasmids including a single copy of a single bacterial or archaeal 16S rRNA gene, amplified with the same primers used for qPCR, were serially diluted and used as standard for DNA quantification.

qPCR reactions were performed using a Chromo4™ Real Time PCR Detection System (Bio-Rad Laboratories), and data were analysed with the MJ Opticon Monitor software (version 3.1). Both samples and standards were analysed in triplicate in PCR strip tubes (Bio-Rad Laboratories), under cycling conditions reported in detail in Annex I. PCR specificity was verified by melting curves analysis and bands visualisation on agarose gel. Standard curves R^2 values were always higher than 0.997, and all the reactions showed efficiencies higher than 85%.

3.3.7 16S rRNA gene fragment sequencing and data processing

The V4-V5 regions of bacterial and archaeal 16S rRNA genes were PCR-amplified using primer pair 515F-Y and 926R (Parada et al. 2015).. PCR reactions were performed in triplicate in a 25 µl reaction volume containing 1x KAPA HiFi HotStart Ready Mix, 0.2 µM forward and reverse barcoded primers and 12 ng of DNA. Cycling conditions, resumed in Annex 3.1, were as described by Parada et al. 2015. After the amplification, PCR products were checked for presence and intensity by electrophoresis on 1.5% agarose gel, and replicates of the same sample were pooled.

Amplicon purification, 16S library preparation and sequencing by MiSeq Illumina (Illumina, San Diego, CA) using a 2 x 300 bp paired-end protocol were performed at the Bristol Genomics Facility (Bristol, UK).

Raw Illumina reads quality was checked with Prinseq (Schmieder and Edwards 2011), and terminal portions with an average quality score below q15 across a 5 bp sliding window were trimmed. Forward and reverse priming regions were removed and sequences resulting shorter than 230 bp after the quality trimming were discarded. Paired-end reads were merged using FLASH (Magoč and Salzberg 2011) and processed sing QIIME pipeline (Caporaso et al. 2010a). Chimeric sequences were detected and removed using UCHIME2 in mode sensitive and Gold database as reference (Edgar 2016). Sequences showing >97 % similarity were grouped in OTUs using uclust (Edgar 2010), and for every cluster the most abundant sequence was chosen as representative. The taxonomic affiliation of the OTUs was assessed by RDP classifier (Wang et al. 2007) against the SILVA 128 database (Quast et al. 2013) at a 97% confidence cut-off, and only representative sequences able to align to SILVA database were conserved and used for OTU table construction. Sequences appearing one time in a single sample were discarded, and one of the soil samples collected in 2015 (G3_15) excluded from the analysis due to the remarkably lower number of available sequences. OTU tables containing bacterial and archaeal sequences were obtained and analysed separately, and sequences deriving from mitochondria or chloroplasts were filtered from the bacterial OTU table.

3.3.8 Diversity and statistical analysis

Rarefaction curves were constructed without applying any normalisation on the number of sequences per sample, while for alpha and beta diversity evaluation, the number of sequences per sample was normalised according to the less abundant sample. Alpha diversity estimators (number of OTUs, Chao1, Shannon and Simpson indexes) were calculated on multiple rarefied OTU tables using QIIME (Caporaso et al. 2010a). The same program was used to align bacterial or archaeal OTU sequences (Caporaso et al. 2010b), to build the corresponding phylogenetic trees (Price et al. 2010), and to compute Bray-Curtis, Weighted and Unweighted UniFrac distance matrixes (Lozupone and Knight 2005) based on similarities at OTU level. Ordination of samples according to the different distance matrixes was performed by non-metric multidimensional scaling (NMDS), and the significance of the separation among environmental matrixes and sampling points was evaluated by PERMANOVA (permutational multivariate analysis of variance) using the adonis function of the 'vegan' package in R (Oksanen et al. 2005). In order to show correlations between prokaryotic community structure and environmental parameters a further NMDS ordination was obtained based on a Bray-Curtis distance matrix built from pooled archaeal and bacterial OTU tables. Vectors representing physicochemical properties of the different environmental matrixes were then fitted onto the biplot and the significance of correlations existing between them and the overall ordination was tested by using the 'vegan' function envfit.

Geochemical data, 16S rRNA genes abundance, alpha diversity estimators and the relative abundance of the most abundant bacterial and archaeal classes were compared among the different sampling points, by analysing separately 2015 and 2016 datasets. Data were transformed if required, normal distribution of residuals and homogeneity of variances were tested and, when appropriate, ANOVA was applied. In presence of significant differences, pairwise comparisons were performed using Tukey's honest significance difference (HSD) test, and differences were considered significant for $P<0.05$. When ANOVA assumptions were violated, Kruskall-Wallis nonparametric test

coupled with Dunn's test as post hoc was applied. Moreover, the presence of differences in the same group of parameters between the two sampling years was tested following a multivariate approach. Untransformed data were standardised and compared using the anosim function ('vegan' package).

Heatmaps reporting the relative abundance of the main bacterial phyla and the dominant taxon-specific OTUs were elaborated with 'pheatmap' package (Kolde et al. 2012). All the other plots (diversity bar plots, boxplots and NMDS plots) were drawn with 'ggplot2' package (Wickham et al. 2009).

3.3.9 Data accessibility

Raw sequencing data were deposited in NCBI SRA database under accession number SRP126235.

3.4 Results

3.4.1 Sediments, soils and porewater geochemistry

All the considered geochemical parameters showed a certain degree of variation in relation to the environmental matrix (Fig. 3.2). Total C and N, as well as DOC and TDN showed significantly lower values in RG samples, intermediate values in the pond sediments and the highest values in soil (G). RG samples were also characterised by the lowest levels of ammonia, while the highest levels were detected in sediment samples. The C/N ratio in RG was higher than in sediments and soil. For nitrates, quite homogeneous values were reported for all the samples but G, RG and S3 in 2015.

Table 3.2 Chemical characterisation of porewaters extracted from pond sediments. The maximum and minimum values measured for each year are reported

	2015	2016
pH	5.70 – 8.50	7.10 – 7.90
Si (mg L^{-1})	0.25 – 3.89	0.62 – 1.82
Na^{+} (mg L^{-1})	0.36 – 1.72	0.37 – 0.75
K^{+} (mg L^{-1})	0.96 – 4.60	1.01 – 4.52
Ca^{2+} (mg L^{-1})	5.61 – 50.9	2.66 – 14.7
Mg^{2+} (mg L^{-1})	2.50 – 22.4	1.02 – 4.02
Cl^{-} (mg L^{-1})	0.68 – 4.98	0.11 – 1.52
NO_2^{-} (mg L^{-1})	0.01 – 0.06	0.01 – 0.03
NO_3^{-} (mg L^{-1})	0.01 – 4.46	< 0.01 – 0.05
SO_4^{2-} (mg L^{-1})	1.67 – 11.4	< 0.01 – 17.6
PO_4^{3-} (mg L^{-1})	0.01 – 0.02	0.01 – 0.04

Focusing on lacustrine sediments, a general porewater characterisation is reported in Table 3.2. When comparing sediments collected at different sampling

points (Fig. 3.2), several geochemical properties appeared to follow a similar trend in both the sampling times. For instance, DOC, TDN, NH_4^+ concentrations and porewater pH were higher in the central, deepest samples (S2), and lower in S1 and S3.

3.4.2 Scanning electron microscopy

The SEM observation of sediment samples revealed the presence of a variety of diatoms-like cells among sediment particles, although in many cases they appeared to be partially damaged (Fig. 3.3); also, a potential damage due to the sample treatment cannot be totally excluded. However, the examination did not allow the visualisation of other microbial cells.

3.4.3 Bacterial and archaeal 16S rRNA genes abundance

16S rRNA genes abundance showed the lowest values in RG samples, with an average of 9.33 ± 0.24 and 7.51 ± 0.07 Log copies per g of dry weight for Bacteria and Archaea, respectively (Fig. 3.2). Soil was characterized by higher abundances: between 9.96 and 11.15 Log copies per g of dry weight for Bacteria, and between 7.99 and 8.88 Log copies per g of dry weight for Archaea. A more heterogeneous situation was reported for sediments, with bacterial abundance ranging between 9.64 and 11.01 Log copies per g of dry weight, and Archaea between 6.97 and 8.89 Log copies per g of dry weight. In general, in all the environmental matrixes 16S bacterial markers outnumbered their Archaea counterpart of at least 1 or 2 orders of magnitude.

3.4.4 Bacterial and archaeal community diversity

A total of 6,131,017 high-quality sequences was obtained after trimming and filtering procedures. 96.5 % of the sequences was classified as Bacteria (63,962 OTUs), 3.5 % as Archaea (2478 OTUs), and a negligible number remained unclassified at domain level.

Analysis of rarefaction curves (Annex II) confirmed that a satisfactory level of coverage (Good's Coverage estimator higher than 94%) was achieved for bacterial sequences in all the samples, while lower coverage values were reported

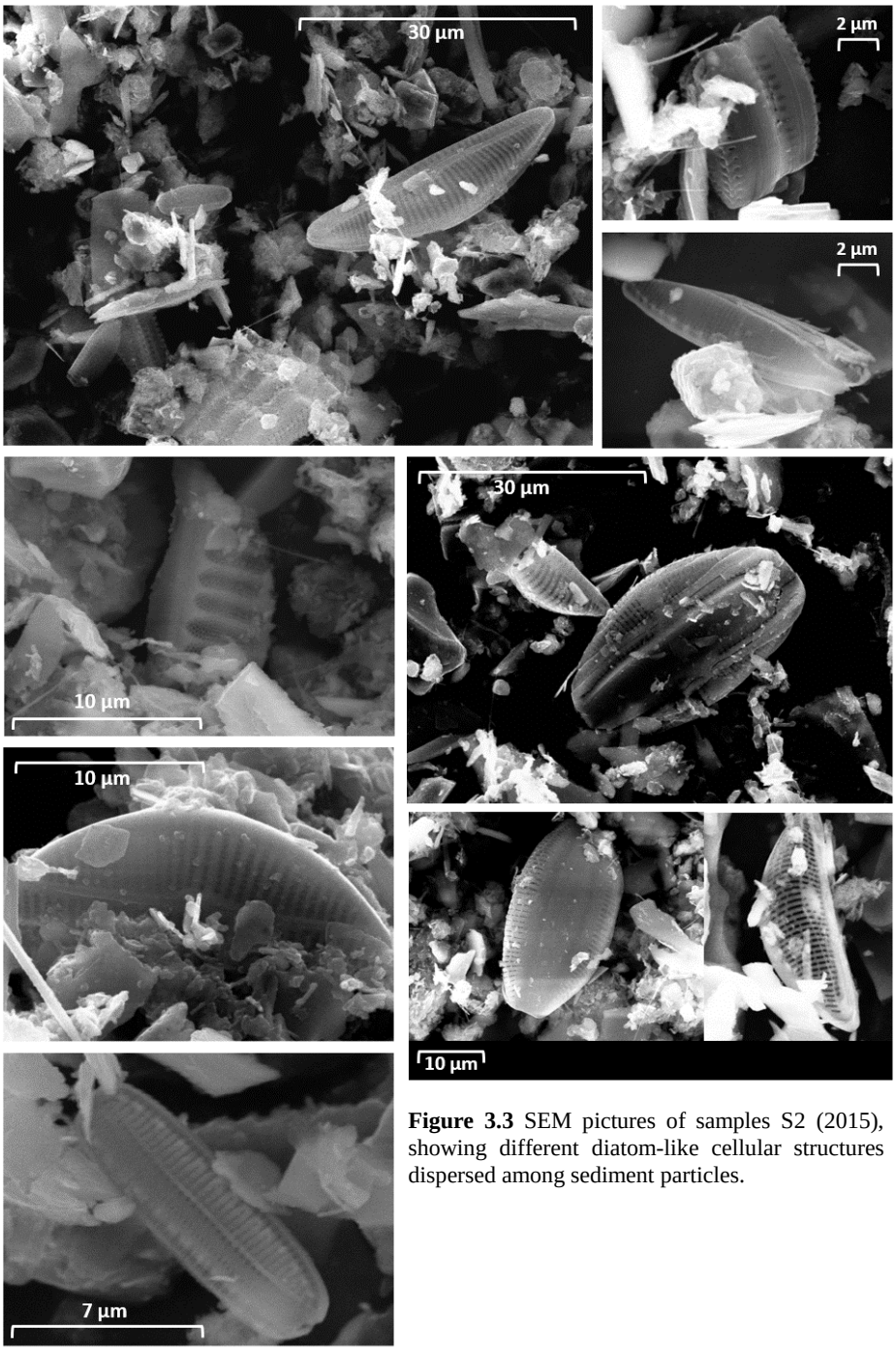


Figure 3.3 SEM pictures of samples S2 (2015), showing different diatom-like cellular structures dispersed among sediment particles.

in sediment samples for Archaea (average 80%). This is coherent with trends in alpha diversity estimators (Table 3.3) applied to the archaeal community,

showing a decrease in richness (OTUs number, Chao1 index), Shannon and Simpson diversity indexes from sediment to soil, to RG communities. However, comparing sediments, lower diversity characterized samples S3, particularly in 2015. Concerning bacteria, shallow sediments (S1, S3) showed higher Chao1 values if compared with RG and G samples, but they did not differ significantly in terms of observed OTUs, Shannon and Simpson indexes. Conversely, sample S2 always reported significantly lower richness and diversity than all the other samples.

Table 3.3 Alpha diversity estimators calculated for archaeal and bacterial communities in the different sampling sites and times. Different letters indicate significant differences among samples collected in the same year ($P<0.05$).

	Observed OTUs	Chao 1	Shannon index	Simpson index	Good's Coverage
Archaea					
S1_15	172 a	416 a	6.36 a	0.97 a	0.75 c
S2_15	175 a	383 a	6.36 a	0.97 a	0.75 c
S3_15	78 b	194 b	4.51 b	0.89 ab	0.91 b
RG_15	20 c	34 c	1.98 d	0.60 b	0.98 a
G_15	23 c	37 c	2.90 c	0.81 b	0.98 a
S1_16	165 a	424 a	6.23 a	0.97 a	0.75 b
S2_16	151 a	356 a	5.78 a	0.94 a	0.78 b
S3_16	130 a	274 a	5.50 a	0.93 a	0.83 b
RG_16	24 b	57 b	1.88 b	0.59 c	0.97 a
G_16	25 b	51 b	2.95 b	0.81 b	0.98 a
Bacteria					
S1_15	5707 a	11018 a	9.38 a	0.99 a	0.94 b
S2_15	4773 b	10202 ab	8.46 b	0.98 b	0.95 ab
S3_15	5141 ab	10173 ab	9.25 a	0.99 a	0.95 ab
RG_15	4610 b	8903 b	9.24 a	0.99 a	0.95 a
G_15	4795 b	8994 b	8.96 ab	0.99 a	0.95 a
S1_16	6078 a	11723 a	9.61 a	0.99 a	0.94 b
S2_16	4412 b	9513 ab	7.74 b	0.95 b	0.95 ab
S3_16	5311 ab	10585 ab	9.43 a	0.99 a	0.95 ab
RG_16	4603 b	8881 b	9.26 a	0.99 a	0.96 a
G_16	4755 b	8851 b	8.89 ab	0.99 ab	0.95 a

For beta diversity, NMDS ordination plots based on different distance estimators all showed a strong separation among communities inhabiting different environmental matrixes, while no apparent differences in terms of sampling year were detectable (Annex III). In particular, for archaeal community Bray-Curtis and Unweighted UniFrac distances defined a sharp separation among sediment, RG and soil samples, while Weighted UniFrac suggested higher similarity between soil and RG communities. For bacteria, all the tested distances produced separate clusters corresponding to different sample typology and were able to divide shallower sediment samples (S1, S3) from the deeper sediment sample S2. PERMANOVA analysis on Bray-Curtis, Unweighted and Weighted UniFrac distance matrixes confirmed the presence of significant differences ($P<0.001$) in archaeal and bacterial community structure among different environmental matrixes. Moreover, samples ordination showed a high degree of correlation with several environmental parameters (Fig. 3.4, Table 3.4). For instance, RG samples were characterised by higher C/N ratios and lower levels of all the other parameters including bacterial and archaeal abundance, while sediment samples were differentiated by the higher concentration of NH_4^+. Only NO_3^- showed no significant correlation with the samples distribution.

Table 3.4 Correlation values and significance between the investigated environmental variables/microbial abundances and the NMDS factors shown in Fig. 3.4.

	r^2	p-Value
TN	0.843	0.001
TOC	0.871	0.001
C/N	0.657	0.001
DOC	0.449	0.001
TDN	0.489	0.001
NO_3^-	0.185	0.061
NH_4^+	0.553	0.001
Arch	0.427	0.002
Bact	0.441	0.003

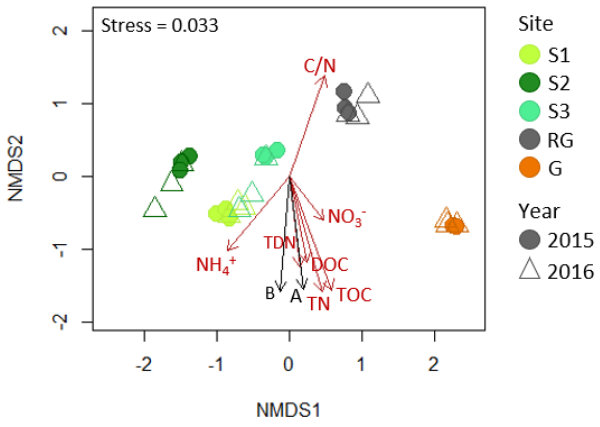


Figure 3.4 NMDS ordination of the five sampling points based on Bray-Curtis distance matrix calculated on pooled archaeal and bacterial OTUs frequencies. Vectors show the direction and strength of environmental variables (red arrows) and 16S rRNA genes abundance (black arrows).

3.4.5 Composition of archaeal and bacterial communities

The taxonomic composition of the archaeal community was clearly differentiated based on sample type (Fig 3.5a, Annex IV). Terrestrial ecosystems were dominated by Thaumarchaeota, representing approximately 90 % and 80 % of RG and G sequences respectively. However, while RG surface material community included mainly members of Soil Crenarchaeotic Group (SCG), in soil a pool of additional classes (e.g. FHMa11terrestrialgroup, SAGMCG-1) was also present. Moreover, by examining in detail the sequences affiliated with SCG, a separation between RG and soil samples in terms of dominant OTUs emerged. Thaumarchaeota were detected also in sediment samples, but only with Marine Group I class, and showed one dominant OTU identified as Cand. *Nitrosoarchaeum* accounting for 80-99% of total Thaumarchaeota sequences. The most represented phyla in sediment samples were Woesearchaeota (49-63 % of sequences per sample) and Euryarchaeota (16-38 %), the latter including mainly Thermoplasmata and Methanomicrobia classes. Despite the diffusion of Thermoplasmata in all the samples, communities associated to different environmental matrixes showed a clear separation at family level.

For bacterial community (Fig. 3.5b, Annex V), all the samples across all habitats harboured essentially the same classes, but variations in their relative abundance distinguished communities belonging to different environmental matrixes. In particular, soil community differed from RG and sediment community due to significantly higher proportions of Alphaproteobacteria, Acidobacteria (Acidobacteria, Solibacteres) and Planctomycetes (Planctomycetacia) and lower proportions of Betaproteobacteria (including mainly Burkholderiales, Nitrosomonadales and SC-I-84 classes). Distinctive features were detectable also comparing RG and sediment samples: sediment community was characterised by higher Cyanobacteria, Deltaproteobacteria and Ignavibacteria relative abundances, while RG samples showed higher proportions of Actinobacteria (Thermoleophilia, Actinobacteria belonging to Frankiales, Micrococcales and Propionibacteriales), Acidobacteria (Blastocatellia) and Bacteroidetes (Sphingobacteriia).

When comparing only sediment samples, some microbial groups appeared to follow peculiar trends. Cyanobacteria proportion, for instance, appeared to be higher in central (S2) than in shallower sediment samples (S1, S3), while an opposite trend was reported for Thaumarchaeota and Acidobacteria. Moreover, some classes (e.g. Gemmatimonadetes, Blastocatellia) exhibited a reduction in percent abundance along a distance gradient moving from the RG front.

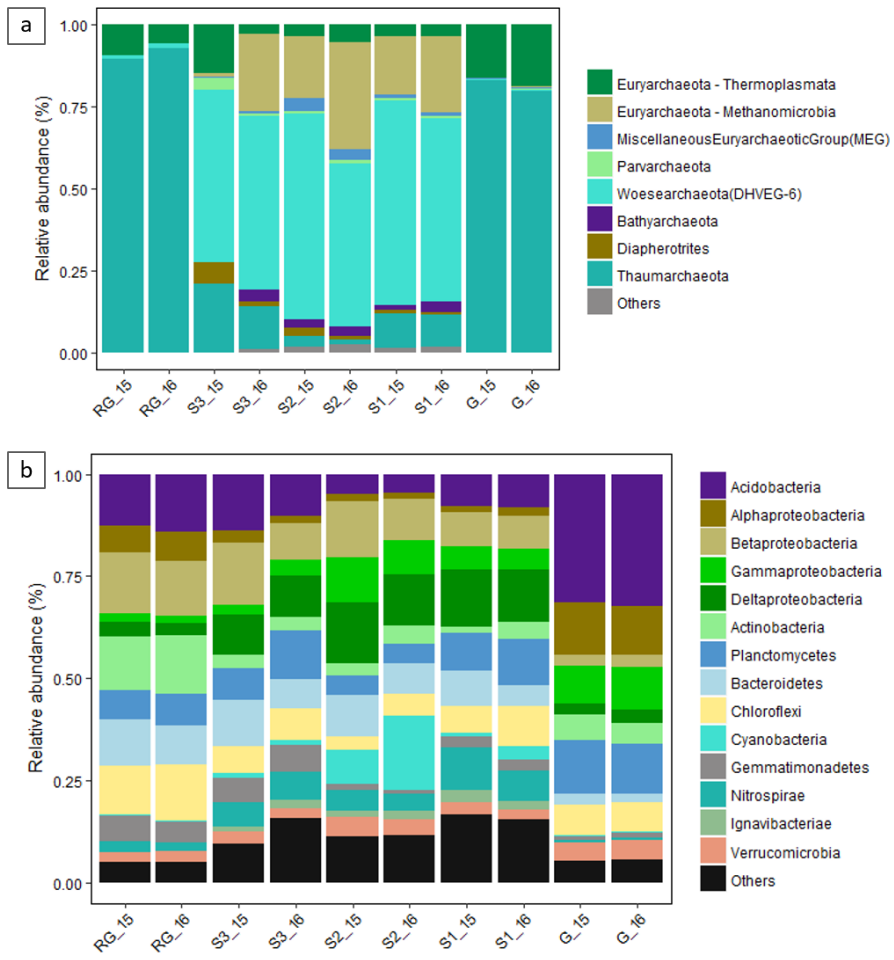


Figure 3.5 Relative abundance of the major archaeal (a) and bacterial phyla (b), with Proteobacteria split in classes, in the different sampling points and times. Each bar represents the average of three replicate samples. The group 'others' include all the taxonomic groups not explicitly reported in the legend.

Finally, samples S3 collected in 2015 displayed anomalous characteristics in terms of community composition, if compared with other sediment samples, including the three collected in 2016. For instance, they harboured lower proportions of Bathyarchaeota and Methanomicrobia, and higher proportions of Thaumarchaeota and Betaproteobacteria belonging to the Oxalobacteraceae family. Moreover, when evaluating similarities in terms of dominant classes relative abundance, the samples S3_15 tended to cluster more strictly with RG samples than with other samples (Fig. 3.6).

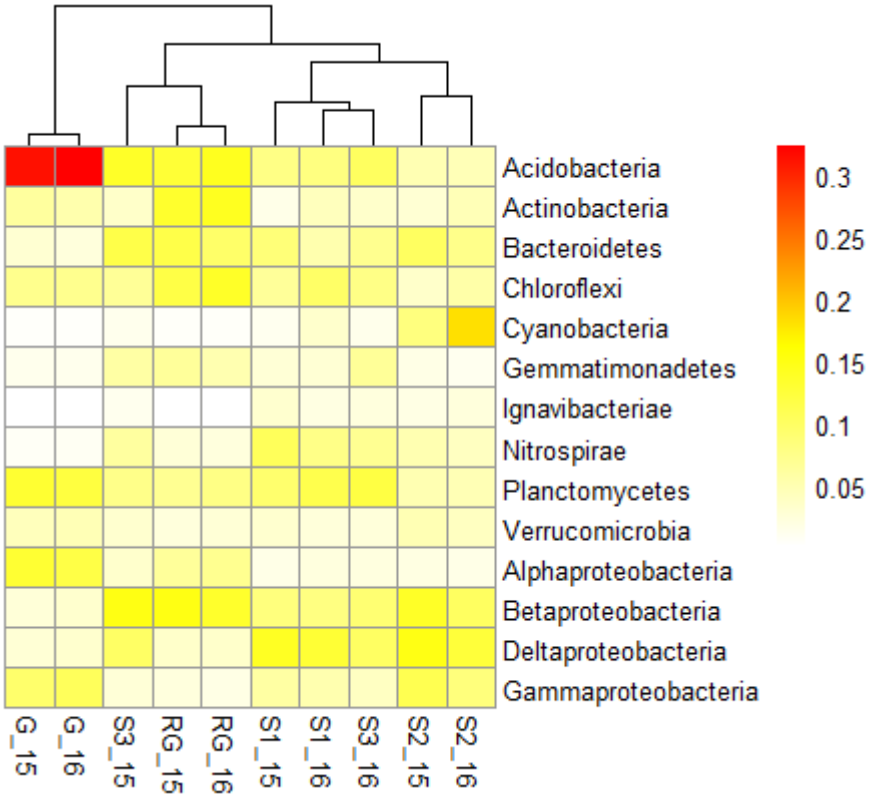


Figure 3.6 Heatmap showing the relative abundance of the major bacterial phyla in the different sampling points and times, with Proteobacteria split in classes. Values in each column represent the average of three replicate samples. Columns were clustered using UPGMA method, based on a Bray-Curtis distance matrix

3.5 Discussion

Despite the increasing interest on the effects of RG thawing, only few studies have considered its consequences on the ecology of downstream ecosystems (Thies et al. 2013, Ilyashuk et al. 2014-2017, Fegel et al. 2016). In this study, we sampled different compartments of an alpine RG-pond system to: 1) investigate their microbial community structure and identify the key microbial groups that characterise each habitat; 2) assess the potential links between microbial diversity and geochemistry; 3) propose microbial markers potentially indicating the presence of RG influence on the connected freshwater ecosystem.

In order to pursue our objectives, the choice of sampling times reflecting the same seasonal condition in both the considered years was fundamental. In this sense, the absence of significant differences in the whole RG-pond system between the two sampling years, as assessed by ANOSIM on a combination of geochemical, microbial abundance and alpha diversity data, seems to confirm the comparability of the 2015 and 2016 datasets.

3.5.1 Microbial community structure in the different habitats of an alpine RG-pond system

In general, beta diversity analysis highlighted a sharp separation among communities associated with different environmental matrixes, for both archaea and bacteria.

Soil bacterial community was dominated by Acidobacteria and Proteobacteria, followed by Planctomycetes, Chloroflexi, Actinobacteria and Verrucomicrobia, as previously reported for several alpine and high latitude tundra soils (Bradley et al. 2014, Jansson and Taş 2014, Lazzaro et al. 2015). However, while these studies included Cyanobacteria among the most represented phyla, in our case cyanobacterial sequences represented less than 1% of the total soil sequences. The archaeal community from soil also corroborated with other studies where a prevalence of Thaumarchaeota sequences, all affiliated with terrestrial groups, was found, followed by Euryarchaeota of the class

Thermoplasmata (Bates et al. 2011, Chroňáková et al. 2015, Siles and Margesin 2016).

Considering lacustrine sediments, a recent survey on 13 freshwater lakes of the Yunnan Plateau (southwestern China) highlighted that bacterial community composition can remarkably vary in terms of dominant phyla among different high elevation freshwater lakes, although factors governing the intra-lake distribution of sediment bacterial and archaeal communities remain unclear (Zhang et al. 2015). However, some common traits appear to be conserved among the abovementioned lakes and the Col d'Olen Rock Glacier pond bacterial community structure, with Proteobacteria (Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria), Planctomycetes (in Olen sediments mainly Phycisphaerae and Planctomycetacia) and Chloroflexi being the dominant groups. Nevertheless, other phyla often poorly represented in the Zhang et al. (2015) study, like Nitrospira, Gemmatimonadetes and Ignavibacteria had higher relative abundances in the Col d'Olen Rock Glacier pond.

Freshwater ecosystems have been reported to harbour, on average, more rich and diverse archaeal communities if compared with soil (Auguet et al. 2009), and this is the case of Olen pond system. Also, differences in dominant archaeal taxonomic groups among alpine lacustrine ecosystems exist and can be greater than those described for bacteria. For instance, sediment communities dominated by Bathyarchaeota and methanogenic Euryarchaeota (Methanomicrobia, Methanobacteria and Thermoplasmata classes) have been described in lakes of the Yunnan and Tibetan Plateau in China (Zhang et al. 2015; Fan and Xing 2016, Liu et al. 2016), or in a number of water systems in the Iberian Peninsula (Fillol et al. 2015, Comte-Port et al. 2017). However, even if 16-38% of total Archaea sequences affiliated to Euryarchaeota (mainly Methanomicrobia), Olen pond showed a quite different situation, with only small proportions of Bathyarchaeota (0.2-4%) and a prevalence of Woesarchaeota (49-63%). Analogous archaeal community composition has previously been described in waters of high-altitude Pyrenean lakes (Ortiz-Alvarez and Casamayor 2016).

The RG fine-grained surface material offered the harshest conditions for life of all the investigated compartments, probably due to a combination of physical instability and lack of vegetation cover. The low TOC and TN and the high C/N ratio, potentially indicating limited rates of mineralization, seem to confirm the impact of such environmental constraints on resident microbial communities. However, these challenging conditions seem to influence Bacteria and Archaea in different ways. Bacterial abundance was significantly lower in the RG samples than in soil or sediments, but community richness and evenness were comparable in all habitats. Conversely, for Archaea no clear differences were detected between RG and sediments for the 16S copy number, but alpha diversity was lower in RG than in sediments. This might suggest the presence of a less diverse but highly specialized archaeal population, able to proficiently colonise the RG surface. For instance, Thermoplasmata in RG samples included mainly representatives of Marine Group II plus two highly abundant unidentified OTUs, while soil was dominated by sequences of the Terrestrial Miscellaneous Group (TMEG) and sediments showed more complex assemblages.

The comparison of the bacterial community showed a RG community more closely related to sediment than soil, where proportions of phyla typically associated to terrestrial habitats like Acidobacteria, Actinobacteria and Alphaproteobacteria were high. If the separation between RG and soil communities may be partially attributed to differences in lithology (serpentine vs calcschist), as demonstrated in other alpine, cryoturbated soils (Mania et al. 2016), the different stage of habitat evolution is also likely to play an important role. The presence of Sphingobacteriales, Actinobacteriaceae affiliated to Micrococcales and Propionibacteriales, and Comamonadaceae in the RG matrix resembled other high elevation, poorly evoluted soil ecosystems, such as unvegetated glacier forefields (Nemergut et al. 2007) and debris-covered glaciers (Franzetti et al. 2013).

3.5.2 Relationships between geochemistry and microbial community structure

In this study, we also aimed to describe variations in microbial community composition and abundance across the pond, in relation to sediment geochemistry. Despite the choice of only three sampling points at two different depths in the pond system, prevented detailed definition and verification of horizontal and vertical trends, some insights on the overall ecosystem functioning were attempted in this study. For instance, our initial hypothesis was that the presence of RG hydrological inputs into the pond documented by Colombo et al. (2017), although limited in time and magnitude, would have led to a differentiation of sediments closest to the RG front from the farther ones, at least in terms of microbial community. Instead, we found out that the strongest differences in terms of both geochemistry and microbial diversity exist between shallow and deep sediments, regardless their distance from the RG front, and this trend seems to be conserved over time. Water depth has been previously reported as one of the factors involved in community shaping in lacustrine and marine sediments (Edlund et al. 2006), but it is interesting to find similar significant differences at a small scale (2 m of variation in water depth, about 30 m of horizontal distance), in a well-mixed, not stratified lacustrine ecosystem. Among the Archaea, the reported separation pond centre-borders is limited to the Thaumarchaeota phylum, showing higher relative abundance in shallow than deep sediments. The absolute prevalence in sediments of sequences affiliated to Marine Group I and identified as Candidatus *Nitrosoarchaeum*, commonly found in freshwater ecosystems (Coci et al. 2015, Fillol et al. 2015), excludes the hypothesis of an accumulation of microorganisms or extracellular DNA from surrounding compartments (richer in Thaumarchaeota sequences, but mainly affiliated to others classes), suggesting that factors regulating their distribution must depend on lake intrinsic dynamics. However, the clearest picture of intra-pond variation is offered by the analysis of the bacterial community structure. In this case, the deepest samples were clearly separated from the others not only in

terms of overall community structure (as shown by NMDS analysis based on both phylogenetic and not phylogenetic distances). They also showed a significantly higher proportion of Cyanobacteria and higher levels of DOC, TDN, NH_4^+ and pH, suggesting the deepest region of the pond as a hot spot of primary production supported by Cyanobacteria, at least during the snow-free season. In addition, the high number of diatom-like cellular structures observed by SEM in the central samples, at least in 2015, give some hints about the potential contribution of diatoms to primary production at the same site. These observations may be consistent with the more stable conditions offered throughout the year by the pond deepest zone, apparently not affected by freezing phenomena that regularly occur in the lateral, shallower areas. However, due to the lack of accurate measurements of sediment temperature dynamics during the year, or data on solar radiation penetration in different points of the pond, these considerations remain of a speculative nature.

3.5.3 Microbial markers in alpine RG-pond systems

In the last part of this study, we have considered possible candidates for microbial markers that can be used to indicate the presence of RG inputs in a connected alpine freshwater ecosystem. For this purpose, we focused on the sampling point closest to the RG inflow (S3), and we investigated whether any microbial group associated with RG habitat was found to be exclusively present, or enriched in terms of relative abundance, in S3 samples with respect to other sediments. However, as discussed above, no modifications in prokaryotic community distinguishing S3 sediments from the others and connecting them to RG habitat were found to be conserved in both the years. This may be due to the absence of an actual influence of RG inputs on sediments microbial community, or to the presence of an influence limited in time, that our approach (DNA-based, focused on a single sampling time per year) was not able to detect. Nonetheless, we did observe a series of anomalies in the S3 samples collected in 2015, suggesting that in some

circumstances the RG influence might be enhanced and become evident. First, a peak in nitrate concentration in sediment pore water was reported for those samples. This is consistent with the increase in electrical conductivity described by Colombo et al. (2017) in Col d'Olen pond waters in the area close to the RG front at the end of the summer 2015, in association with lower water temperatures. Since nitrates are among the solutes most commonly found to enrich waters originating from RG thawing (Williams et al. 2007, Fegel et al. 2016), and considering that we sampled at the end of a phase of high RG hydrological contribution (Colombo et al. 2017), we assume that our samples were influenced by this occurrence.

From a microbiological point of view, the abovementioned anomalies concerned mainly archaeal population, especially in terms of community composition (lower proportions of Bathyarchaeota and Methanomicrobia, higher relative abundance of Diapherotrites and Thermoplasmata). Interestingly, around 96% of Thermoplasmata sequences in samples S3_15 corresponded to a single unidentified OTU, present in lower proportions in all the other pond sediments samples. This and the significant reduction of richness and evenness described for the same samples supports the idea that the registered shift in archaeal community is not linked to the introduction of microorganisms from other compartments, but to a process of selection directly or indirectly driven by the RG influence (e.g. water temperature, nitrates concentration) on the downstream ecosystem. For Bacteria, the samples S3_15 showed higher relative abundance of Oxlobacteraceae, if compared with other sediment samples. In this case, the most abundant OTUs were mainly distributed between S3 and RG samples and, considering that Oxalobacteraceae belongs to Burkholderiales, an order of Betaproteobacteria including many chemolithotrophic and autotrophic microorganisms, frequently associated with glacial and subglacial environment (Ambrosini 2016, Franzetti et al. 2013), this is coherent with the hypothesis of a localised, meltwater-driven microbial enrichment.

In conclusion, our results showed that different habitats of the Col d'Olen RG-pond system harboured different, well separated archaeal and bacterial

communities. They also suggest that the presence of inputs from an active RG has only a limited influence on abundance and diversity of microbial communities inhabiting sediments of an adjacent water body. Indeed, no evidence of a stable modification of microbial ecology or sediments geochemistry in the area closer to the predicted RG inflow has been found, although signals of a response to stronger input events were recognizable. These included modifications in Archaea community composition and diversity and an increase in the relative abundance of Oxalobacteraceae, all likely to be potential markers of subglacial influence on downstream ecosystem. On the other hand, factors involved in stably shaping microbial diversity and distribution in the overall pond system seem to be more linked to dynamics intrinsic to the pond, such as differences in water column depth or the presence of hot spots of primary production, than to RG influences.

3.6 Acknowledgements

We would like to thank Danilo Godone and Davide Viglietti for their help in field work activities and laboratory analyses, Emanuele Costa for the SEM observations and the family Beck-Peccoz, Consorzio di Miglioramento Fondiario di Gressoney (Aosta) and MonteRosa-ski for allowing the access to the study site. This study was partially financed by the UK NERC grant (NE/J02399X/1) to A. M. Anesio.

3.7 References

References of this chapter are integrated into the overall reference section (Chapter 6).

3.8 Annexes

Annex I Primer pairs and PCR conditions used in this study.

Primer	Sequence (5'-3')[a]	Target gene	Fragment length	Reference	Amplification details	Application
515F-Y[b]	GTG YCA GCM GCC GCG GTA A	16S rRNA *Bacteria* + *Archaea*	c 450 bp	Parada et al. 2015	95°C 3 min; 25 cycles: 95°C 45 s 50°C 45 s 68°C 90 s; 68°C 5 min	Illumina sequencing
926R[b]	CCG YCA ATT YMT TTR AGT TT					
338F	ACT CCT ACG GGA GGC AGC AGC AG	16S rRNA *Bacteria*	197 bp	Muyzer et al. 1993	95°C 30s; 40 cycles: 95°C 30 s 50°C 30 s 72°C 45 s	qPCR
518R	ATT ACC GCG GCT GCT GG					
340F	CCC TAY GGG GYG CAS CAG	16S rRNA *Archaea*	660 bp	Gantner et al. 2010	98°C 3 min; 40 cycles: 95°C 30 s 57°C 30 s 72°C 30 s	qPCR
1000R	GAG ARG WRG TGC ATG GCC					

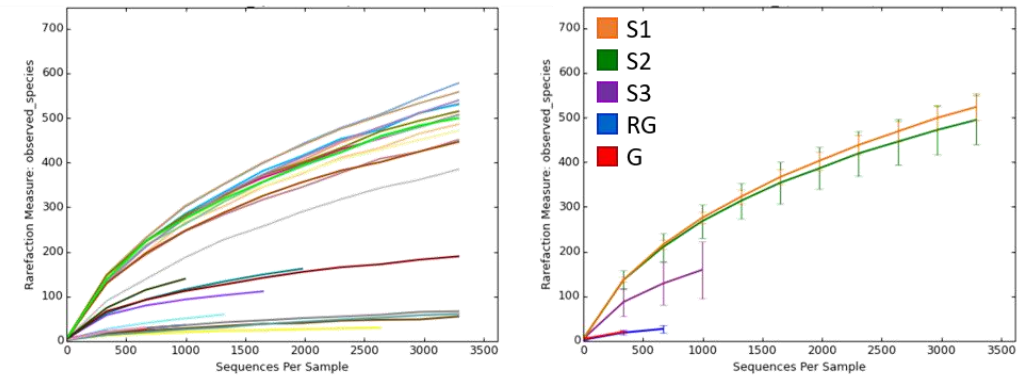
[a] D=G, A or T; H=A, T or C; K=G or T; M=A or C; R=A or G; S=G or C; W=A or T; Y=C or T

[b] Primers linked in 5' to Illumina overhang adapter sequences:
Forward overhang (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG)
Reverse overhang (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG)

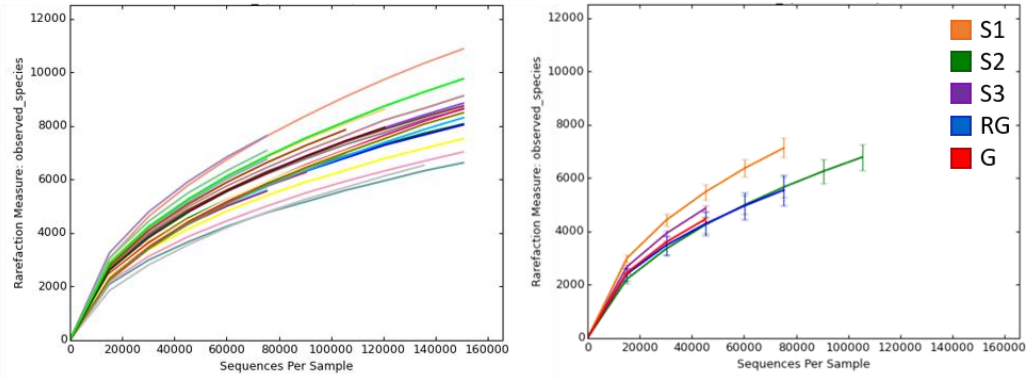
Annex II

Rarefaction curves obtained for Archaea (a) and Bacteria (b) communities, representing all the samples and times (left) or the average per sampling point (right).

a

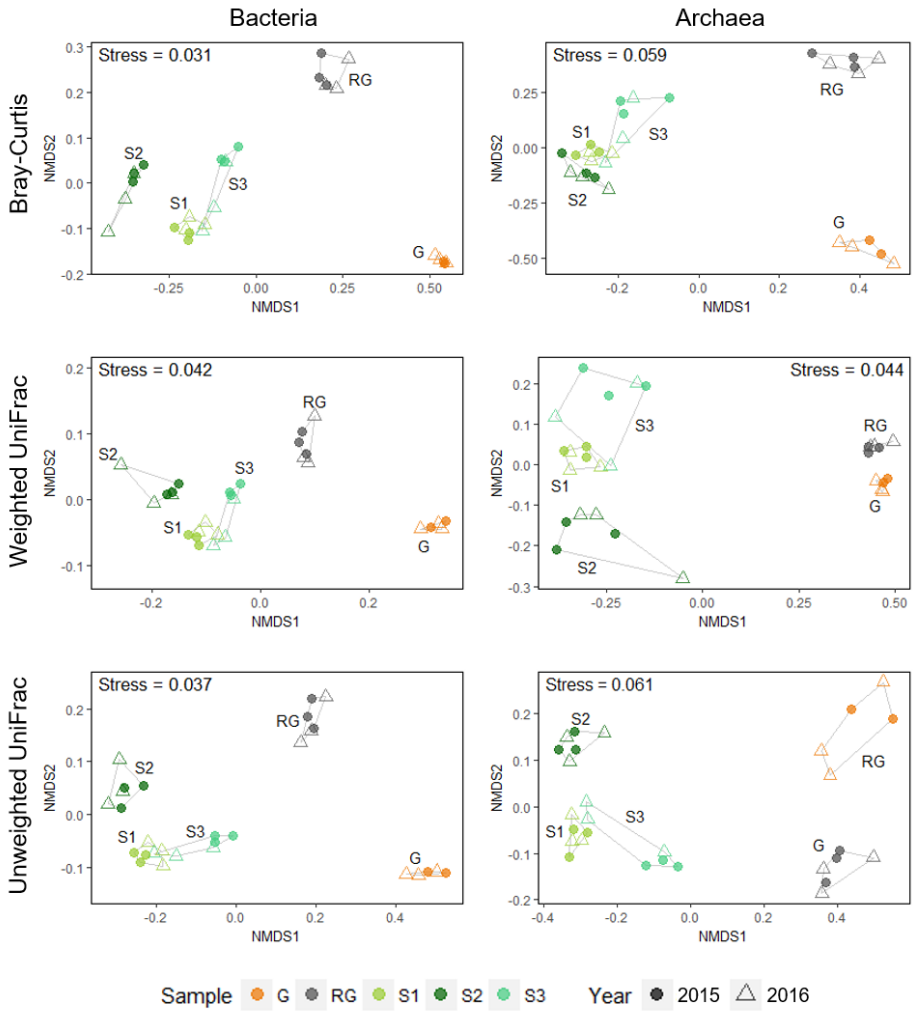


b



Annex III

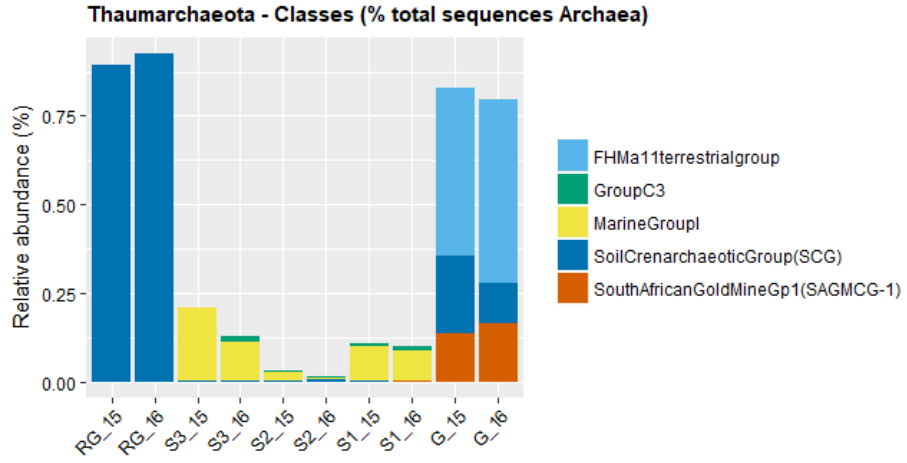
NMDS ordination based on Bray-Curtis, Unweighted and Weighted UniFrac distance matrixes calculated using total archaeal and bacterial OTUs.

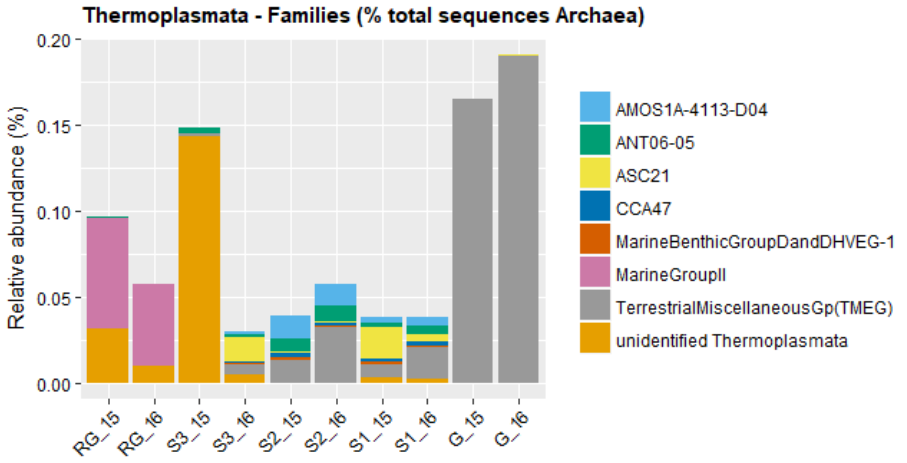


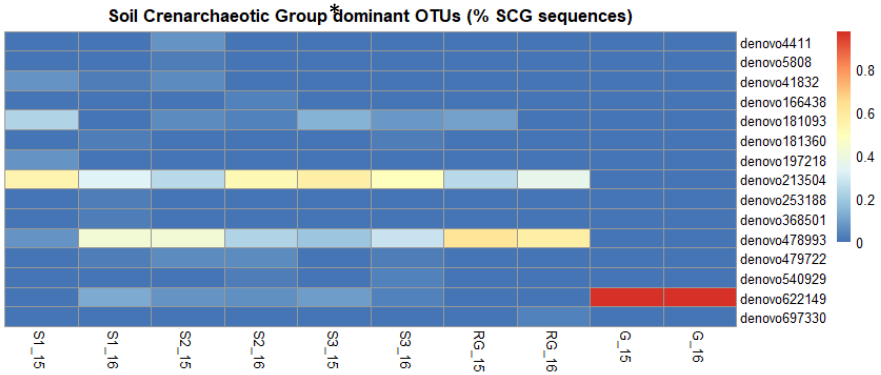
Annex IV

Graphs and tables summarising the composition of selected taxonomic groups among Archaea.

Values reported for each sample (sample_year) represents the average among three replicate samples. For each graph the dataset used to calculate relative abundances and, in case of data filtering, the applied thresholds are specified.







*Only OTUs with relative abundance higher

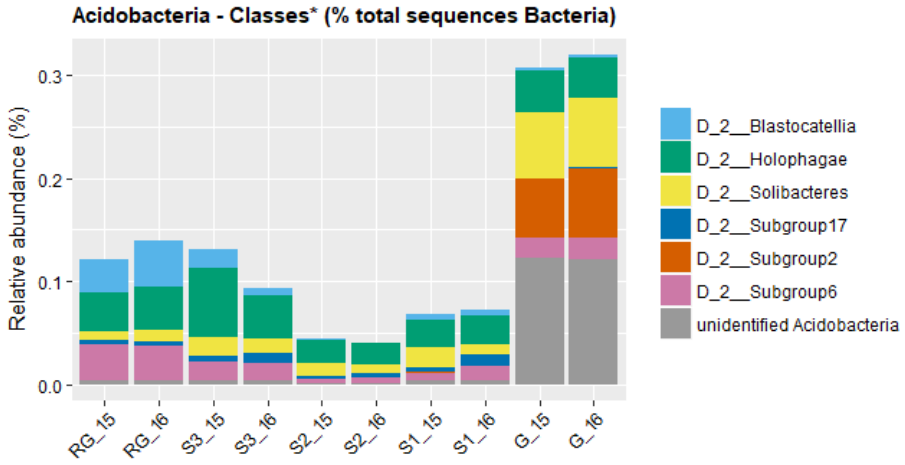
Taxonomic affiliation (97% similarity cutoff) of dominant SCG OTUs

	Order	Family	Genera	Specie
denovo4411	D_3__unculturedbacterium	D_4__unculturedbacterium	D_5__unculturedbacterium	D_6__unculturedbacterium
denovo5808				
denovo41832				
denovo166438	D_3__unculturedcrenarchaeote	D_4__unculturedcrenarchaeote	D_5__unculturedcrenarchaeote	D_6__unculturedcrenarchaeote
denovo181093	D_3__UnknownOrder	D_4__UnknownFamily	D_5__CandidatusNitrososphaera	Ambiguous_taxa
denovo181360	D_3__unculturedeuryarchaeote	D_4__unculturedeuryarchaeote	D_5__unculturedeuryarchaeote	D_6__unculturedeuryarchaeote
denovo197218	D_3__unculturedeuryarchaeote	D_4__unculturedeuryarchaeote	D_5__unculturedeuryarchaeote	D_6__unculturedeuryarchaeote
denovo213504				
denovo253188	D_3__unculturedarchaeon	D_4__unculturedarchaeon	D_5__unculturedarchaeon	D_6__unculturedarchaeon
denovo368501	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
denovo478993				
denovo479722				
denovo540929	D_3__unculturedarchaeon	D_4__unculturedarchaeon	D_5__unculturedarchaeon	D_6__unculturedarchaeon
denovo622149	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
denovo697330	D_3__UnknownOrder	D_4__UnknownFamily	D_5__CandidatusNitrososphaera	D_6__unculturedarchaeon

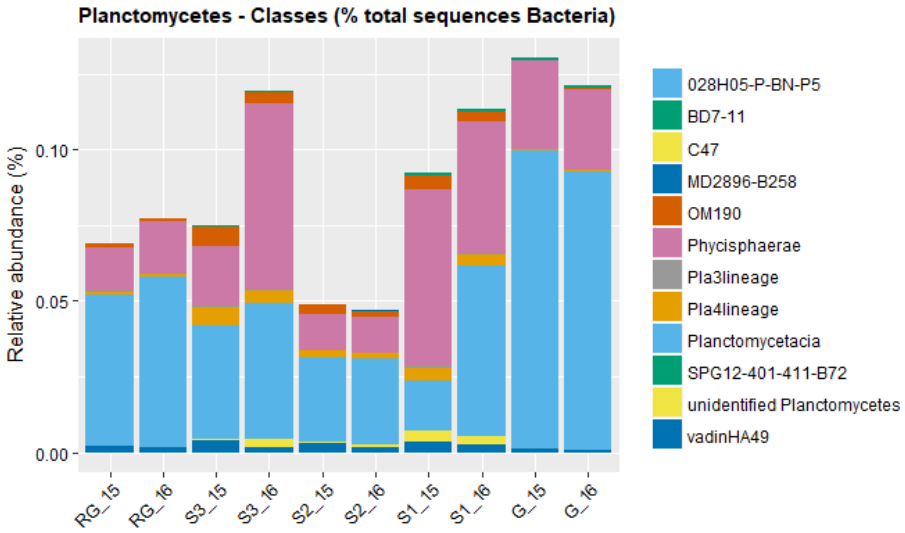
Annex V

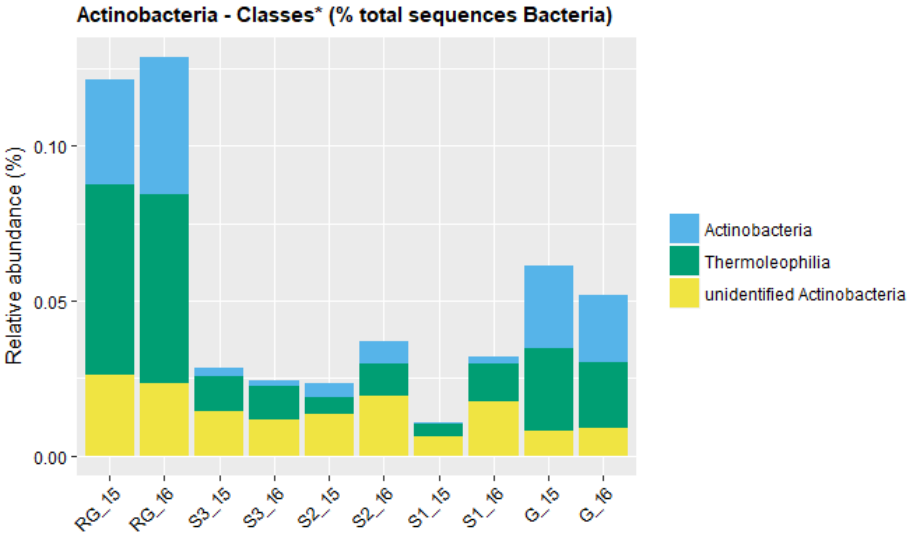
Graphs and tables summarising the composition of selected taxonomic groups among Bacteria.

Values reported for each sample (sample_year) represents the average among three replicate samples. For each graph the dataset used to calculate relative abundances and, in case of data filtering, the applied thresholds are specified.

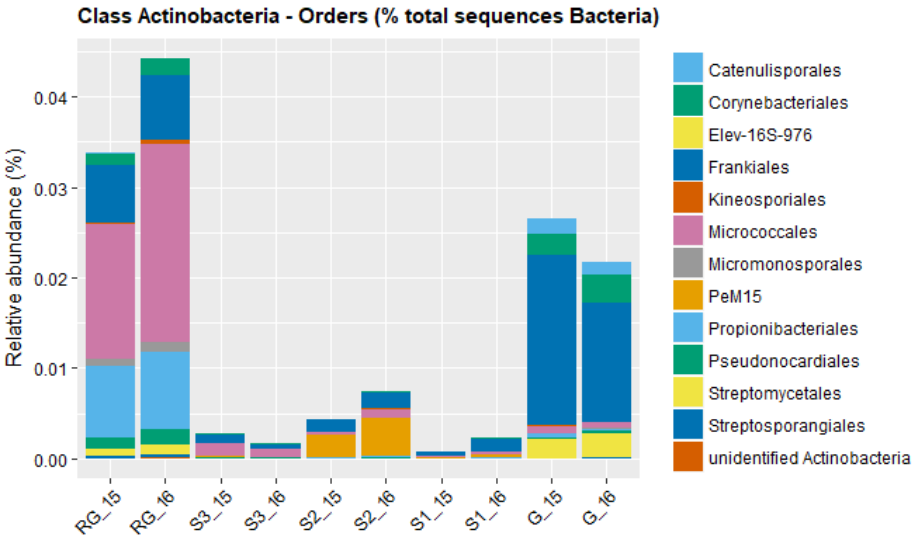


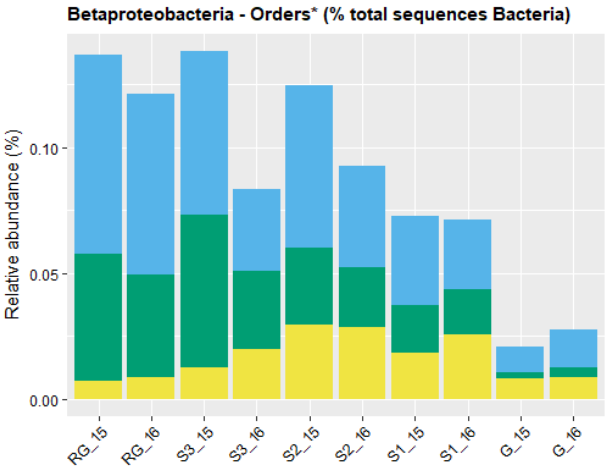
*Only Classes with relative abundance higher than 1% are shown.



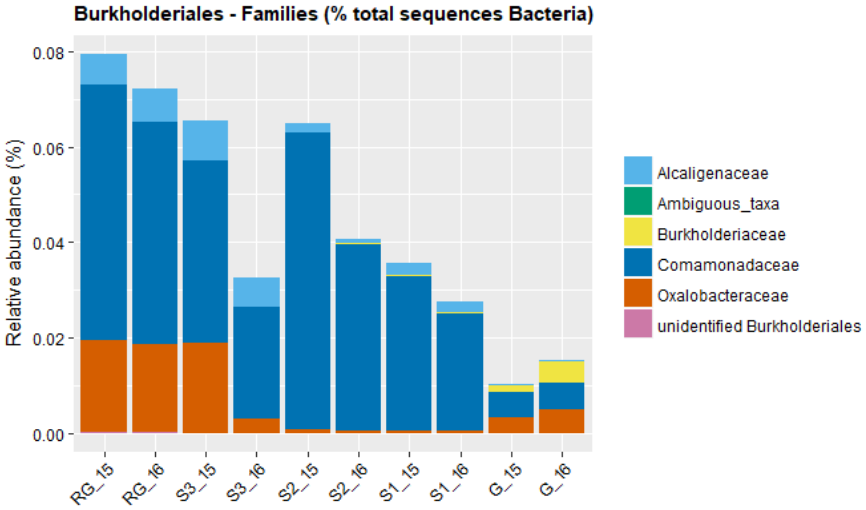


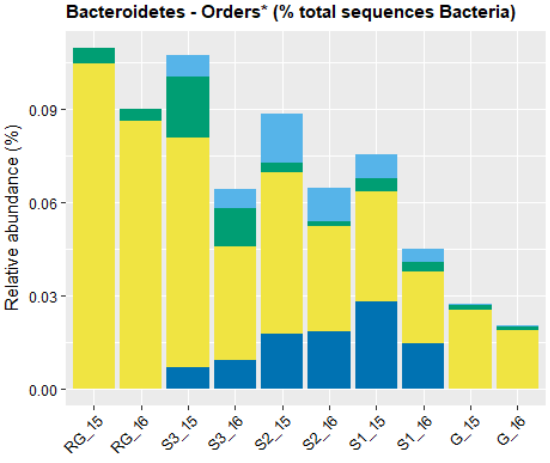
*Only Classes with relative abundance higher than 1% are shown.





*Only Orders with relative abundance higher than 1% are shown.





*Only Orders with relative abundance higher than 1% are shown.

4. General conclusions

With this dissertation some first insights are given into the microbial community inhabiting two typical examples of periglacial features, patterned ground and rock glaciers. Both of them have been studied/described in the last decades in terms of morphology, formative processes and evolution, and relationships with surrounding ecosystems. However, they remained poorly investigated in terms of microbial ecology, at least in the alpine context, thus limiting a detailed understanding of their actual functioning. The aim of this PhD thesis was to give a first overview on the microbiological characteristics of these systems, offering some basic information on microbial abundance and community composition as well as on their link with various environmental drivers.

4.1 Effect of freeze-thaw disturbance on microbial community

The first proposed research question concerned the impact of freeze-thaw succession on microbial community structure and abundance within the selected periglacial landforms, i.e. patterned ground (PG) and rock glacier (RG). On the one hand, in PGs, the micro-topographic heterogeneity produced by cryoturbation processes seems to influence microbial distribution within the single features in terms of microbial abundance, but not in terms of community composition. On the other hand, in the RG-pond system is the habitat type (lacustrine/terrestrial at different degrees of evolution) to mainly determine the community differentiation, although, comparing samples of the same typology, some evidences suggest a possible role of freeze-thaw alternation in community shaping. For instance, the high beta diversity reported for both archaeal and bacterial communities between shallow and deep samples might be the result of a different exposition to freezing stress. Indeed, shallower, peripheral sampling points experience seasonal freezing, that does not affect the central, deepest part of the pond. Obviously, an objection would be that sampling points located at different depths may experiences different light, oxygen and temperature

conditions, leading to a differentiation between communities in shallow and deep sediments; but, since the pond proved to be well mixed, and the maximum depth difference is 2 m, the hypothesis of seasonal freezing as an additional driver involved in community shaping seems reasonable.

4.2 Environmental drivers affecting microbial diversity and abundance

The second aspect considered in this dissertation is the analysis of environmental factors potentially influencing microbial community abundance and composition. In PGs, if altitude has an effect on total abundance of Bacteria and Archaea, as previously reported in literature, the main driver of community structure differentiation is lithology. Nutrients availability, TN and TOC are correlated to bacterial abundance, and seem to follow analogous trends at a small, within-feature scale. However, the presence of opposite trends only in the site characterised by serpentinitic lithology may suggest a hierarchic dominance of the lithology over the other geochemical properties. In the RG-pond system the situation looks, again, more complex. The differences in lithology correspond for terrestrial habitats to differences in the degree of soil evolution (serpentinite in poorly evoluted RG sediment against calcschist in vegetated soil), preventing any consideration about the influence of parent material on the microbial community. Nevertheless, focusing only on sediment samples, a conserved trend consisting of an increase in several geochemical parameters (pH, DOC, TDN, NH_4^+ and Si) in the deepest sampling point emerge, supporting the previous idea of the existence of two partially differentiated niches (central, deepest zone and peripheral, shallower zone) within the pond.

Some final remarks will be dedicated to pH, as one of the environmental factors most commonly found to influence microbial communities in different ecosystems. Indeed, a number of studies reported pH as the best predictor of microbial diversity and community structure in both soils (Lauber et al. 2009, Delgado-Baquerizo et al. 2018) and sediments (Hollister et al. 2010, Xiong et al. 2012, Liu et al. 2015), and also in the investigated PG and RG-pond ecosystem it

follows relevant patterns. In PGs, for instance, it shows a concentric trend, with values decreasing (ΔpH = 0.3-1 point) from the central, more disturbed area to the PG rims, where higher microbial and vegetal biomass is concentrated. This trend is conserved over all the lithologies, suggesting a tighter relationship with freeze-thaw processes than those hypothesized for the other geochemical parameters. In the Olen RG Pond pH is higher in the central, deepest samples, than in the shallower ones, as reported for several other geochemical parameters. This may be consistent with the presence of increased proportions of Cyanobacteria in the deepest samples, since their potential in lowering sediments pH has been reported in different benthic ecosystems (Lopez-Achilla et al. 2004, Reid and Mosley 2015). However, the detection of surprisingly high variability between central and nearshore samples (exceeding 2 pH points) in 2015 while in 2016 the maximum difference was 0.8 points supports the idea that, despite the described trend is characteristic of the system, its intensity may vary on an interannual basis.

4.3 Microbial community controlling factors in periglacial environments: a hierarchic view

Looking at all the overall results of this PhD thesis, some considerations about the hierarchical order of the investigated environmental factors acting on - or influenced by - microbial populations are possible as summarised in the conceptual model below (Fig. 4.1). As an important starting point, all the considered environments are characterised by alpine climate, and thus exposed to cold temperatures, high solar radiation, strong daily and annual temperature fluctuations, presence of snow cover during the winter and occurrence of permafrost. Therefore, climate can be seen as the first, common environmental control.

A second level in the hierarchic structure is represented by habitat, as clearly emerging from the study of the Col d' Olen RG-pond system. Here,

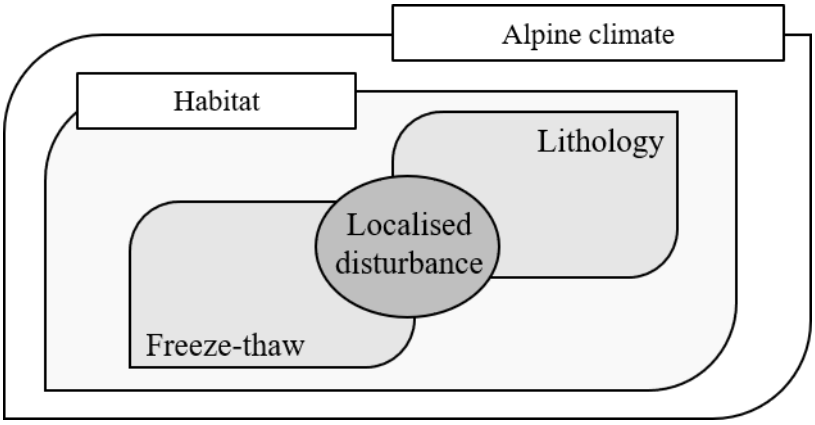


Figure 4.1. Conceptual map summarising the hierarchy of environmental factors acting on microbial communities in periglacial environments, as described in this research.

despite being closely related components of the same system, the three investigated ecological compartments (soil, RG surface material and lacustrine sediments) showed a clear separation in terms of community structure and composition, coherent with the different habitat characteristics. To give an example, Thaumarchaeota community in lake sediments proved to be far more similar to those described in other freshwater ecosystems than to the Olen community associated with soil or RG surface material.

Parent material lithology, as described in detail in chapter 2, may be seen as the third-level controlling factor. Then, within the same habitat and on equal terms of lithology, two other aspects should be considered: the frequency and severity of freeze-thaw processes (stable rims vs cryoturbated centre in PGs, seasonally frozen shallow sediments vs deepest sediments in the Olen pond) and the variation in geochemical properties. However, descending the scale, the direction of the relationships existing between environmental controls and microbial community tends to fade, becoming less clear. For instance, if it is quite straightforward that the composition of the parent material can influence microbial community composition because of the release of certain nutrients or toxic compounds, the correlations reported between geochemistry and microbial-related parameters are often more difficult to interpret.

A last factor that can be superimposed to the framework described so far is localised disturbance, intended as a perturbation limited in time or space that can induce shifts in community characteristics independently from higher-level environmental controls. For instance, in the second study, high similarity in terms of community composition was reported among all the investigated sediment samples collected in the shallower areas of the pond. Nevertheless, only for the samples collected in 2015 in the site closer to RG terminus a series of anomalies (higher NO_3^- concentrations, lower Archaea alpha diversity, higher proportions of Oxalobacteraceae) were found, partially differentiating those samples from the others of the same typology. In that case a link between such anomalies and a phase of high hydrological discharge from the RG, representing the localised disturbance, was hypothesized. Unfortunately, the presence of only two sampling years prevented the verification of the hypothesis. In fact, the identification of localised disturbances and their effect requires a solid knowledge of the investigated ecosystem, deriving from complex monitoring studies, in order to discriminate between disturbance and variability intrinsic to the system.

4.4 Final remarks and future perspectives

The studies presented in this dissertation offer insights in the microbiology of two poorly explored periglacial features such as patterned grounds and rock glaciers. However, as described along the previous chapters, some limitations linked to the sampling strategy and the techniques applied have to be taken into account when considering the obtained results.

In terms of experimental design, both the studies were affected by limitations linked to the small number of available samples. In the case of PGs the possibility of sample only a limited amount of soil at each site, resulting in the lack of true field replicates, prevented a solid assessment of within-feature trends in geochemical and microbiological properties. However, signals of small-scale variations, especially in terms of geochemistry and microbial abundance, were reported and were consistent with gradients in freeze-thaw disturbance, suggesting this aspect as one of the most interesting to be considered in future

studies on analogous ecosystems. In the second study, the choice of a more complex ecosystem including different habitats led us to focus on just one, well characterised site. This makes our conclusions not directly applicable for any RG-pond/lake system but can offer a starting point for the planning of more extensive surveys including different RG-affected water bodies, for instance by indicating that the sampling effort should be concentrated in the areas closest to the RG inflow point in order to avoid the additional effect of depth or freeze-thaw disturbances on microbial community. We offered a snapshot of the prokaryotic community in the late snow-free season, deriving some considerations based on trends conserved or not conserved in the two examined years. Therefore, in order to test any hypotheses on the microbial dynamics occurring in the RG-pond system and their adherence with the variations in meltwater contribution a more temporarily articulated sampling plan would be required.

Another critical point concerns the applied molecular approach. First of all, most of the presented molecular results refer to ribosomal genes. Despite being useful markers for the monitoring of community composition, they give only partial indications about the metabolic potential of the community itself. Thus, if the objective is to explore in detail a particular metabolic group, or a certain environmental function, a metagenomic or a functional genes-based approach would be more effective. Moreover, if the choice of total environmental DNA as molecular marker is suitable for the description of the major microbial components stably associated with an environmental sample, it does not allow robust considerations in terms of active community, even if applied to functional markers. To address these issues, metatranscriptomics, metaproteomics or metabolomics, coupled with appropriate activity tests would be powerful tools, especially if applied after a separation between intracellular and extracellular molecular markers, despite the persistent difficulty in their effective application on complex environmental matrixes.

4.5 Conclusions

In conclusion, in spite of all the limits discussed above, I believe that this PhD thesis can significantly contribute to improve the scientific knowledge on the microbial ecology of periglacial ecosystems. In particular, the ecological relevance of the results obtained is mainly linked to the very limited amount of microbiological data on patterned ground and rock glaciers systems, although they are widespread and considered particularly sensitive to climate change, and thus potentially of great scientific interest. In this context, obtaining new data on microbial abundance, microbial diversity, dominant microbial groups and their relationships with different environmental parameters is the first fundamental step required to understand the ecosystem functioning, and therefore to predict its potential response to environmental pressures. I hope that future studies could take advantage of this information and test the first hypotheses that have been proposed based on it, furtherly increasing our comprehension of such fascinating and fragile ecosystems.

5. Acknowledgements

> ***Caution!*** *You are entering a not revised area. Danger of wrong preposition use, third-person-s-lacking and rambling phrases formulation.*

At the end of these three years – actually three and a half! – I believe that I really need to thank a large group of persons that accompanied and supported me during this experience.

Starting from the conclusion, I would like to thank Dr. Judith Ascher-Jenull and Prof. Sara Borin for revising my thesis manuscript and for providing valuable comments and suggestions for its improvement. Thanks also to Prof. Alberto Agnelli, completing the Dissertation Committee that will escort me through the last meters of this long path, giving me the opportunity to present and discuss the results of my research.

My next acknowledgements go to my advisor Roberta for welcoming me in this department as a bachelor student and keeping me here since then. For both the last thesis an 'acknowledgments' section has not been written in time to be published, so I have to condense in a few lines several years of gratitude. Thus…thanks for teaching me how to get valuable information out of a few nanograms of poor-quality environmental DNA that everyone else would have abandoned without giving it a chance (because dilution is the way); thanks for having a look at every document/manuscript/presentation I sent you seeking help; thanks for trying to teach me that a specific research question should always be addressed to answer issues of wider ecological relevance; thanks for contributing to the evening-submission sessions and to the last minute corrections… and for trying to explain to me that maybe this is not the best way to face the deadlines, also if it finally works. In one sentence: thanks for supporting and encouraging me over all this period. At the same time, I have also to thank Cristian for lending me Roberta's time on many occasions, and for accepting to offer their diving services…in the name of Science?

Thanks to all the people from the Food Microbiology, Food Technology, and Soil Chemistry sectors that directly or indirectly collaborated with me by answering my doubts and questions or helping me with samples analysis and data interpretation.

Thanks to Alex Anesio, the School of Geographical Sciences and the Bristol Glaciology Centre for hosting me in Bristol during six months that have been precious not only as a part of my scientific formation but also as a personal experience…thanks for making it possible!

Thanks to all the co-authors collaborating with me in the production of the two research papers upon which this PhD thesis was built. A special mention here goes to Michele D'Amico and Nicola Colombo, for their patience, competence, and efficiency.

Finally, all my gratitude to the abundant, diverse and seasonally diversified population of students and workers that shared with me laboratories, offices, hopes and troubles during this 3+1 years. Because, if one may think that a strong motivation and a valuable supervisor are the essential elements for successfully complete a PhD…I should point out that having a bunch of good travel companions is a good way to get it without going crazy in the process!

N.B. I will not thank here my family and my home/Turin friends, since too many lines of text are required for this, and the addition of more pages would irreparably alter the precarious formatting of this document. Sorry for this.

6. References

Agnelli A, Ascher J, Corti G, et al. (2004) Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biol Biochem 36:859–868. doi: 10.1016/j.soilbio.2004.02.004

Altschul SF, Gish W, Miller W, et al. (1990) Basic local alignment search tool. J Mol Biol 215:403–410.

Ambrosini R, Musitelli F, Navarra F, et al. (2017) Diversity and assembling processes of bacterial communities in cryoconite holes of a Karakoram Glacier. Microb Ecol 73:827–837. doi: 10.1007/s00248-016-0914-6

Araujo JC, Téran FC, Oliveira RA, et al. (2003) Comparison of hexamethyldisilazane and critical point drying treatments for SEM analysis of anaerobic biofilms and granular sludge. J Electron Microsc (Tokyo) 52:429–433. doi: 10.1093/jmicro/52.4.429

Auguet J-C, Barberan A, Casamayor EO (2009) Global ecological patterns in uncultured Archaea. ISME J 4:182–190. doi: 10.1038/ismej.2009.109

Bajerski F, Wagner D (2013) Bacterial succession in Antarctic soils of two glacier forefields on Larsemann Hills, East Antarctica. FEMS Microbiol Ecol 85:128–142. doi: 10.1111/1574-6941.12105

Ballantyne CK (2013) Patterned ground. In: The encyclopedia of quaternary science, 2nd edn. Elsevier B.V., pp 452–463

Barrett JE, Virginia RA, Wall DH, et al. (2004) Variation in biogeochemistry and soil biodiversity across spatial scales in a polar desert ecosystem. Ecology 85:3105–3118.

Barsch D (1996) Rockglaciers: indicators for the present and former geoecology in high mountain environments. Springer-Verlag, Berlin-Heidelberg

Bates ST, Berg-Lyons D, Caporaso JG, et al. (2010) Examining the global distribution of dominant archaeal populations in soil. ISME J 5:908–917. doi: 10.1038/ismej.2010.171

Berthling I (2011) Beyond confusion: Rock glaciers as cryo-conditioned landforms. Geomorphology 131:98–106. doi: 10.1016/j.geomorph.2011.05.002

Blaud A, Phoenix GK, Osborn AM (2015) Variation in bacterial, archaeal and fungal community structure and abundance in High Arctic tundra soil. Polar Biol 38:1009–1024. doi: 10.1007/s00300-015-1661-8

Bockheim JG, Munroe JS (2014) Organic carbon pools and genesis of alpine soils with permafrost: a review. Arctic, Antarct Alp Res 46:987–1006.

Borin S, Crotti E, Mapelli F, et al. (2008) DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. Saline Systems. doi: 10.1186/1746-1448-4-10

Boyd ES, Cummings DE, Geesey GG (2007) Mineralogy influences structure and diversity of bacterial communities associated with geological substrata in a pristine aquifer. Microb Ecol 54:170–182. doi: 10.1007/s00248-006-9187-9

Boyd WL, Boyd JW (1964) The presence of bacteria in permafrost of the Alaskan Arctic. Can J Microbiol 10:917–919.

Bradley JA, Singarayer JS, Anesio AM (2014) Microbial community dynamics in the forefield of glaciers. Proocedings R Br Soc 281:20140882. doi: 10.1098/rspb.2014.0882

Brown J, Ferrians OJ, Heginbottom JA, et al. (1997) Circum-Arctic map of permafrost and ground-ice conditions. United States geological survey, circum-Pacific map series, CP-45, scale 1:10,000,000

Buraschi E, Salerno F, Monguzzi C, et al. (2005) Characterization of the Italian lake-types and identification of their reference sites using anthropogenic pressure factors. J Limnol 64:75–84.

Buzzini P, Margesin R (2014) Cold-adapted yeasts. Springer

Cameron RE, Morelli FA (1974) Viable microorganisms from ancient Ross Island and Taylor Valley drill core. Antarct J US 9:113–116.

Caporaso GJ, Kuczynski J, Stombaugh J, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. doi: 10.1038/nmeth.f.303

Caporaso JG, Bittinger K, Bushman FD, et al. (2010) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267. doi: 10.1093/bioinformatics/btp636

Cavicchioli R, Charlton T, Ertan H, et al. (2011) Biotechnological uses of enzymes from psychrophiles. Microb Biotechnol 4:449–460. doi: 10.1111/j.1751-7915.2011.00258.x

Cavicchioli R (2002) Extremophiles and the search for extraterrestrial life. Astrobiology 2:281–292.

Cheng G, Dramis F (1992) Distribution of mountain permafrost and climate. Permafr Periglac Process 3:83–91. doi: 104.5-6740/92/020083-09$09.50

Christner BC (2010) Bioprospecting for microbial products that affect ice crystal formation and growth. Appl Microbiol Biotechnol 85:481–489. doi: 10.1007/s00253-009-2291-2

Christner BC, Mikucki JA, Foreman CM, et al. (2005) Glacial ice cores: a model system for developing extraterrestrial decontamination protocols. Icarus 174:572–584. doi: 10.1016/j.icarus.2004.10.027

Chroňáková A, Schloter-Hai B, Radl V, et al. (2015) Response of archaeal and bacterial soil communities to changes associated with outdoor cattle overwintering. PLoS One 10:e0135627. doi: 10.1371/journal.pone.0135627

Classen AT, Sundqvist MK, Henning JA, et al. (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere. doi: 10.1890/ES15-00217.1

Coci M, Odermatt N, Salcher MM, et al. (2015) Ecology and distribution of Thaumarchaea in the deep hypolimnion of Lake Maggiore. Archaea. Article ID 590434. doi: 10.1155/2015/590434

Cocolin L, Bisson LF, Mills DA (2000) Direct profiling of the yeast dynamics in wine fermentations. FEMS Microbiol Lett 189:81–87. doi: 10.1016/S0378-1097(00)00257-3

Colombo N, Salerno F, Gruber S, et al. (2018) Impacts of permafrost degradation on inorganic chemistry of surface fresh water. Glob Planet Change 162:69–83. doi: 10.1016/j.gloplacha.2017.11.017

Colombo N, Sambuelli L, Comina C, et al. (2017) Mechanisms linking active rock glaciers and impounded surface water formation in high-mountain areas. Earth Surf Process Landforms. doi: 10.1002/esp.4257

Compte-Port S, Subirats J, Fillol M, et al. (2017) Abundance and co-distribution of widespread marine archaeal lineages in surface sediments of freshwater

water bodies across the Iberian Peninsula. Microb Ecol 74:776–787. doi: 10.1007/s00248-017-0989-8

Crooke WM, Simpson WE (1971) Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J Sci Food Agric 22:9–10. doi: 10.1002/jsfa.2740220104

Gilichinsky DA, Wilson GS, Friedmann EI, et al. (2007) Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology. Astrobiology 7:275–311.

D'Amico M (2009) Soil ecology and pedogenesis on ophiolitic materials in the western Alps (Mont Avic Natural Park, North-western Italy): soil properties and their relationships with substrate, vegetation and biological activity. Università degli Studi di Milano Bicocca

D'Amico M, Gorra R, Freppaz M (2015) Small-scale variability of soil properties and soil-vegetation relationships in patterned ground on different lithologies (NW Italian Alps). Catena 135:47–58. doi: 10.1016/j.catena.2015.07.005

D'costa VM, King CE, Kalan L, et al. (2011) Antibiotic resistance is ancient. Nature 477:457–461. doi: 10.1038/nature10388

Delmont TO, Francioli D, Jacquesson S, et al. (2014) Microbial community development and unseen diversity recovery in inoculated sterile soil. 1069–1076. doi: 10.1007/s00374-014-0925-8

Ebrahimi A, Or D (2017) Mechanistic modeling of microbial interactions at pore to profile scale resolve methane emission dynamics from permafrost soil. J Geophys Res Biogeosciences 122:1216–1238. doi: 10.1002/2016JG003674

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. doi: 10.1093/bioinformatics/btq461

Edgar RC, Haas BJ, Clemente JC, et al. (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. doi: 10.1093/bioinformatics/btr381

Edlund A, Soule T, Sjöling S, et al. (2006) Microbial community structure in polluted Baltic Sea sediments. Environ Microbiol 8:223–232. doi: 10.1111/j.1462-2920.2005.00887.x

Fan X, Xing P (2016) Differences in the composition of archaeal communities in sediments from contrasting zones of Lake Taihu. Front Microbiol 7:1510. doi: 10.3389/fmicb.2016.01510

Fegel TS, Baron JS, Fountain AG, et al. (2016) The differing biogeochemical and microbial signatures of glaciers and rock glaciers. J Geophys Res Biogeosciences 121:919–932. doi: 10.1002/2015JG003236

Feller G (2003) Molecular adaptations to cold in psychrophilic enzymes. Cell Mol Life Sci 60:648–662. doi: 10.1007/s00018-003-2155-3

Fierer N, Leff JW, Adams BJ, et al. (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci 109:21390–21395. doi: 10.1073/pnas.1215210110

Fillol M, Sànchez-Melesió A, Gich F, et al. (2015) Diversity of Miscellaneous Crenarchaeotic Group archaea in freshwater karstic lakes and their segregation between planktonic and sediment habitats. FEMS Microbiol Ecol 91:fiv020. doi: 10.1093/femsec/fiv020

Francis CA, Roberts KJ, Beman JM, et al. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci U S A 102:14683–14688. doi: 10.1073/pnas.0506625102

Frank-Fahle BA, Yergeau É, Greer CW, et al. (2014) Microbial functional potential and community composition in permafrost-affected soils of the NW Canadian Arctic. PLoS One. doi: 10.1371/journal.pone.0084761

Franzetti A, Tatangelo V, Gandolfi I, et al. (2013) Bacterial community structure on two alpine debris-covered glaciers and biogeography of Polaromonas phylotypes. ISME J 7:1483–1492. doi: 10.1038/ismej.2013.48

French HM (2007) The Periglacial Environment, 3rd edn. Wiley

Frey B, Rime T, Phillips M, et al. (2016) Microbial diversity in European alpine permafrost and active layers. FEMS Microbiol Ecol. doi: 10.1093/femsec/fiw018

Frey KE, McClelland JW (2009) Impacts of permafrost degradation on arctic river biogeochemistry. Hydrol Process 23:169–182. doi: 10.1002/hyp.7196

Gantner S, Andersson AF, Alonso-sáez L, et al. (2011) Novel primers for 16S rRNA-based archaeal community analyses in environmental samples. J Microbiol Methods 84:12–18. doi: 10.1016/j.mimet.2010.10.001

Giardino J, Vitek J, Demorett J (1992) A model of water movement in rock glaciers and associated water characteristics. In: Proceedings of the 22nd Annual Binghamton Symposium in Geomorphology. pp 159–184

Gilichinsky D, Khlebnikova G, Zvyagintsev D, et al. (1989) Microbiology of sedimentary materials in the permafrost zone. Int Geol Rev 31:847–858.

González G, Rivera-Figueroa FJ, Gould WA, et al. (2014) Microorganisms in small patterned ground features and adjacent vegetated soils along topographic and climatic gradients in the High Arctic , Canada. Open J Soil Sci 4:47–55.

Graham DE, Wallenstein MD, Vishnivetskaya TA, et al. (2012) Microbes in thawing permafrost: the unknown variable in the climate change equation. ISME J 6:709–712. doi: 10.1038/ismej.2011.163

Haeberli W (1992) Construction, environmental problems and natural hazards in periglacial mountain belts. Permafr Periglac Process 3:111–124. doi: 10.1002/ppp.3430030208

Haeberli W, Hallet B, Arenson L, et al. (2006) Permafrost creep and rock glacier dynamics. Permafr Periglac Process 17:189–214. doi: 10.1002/ppp.561

Haeberli W, Noetzli J, Arenson L, et al. (2010) Mountain permafrost: development and challenges of a young research field. J Glaciol 56:1043–1058. doi: 10.3189/002214311796406121

Hamerlík L, Svitok M, Novikmec M, et al. (2013) Local, among-site, and regional diversity patterns of benthic macroinvertebrates in high altitude waterbodies: do ponds differ from lakes? Hydrobiologia 723:41–52. doi: 10.1007/s10750-013-1621-7

Harris C, Arenson LU, Christiansen HH, et al. (2009) Permafrost and climate in Europe: monitoring and modelling thermal, geomorphological and geotechnical responses. Earth-Science Rev 92:117–171. doi: 10.1016/j.earscirev.2008.12.002

Harris C, Davies MCR, Etzelmüller B (2001) Remote sensing of rermafrost-related problems and hazards. Permafr Periglac Process 12:145–156. doi: 10.1002/ppp 376

Harris C, Mühll DV, Isaksen K, et al. (2003) Warming permafrost in European mountains. Glob Planet Change 39:215–225. doi: 10.1016/j.gloplacha.2003.04.001

Harris S, French H, Heginbottom JA, et al. (1988) Glossary of permafrost and related ground-ice terms. National Research Council of Canada, Technical Memorandum No. 142

Hollesen J, Elberling B, Jansson PE (2011) Future active layer dynamics and carbon dioxide production from thawing permafrost layers in Northeast Greenland. Glob Chang Biol 17:911–926. doi: 10.1111/j.1365-2486.2010.02256.x

Horowitz N, Cameron N, Hubbard J (1972) Microbiology of the Dry Valleys of Antarctica. Science 176:242–245.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363. doi: 10.1002/bimj.200810425

Hultman J, Waldrop MP, Mackelprang R, et al. (2015) Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. Nature 521:208–212. doi: 10.1038/nature14238

Huss M (2011) Present and future contribution of glacier storage change to runoff from macroscale drainage basins in Europe. Water Resour Res 47:W07511.

Ilyashuk BP, Ilyashuk EA, Psenner R, et al. (2017) Rock glaciers in crystalline catchments: hidden permafrost-related threats to alpine headwater lakes. Glob Chang Biol. doi: 10.1111/gcb.13985

Ilyashuk BP, Ilyashuk EA, Psenner R, et al. (2014) Rock glacier outflows may adversely affect lakes: lessons from the past and present of two neighboring water bodies in a crystalline-rock watershed. Environ Sci Technol 48:6192–6200. doi: 10.1021/es500180c

IPCC (2013) Climate Change 2013: The physical science basis. In: Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA,

Jakosky B, Nealson K, Bakermans C, et al. (2003) Subfreezing activity of microorganisms and the potential habitability of Mars' polar regions. Astrobiology 3:343–350.

James N, Sutherland M (1942) Are there living bacteria in permanently frozen subsoil? Can J Res 20:229–235.

Jansson JK, Taş N (2014) The microbial ecology of permafrost. Nat Rev Microbiol 12:414–425. doi: 10.1038/nrmicro3262

Jones RL, Dreher GB (1996) Silicon. In: Methods in soil analysis, Bartles J. Soil Society of America, Inc., Madison, Wisconsin, USA, pp 627–639

Juck DF, Whissell G, Steven B, et al. (2005) Utilization of fluorescent microspheres and a green fluorescent protein-marked strain for assessment of microbiological contamination of permafrost and ground ice core samples from the Canadian High Arctic. Appl Environ Microbiol 71:1035–1041. doi: doi:10.1128/AEM.71.2.1035–1041.2005

Kolde R (2015) pheatmap: pretty heatmaps

Koven CD, Ringeval B, Friedlingstein P, et al. (2011) Permafrost carbon-climate feedbacks accelerate global warming. Proc Natl Acad Sci 108:14769–14774. doi: 10.1073/pnas.1103910108

Krembs C, Deming JW (2008) The role of exopolymers in microbial adaptation to sea ice. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 247–264

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29:1–27. doi: 10.1007/BF02289565

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chichester, UK, pp 115–175

Larouche JR, Bowden WB, Giordano R, et al. (2012) Microbial biogeography of arctic streams: Exploring influences of lithology and habitat. Front Microbiol 3:1–9. doi: 10.3389/fmicb.2012.00309

Lawley B, Ripley S, Bridge P, et al. (2004) Molecular analysis of geographic patterns of eukaryotic diversity in antarctic soils. Appl Environ Microbiol 70:5963–5972. doi: 10.1128/AEM.70.10.5963

Lazzaro A, Hilfiker D, Zeyer J (2015) Structures of microbial communities in alpine soils: seasonal and elevational effects. Front Microbiol 6:1330. doi: 10.3389/fmicb.2015.01330

Legendre M, Bartoli J, Shmakova L, et al. (2014) Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. Proc Natl Acad Sci 111:4274–4279. doi: 10.1073/pnas.1320670111

Li X, Cheng G, Jin H, et al. (2008) Cryospheric change in China. Glob Planet Change 62:210–218. doi: 10.1016/j.gloplacha.2008.02.001

Liljedahl AK, Boike J, Daanen RP, et al. (2016) Pan-Arctic ice-wedge degradation in warming permafrost and its influence on tundra hydrology. Nat Geosci 9:312–318. doi: 10.1038/ngeo2674

Liu Y, Priscu JC, Xiong J, et al. (2016) Salinity drives archaeal distribution patterns in high altitude lake sediments on the Tibetan Plateau. FEMS Microbiol Ecol 92:fiw033. doi: 10.1093/femsec/fiw033

Lozinski W (1912) Die periglaziale fazies der mechanischen verwitterung. In: Comptes Rendus, XI Congres Internationale Geologie,. Stockholm, pp 1039–1053

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235. doi: 10.1128/AEM.71.12.8228-8235.2005

Mackelprang R, Saleska SR, Jacobsen CS, et al. (2016) Permafrost meta-omics and climate change. Annu Rev Earth Planet Sci 44:439–462. doi: 10.1146/annurev-earth-060614-105126

Mackelprang R, Waldrop MP, Deangelis KM, et al. (2011) Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. Nature 480:368–371. doi: 10.1038/nature10576

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. doi: 10.1093/bioinformatics/btr507

Mancuso Nichols CA, Guezennec J, Bowman JP (2005) Bacterial exopolysaccharides from extreme marine environments with special consideration of the Southern Ocean, sea ice, and deep-sea hydrothermal vents: A review. Mar Biotechnol 7:253–271. doi: 10.1007/s10126-004-5118-2

Mania I, D'Amico M, Freppaz M, et al. (2016) Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps). Biol Fertil Soils 52:1135–1148. doi: 10.1007/s00374-016-1147-z

Margesin R, Feller G (2010) Biotechnological applications of psychrophiles. Environ Technol 31:835–844. doi: 10.1080/09593331003663328

Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Res Microbiol 162:346–361. doi: 10.1016/j.resmic.2010.12.004

Martins Z, Cottin H, Michelle J, et al. (2017) Earth as a tool for astrobiology — a European perspective. Sp Sci Rev 209:43–81. doi: 10.1007/s11214-017-0369-1

McTavish H, Fuchs JA, Hooper AB (1993) Sequence of the gene coding for ammonia monooxygenase in Nitrosomonas europaea. J Bacteriol 175:2436–2444. doi: citeulike-article-id:10421008

Médigue C, Krin E, Bernsel A, et al. (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res 15:1325–1335. doi: 10.1101/gr.4126905

Michaelson GJ, Ping CL, Epstein H, et al. (2008) Soils and frost boil ecosystems across the North American Arctic Transect. J Geophys Res Biogeosciences. doi: 10.1029/2007JG000672

Michaelson GJ, Ping CL, Walker DA (2012) Soils associated with biotic activity on frost boils in Arctic Alaska. Soil Sci Soc Am J 0:0. doi: 10.2136/sssaj2012.0064

Millar CI, Westfall RD (2008) Rock glaciers and related periglacial landforms in the Sierra Nevada, CA, USA; inventory, distribution and climatic relationships. Quat Int 188:90–104. doi: 10.1016/j.quaint.2007.06.004

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide - Biol Chem 5:62–71. doi: 10.1006/niox.2000.0319

Müller SW (1943) Permafrost or permanently frozen ground and related engineering problems. U.S. Engineers Office, Strategic Engineering Study, Special Report No. 62, 136p. (Reprinted in 1947, J.w. Edwards, Ann Arbor, Michigan, 231 p.)

Muyzer G, Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700. doi: 0099-2240/93/030695-06$02.00/0

Muyzer G, Teske A, Wirsen CO, et al. (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol 164:165–172. doi: 10.1007/BF02529967

Methé BA, Nelson KE, Deming JW, et al. (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci U S A 102:10913–10918. doi: 10.1073?pnas.0504766102

Nemergut DR, Anderson SP, Cleveland CC, et al. (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microb Ecol 53:110–122. doi: 10.1007/s00248-006-9144-7

Nemergut DR, Costello EK, Meyer AF, et al. (2005) Structure and function of alpine and arctic soil microbial communities. Res Microbiol 156:775–784. doi: 10.1016/j.resmic.2005.03.004

Nicol GW, Glover AL, Prosser JI (2003) The impact of grassland management on archaeal\n community structure in upland pasture rhizosphere soil. Environ Microbiol 5:152–162.

Nyyssönen M, Hultman J, Ahonen L, et al. (2014) Taxonomically and functionally diverse microbial communities in deep crystalline rocks of the Fennoscandian shield. ISME J 8:126–138. doi: 10.1038/ismej.2013.125

O'Donnel K (1993) Fusarium and its near relatives. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Wallingford, UK, pp 225–233

Oksanen J, Blanchet FG, Friendly M, et al. (2017) vegan: community ecology package. R Packag version 24-3 https://CRAN.R-project.org/package=vegan

Oliver JD (2005) The viable but nonculturable state in bacteria. J Microbiol 43 Spec No:93–100.

Ollivier J, Yang S, Dörfer C, et al. (2014) Bacterial community structure in soils of the Tibetan Plateau affected by discontinuous permafrost or seasonal freezing. 555–559. doi: 10.1007/s00374-013-0869-4

Ortiz-Alvarez R, Casamayor EO (2016) High occurrence of Pacearchaeota and Woesearchaeota (Archaea superphylum DPANN) in the surface waters of oligotrophic high-altitude lakes. Environ Microbiol Rep 8:210–217. doi: 10.1111/1758-2229.12370

O'Sullivan LA, Webster G, Fry JC, et al. (2008) Modified linker-PCR primers facilitate complete sequencing of DGGE DNA fragments. J Microbiol Methods 75:579–581. doi: 10.1016/j.mimet.2008.08.006

Øvreås L, Forney L, Daae FL, et al. (1997) Distribution of bacteroplankton in meromictic lake Sælenvannet, as deteremined by denaturing gradient gel electrophorresis of PCR-amplified gene fragments coding for 16S rRNA. Appl Environ Microbiol 63:3367–3373. doi: 0099-2240/97/$04.0010

Ozerskaya S, Kochkina G, Ivanushkina N, et al. (2009) Fungi in permafrost. In: Margesin R (ed) Permafrost soils. Springer, Berlin, Heidelberg, pp 85–95

Panikov NS, Sizova MV (2007) Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to -35°C. FEMS Microbiol Ecol 59:500–512. doi: 10.1111/j.1574-6941.2006.00210.x

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities , time series and global field samples. Environ Microbiol 18:1403–1414. doi: 10.1111/1462-2920.13023

Pester M, Schleper C, Wagner M (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. 300–306. doi: 10.1016/j.mib.2011.04.007

Petrova MA, Gorlenko ZM, Soina VS, et al. (2008) Association of the strA-strB genes with plasmids and transposons in the present-day bacteria and in bacterial strains from permafrost. Russ J Genet 44:1116–1120. doi: 10.1134/S1022795408090160

Péwé T (1969) The periglacial environment. In: Péwé T (ed) The periglacial environment: past and present. McGill-Queen's University Press, Montreal, pp 1–9

Phadtare S (2004) Recent developments in bacterial cold-shock response. Curr Issues Mol Biol 6:125–136.

Phadtare S, Severinov K (2010) RNA remodeling and gene regulation by cold shock proteins. RNA Biol 7:788–795.

Pietramellara G, Ascher J, Borgogni F, et al. (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. 219–235. doi: 10.1007/s00374-008-0345-8

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – Approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490. doi: 10.1371/journal.pone.0009490

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia- oxidisers in soil: the quest for niche specialisation and differentiation. Trends Microbiol 20:523–531. doi: 10.1016/j.tim.2012.08.001

Qin H, Yuan H, Zhang H (2013) Ammonia-oxidizing archaea are more important than ammonia-oxidizing bacteria in nitrification and NO 3 − -N loss in acidic soil of sloped land. 767–776. doi: 10.1007/s00374-012-0767-1

Quast C, Pruesse E, Yilmaz P, et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:590–596. doi: 10.1093/nar/gks1219

Rappé MS, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394. doi: 10.1146/annurev.micro.57.030502.090759

Reith F, Zammit CM, Pohrib R, et al. (2015) Geogenic factors as drivers of microbial community diversity in soils overlying polymetallic deposits. Appl Environ Microbiol 81:7822–7832. doi: 10.1128/AEM.01856-15

Reysenbach AL, Pace NR (1995) Thermophiles. In: Robb FT, Place AR (eds) Archaea: a laboratory manual. Cold Spring Harbour Press, pp 101–107

Romanovsky VE, Smith SL, Christiansen HH (2010) Permafrost thermal state in the polar northern hemisphere during the international polar year 2007-2009: a synthesis. Permafr Periglac Process 21:106–116. doi: 10.1002/ppp.689

Russell NJ (2008) Membrane components and cold censing. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 177–190

Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. doi: 10.1093/bioinformatics/btr026

Schulz S, Brankatschk R, Dümig A, et al. (2013) The role of microorganisms at different stages of ecosystem development for soil formation. Biogeosciences 10:3983–3996. doi: 10.5194/bg-10-3983-2013

Schuur EAG, Vogel JG, Crummer KG, et al. (2009) The effect of permafrost thaw on old carbon release and net carbon exchange from tundra. Nature 459:556–559. doi: 10.1038/nature08031

Shivaji S, Prakash JSS (2010) How do bacteria sense and respond to low temperature? Arch Microbiol 192:85–95. doi: 10.1007/s00203-009-0539-y

Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–433. doi: 10.1146/annurev.biochem.75.103004.142723

Siles JA, Margesin R (2016) Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors? Microb Ecol 72:207–220. doi: 10.1007/s00248-016-0748-2

Sleator RD, Hill C (2001) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71.

Slemmons KEH, Saros JE, Simon K (2013) The influence of glacial meltwater on alpine aquatic ecosystems: a review. Environ Sci Process Impacts 15:1794–1806. doi: 10.1039/c3em00243h

Steven B, Léveillé R, Pollard WH, et al. (2006) Microbial ecology and biodiversity in permafrost. Extremophiles 10:259–267. doi: 10.1007/s00792-006-0506-3

Steven B, Niederberger TD, Whyte LG (2009) Bacterial and archaeal diversity in permafrost. In: Margesin R (ed) Permafrost soils. Springer, Berlin, Heidelberg, pp 99–106

Thies H, Nickus U, Tolotti M, et al. (2013) Evidence of rock glacier melt impacts on water chemistry and diatoms in high mountain streams. Cold Reg Sci Technol 96:77–85. doi: 10.1016/j.coldregions.2013.06.006

Timling I, Walker DA, Nusbaum C, et al. (2014) Rich and cold: diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Mol Ecol 23:3258–3272. doi: 10.1111/mec.12743

Torti A, Lever MA, Jørgensen BB (2015) Origin, dynamics, and implications of extracellular DNA pools in marine sediments. Mar Genomics 24:185–196. doi: 10.1016/j.margen.2015.08.007

Tricart J (1968) Geomorphology. Encyclopedia of Earth science. Springer, Berlin, Heidelberg,

Vetriani C, Jannasch HW, MacGregor, et al. (1999) Population structure and phylogenetic characterization of marine benthic Archaea in deep-sea sediments. Appl Environ Microbiol 65:4375–4384.

Vishnivetskaya TA, Spirina E, Shatilovich AV, et al. (2003) The resistance of viable permafrost algae to simulated environmental stresses: implications for astrobiology. Int J Astrobiol 2:171–177. doi: 10.1017/S1473550403001575

Vishnivetskaya TA (2009) Viable cyanobacteria and green algae from the permafrost darkness. In: Margesin R (ed) Permafrost soils. Springer, Berlin, Heidelberg, pp 73–84

Viviroli D, Archer DR, Buytaert W, et al. (2011) Climate change and mountain water resources: overview and recommendations for research, management and policy. Hydrol Earth Syst Sci 15:471–504. doi: 10.5194/hess-15-471-2011

Vorobyova E, Minkovsky N, Mamukelashvili A, et al. (2001) Micro-organisms and biomarkers in permafrost. In: Paepe R, Melnikov V (eds) Permafrost response on economic development, environmental security and natural resources. Kluwer Academic Publishers, pp 527–541

Vorobyova E, Soina V, Gorlenko M, et al. (1997) The deep cold biosphere: facts and hypothesis. FEMS Microbiol Lett 20:277–290.

Wagner D, Lipski A, Embacher A, et al. (2005) Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. Environ Microbiol 7:1582–1592. doi: 10.1111/j.1462-2920.2005.00849.x

Walker DA, Epstein HE, Romanovsky VE, et al. (2008) Arctic patterned-ground ecosystems: a synthesis of field studies and models along a North American Arctic Transect. J Geophys Res. doi: 10.1029/2007JG000504

Walker DA, Kuss P, Epstein HE, et al. (2011) Vegetation of zonal patterned-ground ecosystems along the North America Arctic bioclimate gradient. Appl Veg Sci 14:440–463. doi: 10.1111/j.1654-109X.2011.01149.x

Wang Q, Garrity GM, Tiedje JM et al. (2007) Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. doi: 10.1128/AEM.00062-07

Ward NL, Challacombe JF, Janssen PH, et al. (2009) Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl Environ Microbiol 75:2046–2056. doi: 10.1128/AEM.02294-08

Washburn (1979) Geocryology. Edward Arnold, London

Webster G, Parkes RJ, Cragg BA, et al. (2006) Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. FEMS Microbiol Ecol 58:65–85. doi: 10.1111/j.1574-6941.2006.00144.x

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer-V. New York

Wilhelm RC, Radtke KJ, Mykytczuk NCS, et al. (2012) Life at the wedge: the activity and diversity of arctic ice wedge microbial communities. Astrobiology 12:347–360. doi: 10.1089/ast.2011.0730

Williams MW, Knauf M, Cory R, et al. (2007) Nitrate content and potential microbial signature of rock glacier outflow, Colorado Front Range. Earth Surf Process Landforms 32:1032–1047. doi: 10.1002/esp.1455

Williams MW, Knauf M, Caine N, et al. (2006) Geochemistry and source waters of rock glacier outflow, Colorado Front Range. Permafr Periglac Process 17:13–33. doi: 10.1002/ppp.535

Woo M (2012) Permafrost hydrology. Springer Science & Business Media, Berlin

Yang Y, Wu Q, Hou Y, et al. (2017) Unraveling of permafrost hydrological variabilities on Central Qinghai-Tibet Plateau using stable isotopic technique. Sci Total Environ 605–606:199–210. doi: 10.1016/j.scitotenv.2017.06.213

Yergeau E, Hogues H, Whyte LG, et al. (2010) The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J 4:1206–1214. doi: 10.1038/ismej.2010.41

Zhang J, Yang Y, Zhao L, et al. (2015) Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. Appl Microbiol Biotechnol 99:3291–3302. doi: 10.1007/s00253-014-6262-x