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Influence of different environmental parameters on diversity and distribution of polar microbial communities

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Contents

Abstract

In recent years, polar regions have undergone the dramatic effects of global warming. Determining the current composition of the microbial communities of these environments and defining the environmental parameters that are crucial for their spread is fundamental to monitor future changes and predict the possible effects of the variation of environmental conditions. For these purposes, we used the metabarcoding approach, that represents a rapid and reliable method for studying microbial communities. Given the greening and shrub encroachment reported all over the Arctic, we studied both the fungal and bacterial communities associated to three different soil habitats in Western Greenland, representative of a vegetation increasing coverage. The type of coverage was the main predictor of community composition and the shrub composition a fundamental parameter in vegetated plots. The results showed a high number of species unique of the simplest ecosystems, that are expected to be outcompeted with the expansion of shrubs, suggesting a possible loss of local diversity. In Antarctica, we took into account two different ecosystems among the most representatives of this environment. Firstly, we analyzed the bacterial composition of cryptoendolithic lichen-dominated communities in a gradient of increasing environmental pressure; the structure of the bacteria communities showed to be correlated with the fungal counterpart and, among the environmental parameters considered, the altitude was found to be the main factor influencing the bacterial biodiversity. Later, we analyzed the fungal component of biological soil crusts spreading along the Victoria Land coasts during austral summer. The results showed a stronger effect of the abiotic factors on the diversity and community composition. The strong effect of pH and cation exchange capacity on fungal community's composition let us hypothesize that in such nutrient limiting environments, even small changes in the external conditions can dramatically affect the nutrient availability and thus the balance of these ecosystems.

Chapter 1

Introduction

Determining the factors driving species survival, distribution and resilience still remains a major challenge in ecology. Organization of communities may be explained by a combination of deterministic (i.e., environmental conditions, species interactions) and stochastic (i.e., chance colonization, ecological drift) mechanisms (Leibold and McPeek, 2006; Chase and Myers, 2011), but many evidences suggest that environmental filtering is a major driver of the distribution of species and the development of their functional traits from the tropics to the poles (e.g., Hillebrand, 2004). This concept relies on the idea that species are selected in different environments based on their functional traits and their adaptations to dominating environmental conditions (Chase and Leibold; 2003). For example, organisms that cannot tolerate freezing temperatures are excluded from alpine or polar regions (Körner, 2003) and those that cannot survive at high temperatures are bounded to higher elevations (e.g., Wilson *et al.*, 2007). In this optic, trait-based approaches can provide insights into the mechanisms that drive community organization, ecosystem functioning and species coexistence and it is increasingly recognized that robust tests of community assembly need to incorporate information about the functional characteristics of species (e.g., McGill *et al.*, 2006).

Free-living and symbiotic microorganisms may be affected by both dispersal limitation and environmental filtering, including host specificity or habitat and whether they are more strongly driven by niche or neutral processes might depend on their ecology. For example, evidences for environmental filtering by soil nutrients and pH exists for both fungi (Dumbrell *et al.*, 2010; Tedersoo *et al.*, 2014; Erlandson *et al.*, 2016) and bacteria (Griffiths *et al.*, 2011; Jones *et al.*, 2009). However, while it is clear that bacteria are more strongly driven by pH (Fierer and Jackson, 2006), the factors that predominate in structuring symbiotic and free-living fungal communities are still not completely defined (Rousk *et al.*, 2010).

Microbial communities, because of their high phylogenetic and functional diversity and their central role in many ecological processes, are considered excellent models to examine the drivers of communities assembly. In fact, microbes are essential in driving many ecosystem processes, like decomposition and carbon cycling (van der Heijden *et al.*, 2008; Martiny *et al.*, 2016), and play

critical roles in symbiosis, as mycorrhizal fungi that may drive the initial terrestrial colonization by plants (Humphreys *et al.*, 2010). In particular, the relatively simplified structure of Antarctic terrestrial communities provides a tractable system to understand the possible biotic and abiotic drivers of community survival and resilience (Barret *et al.*, 2006; Hogg *et al.*, 2006; Convey *et al.*, 2014).

Additionally, soil microbes mediate carbon and nitrogen balance and other biogeochemical cycles of global importance (van der Heijden *et al.*, 2008). Therefore, understanding the ecological drivers that shape microbial communities may be a key for understanding how biogeochemical cycles will respond to human-induced environmental changes. In the Arctic, the effects of warming on microbial decomposition of vast carbon pools in permafrost soils have the potential to cause a significant positive feedback to global climate change (Lashof, 1989; Schuur *et al.*, 2008). Instead, in Antarctica, climate change is firstly expected to result in the loss of unique and highly adapted ecosystems, mainly because of shifts in temperature and precipitation regimes, as well as longer term changes in edaphic profiles (Schuur *et al.*, 2008) and the invasion of allochthonous more competitive species.

1.1 Greenland

Greenland is located between 59° and 83° N, northeast of Canada and northwest of Iceland, between the Arctic Ocean and the North Atlantic Ocean. The territory is composed of a main island, the largest in the world, covering about 2.17 million square kilometres, and more than a hundred other smaller islands. About 80% of the surface is covered by the ice, with an ice sheet of about 1.710.000 square kilometres surface and up to 3 kilometres thickness, that is the most extended one outside Antarctica (figure 1.1). The ice sheet presses the central area of the continent 300 meters below the see level, while the elevation rapidly increases near the coasts. The climate is arctic to subarctic: the average daily temperature of Nuuk, the capital, located southwest of the island, varies seasonally from −5.1 to 9.9 °C. The lowest mean annual temperatures, about −31 °C, occur on the north-central part of the north ice-sheet, and temperatures in the south are about −20 °C.

In the last years, temperatures are rapidly increasing all over the Arctic, at twice the rate of the rest of the world (Hassol, 2004; Serreze and Francis, 2006; Kaufman *et al.*, 2009; Screen and Simmonds, 2010). These phenomena are of particular concern for Greenland, where it is estimated that a complete melting of the ice sheet could result in an increase of global see level of about 7 meters.

Figure 1.1 Map of Greenland

Ice loss in Greenland is being monitored through the satellite mission Gravity Recovery and Climate Experiment (GRACE) (see for example Wouters *et al.*, 2014; van den Broeke *et al.*, 2016). From 2000 to 2010 ice loss was mainly concentrated in the southeast and northwest margins of the ice sheet, in large part due to the increasing discharge from marine-terminating glaciers. However, the largest (in the last 10 years) acceleration detected by GRACE occurred in southwest Greenland. The sustained acceleration and the subsequent, abrupt, and even stronger deceleration were mostly driven by changes in air temperature and solar radiation, demonstrating that recent Greenland deglaciation is a response to both oceanic and atmospheric factors. Additionally, with recent models it is possible infer that within two decades the southwest portion of Greenland ice sheet will be the major contributor to sea level rise (Bevis *et al.*, 2019).

Temperature increase in the Arctic is generally resulting in an increase of the length of ice-free periods and in an overall greening of terrestrial areas (Goetz *et al.*, 2005; Bhatt *et al.*, 2010). In particular, lot of studies are concentrated on the alterations of the structure and function of ecosystems (Chapin *et al.*, 1995; Shaver *et al.*, 2000; Mack *et al.*, 2004), reporting significant increases in the cover and height of shrubs (e.g. *Betula nana* and *Salix pulchra*), combined with significant decreases in the coverage of bryophytes, lichens (Mercado-Diaz, 2011; Pattison and Welker, 2014) and graminoid (Forbes *et al.*, 2010; Elmendorf *et al.*, 2012), most likely due to the competitive exclusion by shrubs (Cornelissen *et al.*, 2001; Jagerbrand *et al.*, 2009). Reports of these phenomena are also present for the area of Western Greenland (Hollesen *et al.*, 2015).

The mechanisms that drive shrub expansion are still not completely clear. One of the hypothesis is that increased snow accumulation during the winter may enhance shrubs dominance and growth through changes in the abiotic environment, as warmer temperatures (Grogan and Jonasson 2003; Schimel *et al.*, 2004; Wahren *et al.*, 2005) and higher water availability (Sturm *et al.*, 2005; Pomeroy *et al.*, 2006). These changes also affect soil microorganisms (Romanovsky and Osterkamp 2000; Mikan *et al.*, 2002), regulating the rate of organic matter degradation by bacteria and fungi, resulting in higher rates of nitrogen supply to plants. However, deciduous shrubs, such as *Betula nana* biomass, decompose three times slower than leaves and one to eight times slower than leaves and stems from graminoids and evergreen shrubs (Hobbie, 1996). Thus, a compositional shift to more abundant deciduous shrubs may alter nutrient turnover by a shift toward larger inputs of slowly decomposing litter (Buckeridge *et al.*, 2010), resulting in a slower shrub expansion. These phenomena are of particular concern given their potential impact on the highly vulnerable Arctic vast carbon stocks that may be exposed to microbial degradation (Crowther *et al.*, 2016). Changes in litter quality and quantity is expected to have stronger effects on the microorganisms operating litter decomposition and nutrient release, consequently increasing the release of greenhouse gases from soils to the atmosphere and amplifying climate feedbacks (Wieder *et al.*, 2013; Abbot *et al.*, 2016). Thus, the impact of warming will depend on how efficiently this organic matter is incorporated into the soil or released to the atmosphere as carbon dioxide (Cotrufo *et al.*, 2013). In addition, plant litter and root exudates can select microorganisms with enhanced decomposition abilities for old stocks of organic matter, in a process known as priming (Fontaine *et al.*, 2003; Walker *et al.*, 2015).

1.2 Antarctica

Antarctica is the fifth most extended and the southernmost continent; placed at the south pole and completely surrounded by the Ocean, it is the physically most isolate continent of the planet, also because of the Antarctic convergence, considered the geographic boundary of the continent, where the cold Antarctic waters northward-flowing meet the relatively warmer waters of the sub-Antarctic region (figure 1.2). It is geographically divided in two unequal portion, East Antarctica and West Antarctica, by the Transantarctic Mountains, extended from the Ross Sea to the Weddell Sea. East Antarctica is the most extended region, covering about 10.2 million square kilometres, representing 73% of the continent. It is characterized by a cratonic rock body almost entirely covered by the so-called East Antarctic Ice Sheet, with an average thickness of 2400 meters. Instead West Antarctica, including the Antarctic Peninsula, is composed of small fragments of continental crust, extended for about 2.5 million square kilometres, covered by the West Antarctica Ice Sheet, causing underlying rock to sink by between 0.5 and 1 kilometre.

The position, the long periods of winter darkness, the peculiar geographic characteristics, make Antarctica the coldest and driest continent on Earth (Onofri *et al.*, 2004).

Climatic conditions are quite different within the continent. The *sub-Antarctic region*, comprising the islands surrounding the continent at a latitude lower than 60° S, is characterized by a milder oceanic climate and an extended vegetation of vascular plants, mosses and lichens. The *maritime Antarctic region* is characterized by a cold maritime climate, humid in the northern area and drier in the southernmost portion. Here only two vascular plants species are endemic, *Colobanthus quitensis* (Caryophillaceae) and *Deschampsia antartica* (Poaceae), and the vegetation is dominated by a high variety of lichens belonging to the genera *Acarospora*, *Buellia*, *Caloplaca*, *Lecanora*, *Lecidea* and *Rhizoplaca*. Finally, the *continental Antarctic region* is characterized by an extremely cold climate, where low temperatures make water mostly unavailable for life. Here the growth of lichens and mosses is restricted to some areas on the fringe of the continent and life forms are dominated, more than other continents, by microorganisms that adopt astonish adaptations to survive and thrive in this limiting environment (Nienow and Friedmann, 1993).

Life forms are restricted to scattered ice-free regions, accounting for about 2% of the continent; they mainly occur during the warmer austral-summer months, are limited in extent, patchily distributed, and mostly confined in coastal regions or isolated nunataks and mountain peaks protruding from the ice sheet (Barrett *et al.*, 2006).

Figure 1.2 Map of Antarctica

The unique conditions of this continent, as the combination of freezing temperatures, frequent freeze-thaw cycles, low nutrient availability, severe drought, high incidence of solar and UV radiation during the austral summer, and locally high level of salinity, make the study of the life forms adapted to survive and thrive in such limiting conditions extremely interesting to understand processes related to evolution and adaptation, given also the extremely limited human perturbation. Also, for this reason, since 1959, Antarctica is considered a protected area, completely devoted to scientific research activities.

Victoria Land spreads along a latitudinal gradient spanning 8° from Cape Adare (71°S) to Darwin Glacier (79°S), on the western side of the Ross Sea and the Ross Ice Shelf. This region comprises the widest ice-free areas of Continental Antarctica, with a wide range of ecosystems. A milder climate characterizes the Northern Victoria Land (NLV) coasts, a sort of "Antarctic oases" (Pickard, 1986) where wet, seasonally ice-free soils and rock surfaces are colonized by abundant mosses, lichens and algae (Ochyra *et al.*, 2008), and are influenced from marine organic matter deposits. These organisms, associate with soil particles and microorganisms, make up the so-called

Biological Soil Crusts. Instead, inland locations show less permissive conditions with the epilithic life forms gradually disappearing with the increasing sea distance and altitude (Zucconi *et al.*, 2016).

Southern Victoria Land (SVL) hosts the widest desert of Antarctica, the McMurdo Dry Valleys, that constitute the most extensive ice-free area in the continent (Friedmann and Ocampo, 1976). Here the mean annual air temperatures ranges from -20 to -35°C and precipitations from less than 10 to 100 mm (Bockheim and McLeod, 2008). Moreover, katabatic winds, descending from the Antarctic ice plateau into the valleys, contribute to the maintenance of desert conditions (Friedmann, 1982). These valleys, for their extremely low temperatures and extraordinary aridity, are considered one of the best analogues of Martian environment, making the studies of life forms inhabiting these areas interesting not only for their adaptations to extreme conditions, but also as models for understanding the possible evolution of life outside our planet (Friedmann and Ocampo, 1984; Onofri *et al.*, 2004).

In these areas, when conditions became too harsh for epilithic life, rocks are one of the main substrata for colonization, as a refuge from external stressors for highly adapted species (Nienow and Friedmann, 1993). In Antarctica, different endolithic communities have been described, depending on the nature and proportions of primary producers present. The most widely distributed communities in Victoria Land are those dominated by lichens (Friedmann, 1982). This type of communities, named cryptoendolithic lichen-dominated communities, can be clearly identified thanks to the presence inside the rock substratum of different layers, that are due to the light regime in the habitat (Nienow *et al.*, 1988). The uppermost one is usually black, due to the presence of melanized fungi; underlying this, there is a white layer with lichens, and the deepest layer comprises free-living chlorophyte algae and cyanobacteria (Friedmann, 1982; de los Rìos *et al.*, 2014) (figure 1.3).

The composition of these communities has been widely studied by cultural (e.g. Friedmann *et al.*, 1988; Siebert *et al.*, 1996; Selbmann *et al.*, 2005; 2008; 2010; 2014; Egidi *et al.*, 2014) and more recently molecular approaches (e.g. de la Torre *et al.*, 2003; Cary *et al.*, 2010; Selbmann *et al.*, 2017; Archer *et al.*, 2017; Coleine *et al.*, 2018a and b). In particular, lot of cultural studies focussed on the portion of black fungi, leading to the definition of 7 new genera and 12 new species (Selbmann *et al.*, 2005; 2008; 2014; 2015; Egidi *et al.*, 2014). Molecular studies lead to the definition of sandstone as the best substratum for colonization and shed light on the high degree of adaptation of these communities, making them very low resilient to any external perturbation, as the possible changes of the environmental condition due to climate changes and the connected possible invasion of external species (Selbmann *et al.*, 2017; Coleine *et al.*, 2018a).

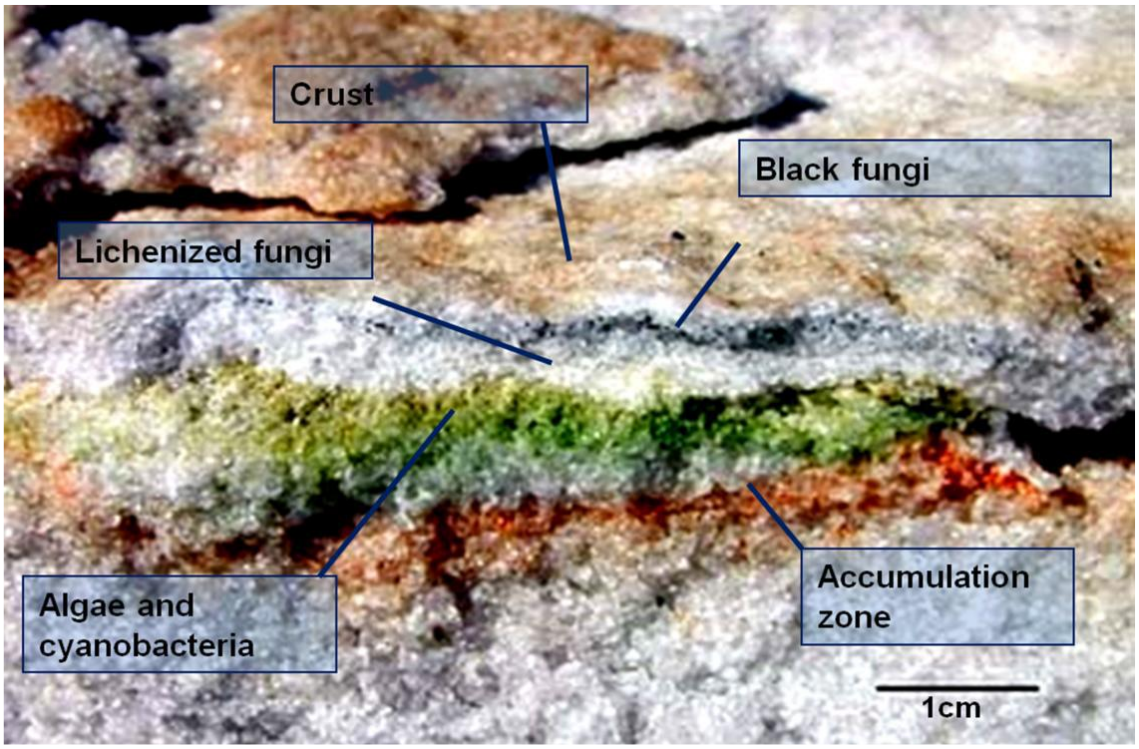

Figure 1.3 Cryptoendolithic community with the description of all the layer present

1.3 Biological soil crusts

Biological soil crusts (BSCs) are a key biotic component of terrestrial ecosystems, deriving from the intimate association among soil particles and cyanobacteria, algae, microfungi, lichens and bryophytes, living within or immediately above the most superficial millimetres of the soil (Belnap and Lange, 2013). Soil particles are aggregated thanks to the presence and activity of these biological components, resulting in a crust that covers the soil surface forming a uniform layer (Belnap *et al.*, 2001) (figure 1.4).

BSCs originate by long succession processes which, through the creation of complex topological and functional relationships between the mineral and biological components, lead to the stabilization of the substrate and a possible development of the soil, with an increase in fertility and resilience to erosion (de los Rios *et al.*, 2014).

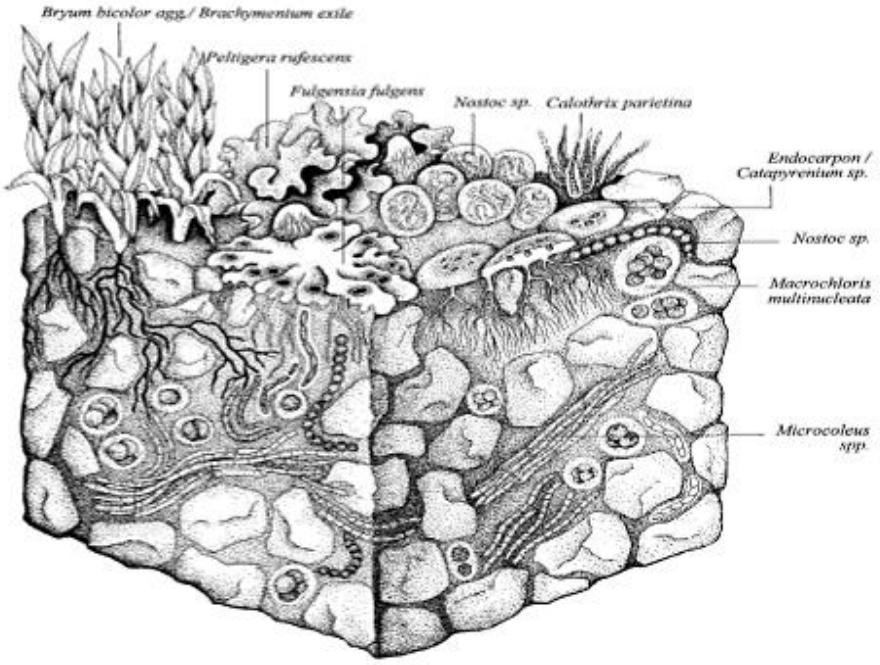

Figure 1.4 Scheme of a biological soil crust with typical colonizers. Layer thickness of about 3 mm, organisms are not in scale (illustration by Renate Klein-Rödder; Belnap *et al.*, 2001)

The ecological importance of biocrusts relies on the fact that cyanobacteria filaments, micro-fungal hyphae, lichens and the rhizoids of the bryophytes aggregate together in the thin layer of the soil below the crust surface, promoting the evolution, fertility and stability of the soil, acting as protection from erosion and increasing the potential for colonization by higher plants (Belnap *et al.*, 2004; Bowker *et al.*, 2006; Pointing and Belnap, 2012). For these reasons, in the last years, the study of these ecosystems is attracting an increasing attention for their potential applications in controlling and reverting desertification process due to climate changes and in the recovery of polluted soils (Xiao *et al.*, 2015; Guan *et al.*, 2018).

BSCs are widely diffused in polar regions or in arid and semi-arid landscapes, where often represent the only vegetation cover. In the Arctic tundra, in maritime Antarctica or along the coasts of continental Antarctica, where water is available during the summer, an abundant growth of mosses and lichens occur (Belnap *et al.*, 2001). In the Arctic, lot of studies focussed on the nitrogen fixing components of these ecosystems, as nitrogen availability is one of the main limiting factors for life in this region (Patova *et al.*, 2016; Diáková *et al.*, 2016; Williams *et al.*, 2017) and lot of studies on the total soil communities highlighted their sensitivity to global changes and the impacts of their possible reduction on global carbon fluxes (Yoshitake *et al.*, 2010). In Antarctica, many studies on BSCs have been carried out on the continental region (Büdel *et al.*, 2014), where most of the studies

concerns the contribution of BSCs in the carbon cycle, while composition of communities or their distribution have rarely been taken into account (Wojtun *et al.*, 2013). Additionally, most of these studies only took into account the primary producers supporting these ecosystem (Colesie *et al.*, 2014a; b and 2016; Pushkareva *et al.*, 2018), while studies on the role of symbiotic and free-living fungi are completely lacking.

1.4 DNA metabarcoding

Recently, a series of high-throughput sequencing technologies based on different chemistries and detection techniques have been introduced, allowing to generate several thousands of millions of sequencing reads in parallel. This massive parallel throughput sequencing capacity can generate sequence reads from fragmented libraries of a specific genome or from a pool of PCR amplified molecules (i.e. amplicon sequencing). Metabarcoding approach relies on the selection of taxonomic informative (barcode) genes for which universal primers are available to amplify short sequences, with sufficient phylogenetic signal and availability of comprehensive taxonomic reference database, that can be sequenced in parallel without the necessity for cloning. NGS technologies have facilitated the analysis of environmental samples from a variety of ecosystems including freshwater, marine, soil, terrestrial and gut microbiota, with the main aim to answer the question of what is present in a given environment. Additionally, this technology allowed to observe slight changes in community structure that may occur due to anthropogenic or natural environmental fluctuations, that were not discernible with less sensitive, traditional, molecular tools such as Sanger sequencing (Sogin *et al.*, 2006; Huse *et al.*, 2010; Xu *et al.*, 2012). Despite the known possible biases introduced during PCR amplification and the development of techniques as metagenomics and metatranscriptomics able to investigate variations in communities functionality, amplicon sequencing, for its rapidity and its reduced costs, is still the most common approach for investigating environmental diversity and to describe the structure of microbial communities (Smith and Peay, 2014). Nowadays, amplicon-based approaches targeting variable regions of specific markers (e.g., 16S, ITS, or 18S) are widely used to describe bacterial, archaeal, fungal (Lindahl *et al.*, 2013), and micro-eukaryote community composition (Lentendu *et al.*, 2014).

1.5 Aims of the thesis and chapters description

The main aim of this work was to provide a taxonomic and functional characterization of soil microbial communities of polar environments, to understand how diversity, composition and functionality are affected by biotic and abiotic parameters. Our goal was to give some insights into the landscape-level dynamics of microbial communities in relation to the extreme environments to which they are adapted, also in the light of climate changes that are threatening polar regions.

The thesis has been organized into six main parts which here correspond to 6 chapters. The first chapter represents an introduction of all the environments studied within the thesis.

Chapters 2 and 3, entitled "*Vegetation, pH and water content as main factors for shaping fungal richness, community composition and functional guilds distribution in soils of Western Greenland*" and "*Expansion of shrubs could result in local soil bacterial diversity loss in Western Greenland*", respectively, correspond to two papers, the former published in *Frontiers in Microbiology* and the second one under review in *FEMS Microbiology Ecology*. In these two chapters we analysed the taxonomic and functional composition of fungal and bacterial communities respectively, associated to three different soil habitats in Western Greenland, where a warming-induced expansion of shrubs vegetation, at the expenses of other soil biomes, has been reported. The three soil habitats are representative of an increasing vegetational coverage, starting from bare grounds without any apparent coverage, to biological soil crusts, dominated by mosses and lichens, and ending with vascular vegetation covered soils. This area is of great interest due to the presence of a vegetation transect monitored since more than ten years, but for which information about the effects of the underlying microbial communities on vegetation dynamics were completely lacking. In both the chapters we studied how diversity and composition of fungal and bacterial communities are determined by the different coverage and edaphic parameters. Additionally, taking into account only vascular vegetation covered soils we aimed to understand the effect of different shrub genera coverage on the structure of the communities.

The fourth and the fifth chapters deal with the Antarctic continent. In the fourth chapter, entitled "*Altitude and fungal diversity influence the structure of Antarctic cryptoendolithic Bacteria communities*", corresponding to a paper published in *Environmental Microbiology Reports*, we took into account the cryptoendolithic Antarctic communities, which is the highest standing biomass of this environment, generally considered as an optimal model for studying the adaptations of microbial communities to extreme conditions. We studied the effect of altitude and see distance on the prokaryotic component of these communities on one hand, on the other side we tried to define the

effect of the associated fungal compartment on the diversity and composition of the prokaryotic component of the same communities.

In chapter 5, we aimed to study the fungal communities associated to the biological soil crusts spreading along the coasts of Northern Victoria Land during the austral summer, never characterized before in the Continental Antarctica. This chapter, entitled "*Exchangeable cations and pH drive diversity and functionality of fungal communities in biological soil crusts from coastal sites of Victoria Land, Antarctica*" represents a manuscript accepted for publication in *Fungal Ecology*. We used the extremely simplified ecosystems of the coastal continental Antarctic area to verify the impact of different physicochemical parameters on both free-living and symbiotic fungi and we aimed to disentangle the separate effect of the geographic distance from the effect of edaphic parameters.

In chapter 6 final general conclusions and future perspectives are reported.

Chapter 2

Vegetation, pH and water content as main factors for shaping fungal richness, community composition and functional guilds distribution in soils of Western Greenland

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Abstract

Fungi are the most abundant and one of the most diverse components of arctic soil ecosystems, where they are fundamental drivers of plant nutrient acquisition and recycling. Nevertheless, few studies have focused on the factors driving the diversity and functionality of fungal communities associated with these ecosystems, especially in the scope of global warming that is particularly affecting Greenland and is leading to shrub expansion, with expected profound changes of soil microbial communities. We used soil DNA metabarcoding to compare taxonomic and functional composition of fungal communities in three habitats (bare ground, biological soil crusts and vascular vegetation coverage) in Western Greenland. Fungal richness increased with the increasing complexity of the coverage, but bare grounds and biological soil crusts samples showed the highest number of unique OTUs. Differences in both fungal community composition and distribution of functional guilds identified were correlated with edaphic factors (mainly pH and water content), in turn connected with the different type of coverage. These results suggest also possible losses of diversity connected to the expansion of vascular vegetation and possible interactions among the members of different functional guilds, likely due to the nutrient limitation, with potential effects on elements recycling.

Keywords: Metabarcoding, functional guilds, shrub encroachment, edaphic factors, ITS1

2.1 Introduction

Almost all plants are highly dependent on mutualistic relationships with mycorrhizal fungi, including ectomycorrhizae, ericoid and arbuscular mycorrhizae (Väre *et al.*, 1992; Michaelson *et al.*, 2008; Newsham *et al.*, 2009) that can allow more efficient nutrient and water uptake (Gardes and Dahlberg, 1996; Hobbie and Hobbie, 2006; Hobbie *et al.*, 2009; Bjorbaekmo *et al.*, 2010). Plants release many root compounds that are fundamental in selecting microorganisms colonizing the rhizosphere (Mendes *et al.*, 2013); in the main time, endophytic and mycorrhizal fungi may promote plant growth (Mucciarelli *et al.*, 2003) and their resistance to abiotic and biotic stress factors (Rodriguez *et al.*, 2008). Moreover, degradation of organic matter predominantly relies on fungi that are the major drivers of nutrient recycling (Dahlberg and Bültmann, 2013). This is particularly true for cold and nutrient-poor arctic terrestrial environments, where fungi dominate the microbial biomass and play key roles in ecosystem functioning as symbionts (mycorrhizae, endophytes, lichens), pathogens and decomposers (Gardes and Dahlberg, 1996; Hobbie *et al.*, 2009; Newsham *et al.*, 2009).

Given the above-mentioned intimate relationships of fungi and plants, many specific associations between both fungal and plant communities composition have been reported. The most relevant published studies indicated the importance of abiotic factors, such as climate at regional scale (Timling *et al.*, 2012), edaphic factors and microclimate driven by micro- and mesotopography at landscape scale (Blaalid *et al.*, 2014; Mundra *et al.*, 2015; Timling *et al.*, 2014; Geml *et al.*, 2016; Grau *et al.*, 2017). Among these mentioned works, Mundra *et al.* (2015) observed that fungal communities associated with *Bistorta vivipara* were driven by different factors, such as periglacial processes, more than the above-ground vegetation, while in the other studies the total soil fungal community composition seems to correlate with vegetation (or habitat) types.

Arctic regions have been experiencing some of the highest rates of warming (Turner *et al.*, 2007), particularly evident in Greenland (Bevis *et al.*, 2019), with an average increase of temperature of about 0.1 °C per year since the 1980s (McBean *et al.*, 2005). Temperature increase is influencing sea ice cover and the length of ice-free periods, resulting in an overall greening of terrestrial Arctic regions (Goetz *et al.*, 2005; Bhatt *et al.*, 2010). In particular, long-term experimental warming studies carried out in vegetation plots have indicated significant increases in the cover and height of shrubs (e.g. *Betula nana* and *Salix pulchra*), combined with a significant decrease of bryophytes and lichens (Mercado-Diaz, 2011; Pattison and Welker, 2014), most likely due to the competitive exclusion by shrubs (Cornelissen *et al.*, 2001; Jagerbrand *et al.*, 2009). Additionally, an analysis of the responses of tundra vegetation to experimental warming conditions across the Arctic suggested that these

phenomena might continue in the future. In fact, abundance and height of shrubs have increased markedly in the last two decades, particularly in the Low Arctic (Elmendorf *et al.*, 2012). These alterations are expected to be coupled with changes in soil fungal communities (Dahlberg and Bültman, 2013), especially regarding mycorrhizal and root-associated fungi (Hobbie and Hobbie, 2006; Buckeridge and Grogan, 2008; Hobbie *et al.*, 2009). Recently, several papers documented marked changes in the fungal community composition in long-term summer warming and increased snow pack (winter warming) experiments in the Arctic (Deslippe *et al.*, 2011; Geml *et al.*, 2015, 2016; Morgado *et al.*, 2015, 2016; Semenova *et al.*, 2015, 2016). While some compositional changes in soil fungal communities were likely caused by alterations in abiotic factors and edaphic processes, some trends, e.g. the decrease of lichen richness and abundance in the warmed plots, seemed to be a direct results of the increased growth of shrubs. In this regard, soil fungi could be viewed both as sentinels and as amplifiers of global change (Vincent, 2010). One of the most widespread hypotheses is that shifts in fungal communities composition are likely to affect carbon and nitrogen cycles in soil and promote the breakdown of organic matter (Clemmensen *et al.*, 2006; Deslippe and Simard, 2011; Zhang *et al.*, 2014; Treseder *et al.*, 2016), increasing the release of greenhouse gases from soils to the atmosphere (Commane *et al.*, 2017; Crowther *et al.*, 2016), and consequently amplifying climate feedbacks (Zhou *et al.*, 2009; Wieder *et al.*, 2013; Abbot *et al.*, 2016).

Knowledge on vegetation composition is fundamental to understand the functionality of any ecosystem, mainly for interspecific variations in productivity (Ward *et al.*, 2013; Walker *et al.*, 2015), root and litter inputs (Cornelissen *et al.*, 2007; Ward *et al.*, 2015), and plant–microbe associations (Read *et al.*, 2004). For example, it has been hypothesized that the presence of different shrub species, such as *Betula nana* (McLaren *et al.*, 2017), characterized by rapid growth and easily decomposable litter, could lead to a faster carbon turnover rate (Ward *et al.*, 2009; 2015).

In this paper, we compared richness and composition of functional guilds of soil fungal communities in three different habitats in West Greenland. The three habitats can be viewed as a gradient of vegetation complexity: bare ground (BG) without any apparent vegetation growth, biological soil crusts (BSCs) dominated by bryophytes and lichens, and soil covered with a vascular vegetation (VV), e.g. *Empetrum nigrum*, *Vaccinium uliginosum*, *Betula nana*, and *Salix glauca*. In this context, the main aims were (i) to understand how differences in functional profiles of fungal communities relate to different habitats and to biotic and abiotic variables, and (ii) to gain insights into the landscape-level dynamics of vegetation and soil fungi in Western Greenland, in light of shrubs expansion, particularly *Betula nana* (Hollesen *et al.*, 2015).

2.2 Materials and methods

2.2.1 Sampling area

The study area was located in Kobbefjord, Nuuk, West Greenland (64°08' N, 51°23' W). The climate of the area was classified as low Arctic, subzone D (Jonasson *et al.*, 2000). The mean annual air temperature of the area in the period 2008-2010 was 0.7 °C and the mean air temperature of the warmest month, July, was 10.7 °C. In winter, the average air temperatures was -30 °C. The total annual precipitation was 838–1127 mm and an average of 25–50 % of the total annual precipitation fell as snow during the winter period (Søndergaard *et al.*, 2012).

Sampling was carried out in July 27-31, 2017 in an area close the NERO line (Bay *et al.*, 2008), a vegetation transect where plant compositions have been monitored for more than ten years. In total, twenty 2-m^2 plots scattered in the landscape, representing the three above-mentioned habitat types, were sampled: 5 in BGs, 6 in BSCs, and 9 in VVs. BG and BSC plots were generally adjacent to the vegetation and in some cases small patches were dispersed among the vegetation. Exact coordinates, elevation, and shrub genera composition for each plot are listed in Supplementary Table 2.1. In each plot, three replicates of soil samples were collected aseptically at a depth of 5 cm, after removing the top of the soil, resulting in a total of 60 samples. Samples were stored at -20 °C in sterile bags until molecular analyses.

2.2.2 Soil characteristics

Soil water content was measured with a gravimetric method, starting from 5 grams of soil (Reynolds, 1970) and measuring the weight before and after drying in oven, repeating the measurements on dried samples until no variation in weight was observed. pH was measured in a 2.5:1 suspension of dried soil in deionized water, with a HI9321 pH meter (Hanna Instruments Woonsocket, Rhode Island, United States). For each sample, water content and pH were measured in independent triplicates and the mean of the three measurements was considered for the final value.

Phosphorous (P), Carbon (C) and Nitrogen (N) content analyses have been carried out at Eszterházy Károly University, Eger, Hungary. P content was measured via Microwave Plasma Atomic Emission Spectrometry (MP-AES) and C and N content via CNS elemental analyser.

2.2.3 DNA extraction, amplification and sequencing

For each sample, metagenomic DNA was extracted from 0.5 g of soil using DNEasy Powersoil kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The ITS1 region was PCR amplified using ITS1F (Gardes and Bruns, 1993) and ITS2 (White *et al.*, 1990)

primers as described in Smith and Peay (2014). The equimolar pool of uniquely barcoded amplicons was paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform at the Vincent J. Coates Genomics Sequencing Laboratory at University of California, Berkeley.

2.2.4 Bioinformatic analyses

Bcl files were converted to Fastq files and were demultiplexed and primer were removed using bcl2fastq (v 2.18). Dual-matched 8-bp indexes were used to eliminate the occurrence of "barcode bleed" (or tag-switching) between samples.

ITS1 demultiplexed sequences were processed with the Amplicon ToolKit (AMPtk) for NGS data (formally UFITS) v.1.2.1 (Palmer *et al.*, 2018). 6 813 346 starting reads were subjected to quality trimming and PhiX screening using USEARCH v. 9.2.64 (Edgar, 2010) with default parameters. Reads with less than 100bp were removed, reads longer than 300 bp were trimmed and paired-end reads were merged in one step. We obtained 3 405 965 quality-filtered contigs. Individual sample sequence files were merged into a single file and clustered into Operational Taxonomic Units (OTUs) with a 97% identity threshold using VSEARCH v. 2.7.0 (Rognes *et al.*, 2016), simultaneously removing putative chimeras. A total of 3 129 891 (92%) reads have been mapped in 3 491 OTUs. Singletons and rare OTUs (< 5 reads) were removed as recommended by Lindahl *et al.* (2013), resulting in 2 938 OTUs retained. We assigned OTUs to taxonomic groups based on the curated UNITE+INSD reference database dynamic Species Hypotheses (SH) (UTAX release of October 10, 2017) using USEARCH v. 9.2.64 (Edgar, 2010). OTUs with < 70% identity to a fungal sequence were excluded from the following analyses, resulting in 2661 OTUs retained. Representative sequences of fungal OTUs have been submitted to GenBank (BioProject PRJNA526618). The OTU table was normalized for subsequent statistical analyses by rarefying the number of high-quality fungal sequences to the smallest library size (33 870 reads) using the rrarefy function implemented in the vegan package v. 2.5-2 (Oksanen *et al.*, 2018) in R (R Core Team, 2018). Only OTUs with > 90% similarity to a fungal SH with known ecological functions were assigned to one of the following functional guilds: animal pathogens, ectomycorrhizal (ECM) fungi, ericoid mycorrhizal (ERM) fungi, lichenized fungi, mycoparasites, plant pathogens, other root-associated fungi (non-ECM fungi, non-ERM fungi and root endophytes) and saprotrophs. The initial functional assignments were made by FunGuild (Nguyen *et al.*, 2016) and manually checked afterwards based on ecological metadata of the corresponding SHs in UNITE for genera that are known to comprise species with diverse functions.

2.2.5 Statistical analyses

Unless otherwise specified, all analyses were carried out with the vegan package v. 2.5-2 (Oksanen *et al.*, 2018) in R (R Core Team, 2018). Total fungal richness, richness of functional guilds and their relative abundances were compared among the three habitats using ANOVA and Tukey's HSD test. In addition, linear regression analyses were used to examine relationships between the above mentioned edaphic factors and richness of the total community, of the functional guilds, and their relative abundance. Linear regression was also used to examine the relations among the community composition differences of the total fungal community and of the components of the eight functional guilds (Bray-Curtis distance between pair of samples of Hellinger-transformed OTU table) and the corresponding differences in the soil parameters. We ran non-metric multidimensional scaling (NMDS) on the Hellinger-transformed OTU table. Ordinations were run separately for functional guilds as well as for all fungi with the following specifications: distance measure = Bray-Curtis, dimensions = 2, initial configurations = 100, model = global, maximum number of iterations = 200, convergence ratio for stress = 0.999999. We used the *envfit* R function to fit edaphic variables and the relative abundance of the shrub genera (*Betula*, *Empetrum*, *Salix*, and *Vaccinium*; Supplementary Table 2.1) onto the NMDS ordinations. In addition, we tested whether fungal communities were statistically different among habitat types using the multi response permutation procedure (MRPP) and we determined any preferences of individual OTUs for each habitat using indicator species analysis (Dufrêne and Legendre 1997) in PC-ORD v. 6.0 (McCune *et al.*, 2002).

Permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001) was carried out on Bray-Curtis distance matrices obtained of Hellinger-transformed OTU tables with 9999 permutations, with the *adonis* function, in order to determine the effect of each soil physico-chemical characteristic, as well as the type of coverage (BG, BSC or VV), on the observed variance of the total community and of the functional guilds identified. To account for correlations among environmental variables, we performed a forward selection of parameters, including only significant environmental variables in the final models. The same approach was used also taking into account only 27 samples of VV plots in order to assess the effect of the edaphic characteristics, and the relative abundance of the four dominant shrub genera (*Salix*, *Betula*, *Vaccinium* and *Empetrum*), on the variance of the total community and of the different functional guilds.

2.3 Results

2.3.1 Fungal richness and abundance patterns

The quality-filtered and rarefied dataset contained 2661 fungal OTUs. The proportions of OTUs found exclusively in VV was the highest (23.6%) compared to BSC and BG samples (7.8% and 16%, respectively), whereas the BG samples showed the highest number of indicator OTUs (266 OTUs, compared with 146 OTUs for VV and 215 for BSC samples, respectively; Supplementary Table 2.2).

The richness of the total fungal community showed a slight increase ranging from BG to vegetation covered soil plots (figure 2.1A). This trend seemed to be driven mainly by Ascomycota, the most abundant phylum (1303 OTUs, 49% of the total) in BSC samples, instead VV samples had a significative higher proportion of Basidiomycota, the second most abundant phylum (775 OTUs, 30% of the total) (Supplementary figure 2.1).

With respect to functional assignments, 1105 OTUs (41.53% of the rarefied dataset) had > 90% similarity to a fungal SH with known ecological function and were assigned to functional guilds. Five out of the eight functional guilds examined, namely ERM fungi, mycoparasites, plant pathogens, animal pathogens and saprotrophic fungi, showed an increase in richness from BG to VV plots (figure 2.1 C and F-I). Among these guilds, the increase of richness in VV plots respect to BSCs was significant only for saprotrophs. ECM fungi showed an increase of richness in VV plots in respect to BSCs, but a not significant difference between VV and BG plots (figure 2.1B). For the remaining root associated fungi, we did not find significant differences in richness among the three habitats (figure 2.1D). As expected, lichenized fungi showed the highest richness in BSC samples (figure 2.1E), where bryophytes and lichens are dominant. When significant, the trends were the same also for the relative abundance of the components of the guilds (Supplementary figure 2.2), except for root-associated fungi (not significant for the richness) that showed an increase from BG to VV and BSC plots (Supplementary figure 2.2C).

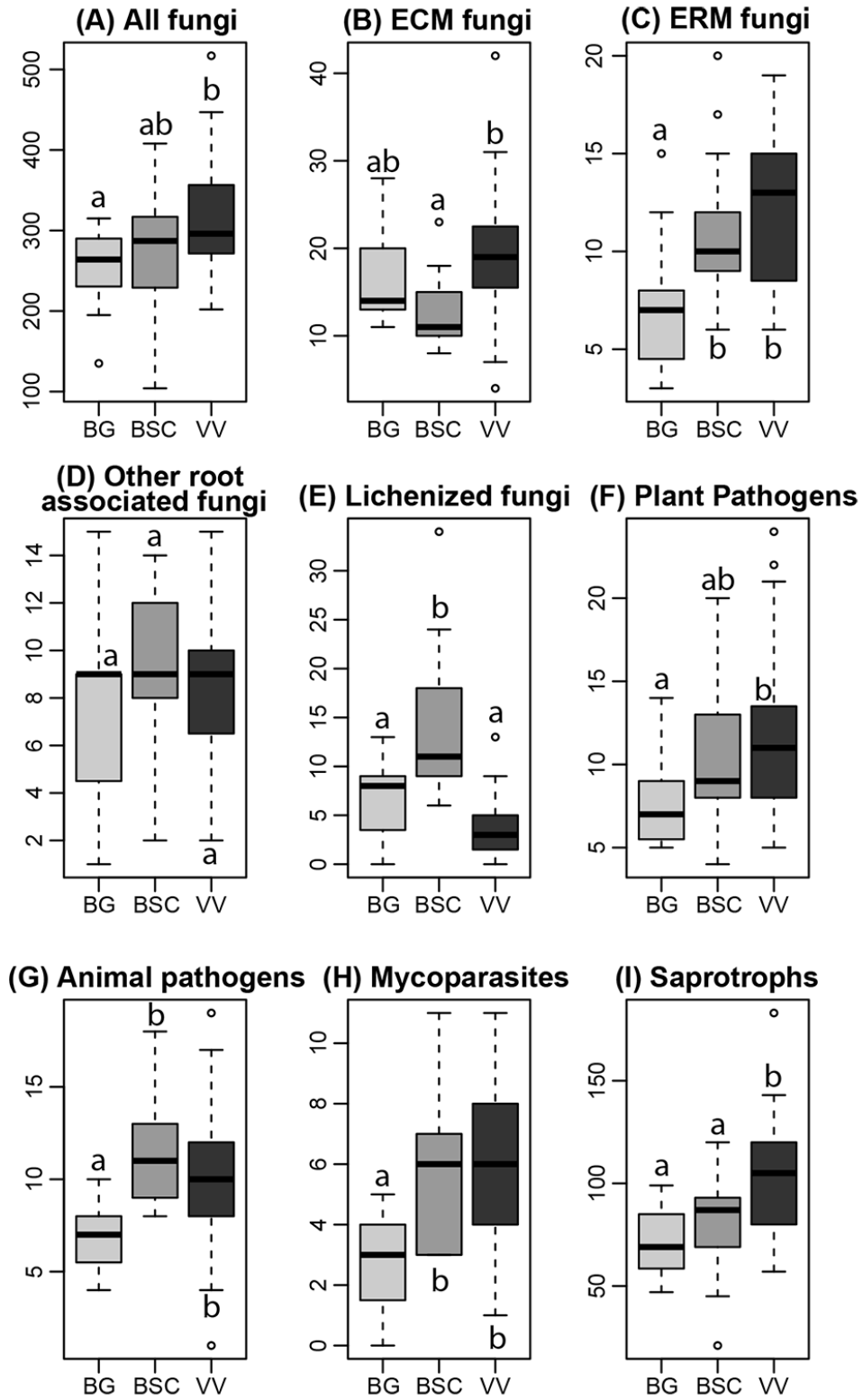


Figure 2.1 Richness of the total fungal communities (A) and of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, plant pathogenic, animal pathogenic, mycoparasites and saprotrophic fungi (B-I), in each habitat (light grey, Bare Grounds plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc TukeyHSD test (significant for $p<0.05$).

2.3.2 Correlation between richness and relative abundance of fungal guilds to soil parameters

Edaphic parameters measured had significant differences among the three habitats, with the water relative content increasing from BG samples to BSC and VV plots and the pH showing an opposite trend, with the highest values in BG samples (figure 2.2 A and B). An increasing trend from BG to BSC and VV plots has been shown for the P, C and N content, and for the ratio of these last two (figure 2.2 C-F). However, C content and C/N ratio were higher in BSC plots compared to VV ones.

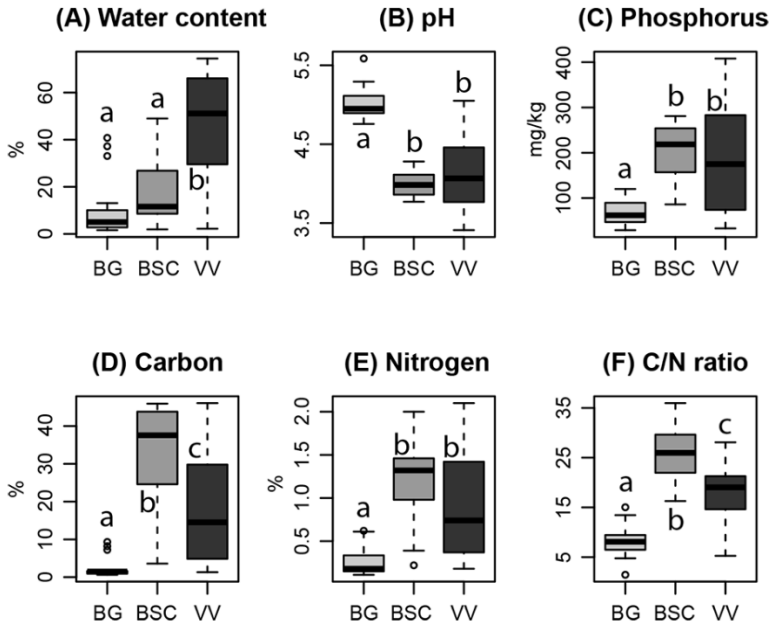


Figure 2.2 Variation of soil parameters in each habitat considered (light grey, Bare Ground plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc TukeyHSD test (significant for $p<0.05$).

The richness of the fungal communities had a significative positive correlation with the water content of the soil samples (figure 2.3; Supplementary table 2.3). The same trend was significant also for many fungal functional guilds, as ECM fungi, ERM fungi, plant pathogens, mycoparasites and saprotrophs, and was the opposite for lichenized fungi that showed a lower diversity for the wettest samples (figure 2.3; Supplementary table 2.3). The same trends were marginally significant only for the abundance of ERM fungi and saprotrophs, and opposite in respect to the trend of plant pathogens abundance (figure 2.4; Supplementary table 2.3).

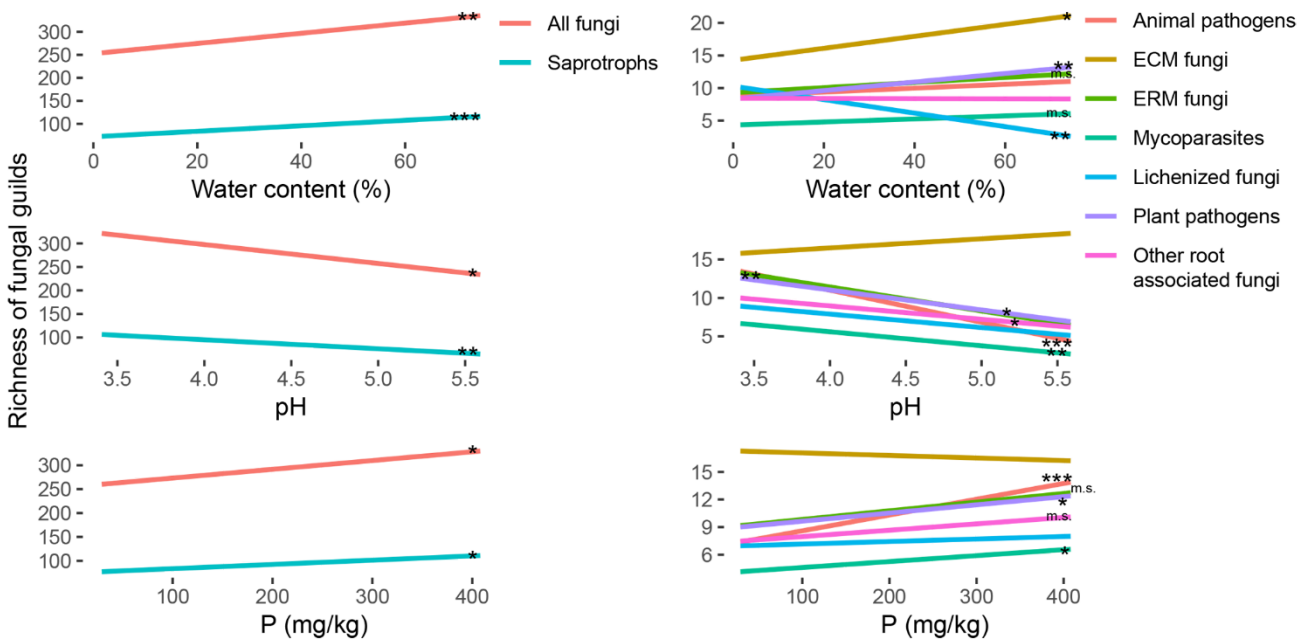


Figure 2.3 Regression lines for the variation of richness (y-axis) of the total fungal community and of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, plant pathogenic, animal pathogenic, mycoparasites and saprotrophic fungi, in response to soil parameters (water content, pH and P content; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m. s. (marginally significant) $p<0.1$. Single graphs of the regressions with the points corresponding to all the samples are reported in Supplementary figure 2.3. All the slopes and r^2 values for statistically significant regressions are reported in supplementary table 2.3.

Regarding the effect on the richness of the total fungal community, the increasing pH had a negative impact, with a decrease of biodiversity (figure 2.3; Supplementary table 2.3). Going deeper in the different guilds (figure 2.3), the richness for ERM fungi, root associated fungi, plant pathogens, animal pathogens, mycoparasites and saprotrophs was negatively correlated with the pH (Supplementary table 2.3). Regarding the abundance (figure 2.4), the same trend was also significant for ERM fungi, root associated fungi, and mycoparasites (Supplementary table 2.3). ECM fungi abundance was positively correlated with soil pH (Supplementary table 2.3), but their richness was not. Total richness was positively correlated with phosphorus content also (figure 2.3; Supplementary table 2.3), as well as the richness of many guilds: ERM fungi, root associate fungi, animal pathogens, mycoparasites, and saprotrophs (Supplementary table 2.3). Among the functional guilds, ERM fungi abundance was marginally negatively correlated with P content (figure 2.4; Supplementary table 2.3).

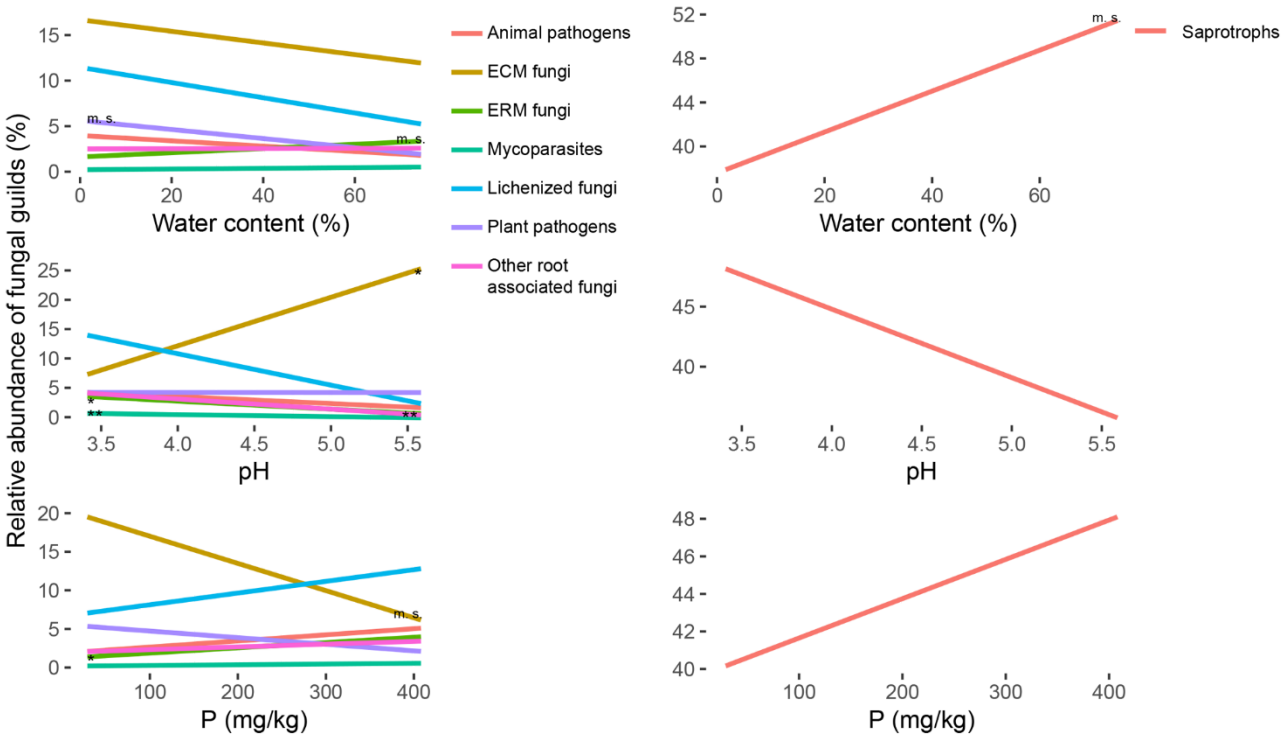


Figure 2.4 Regression lines for the variation of relative abundance (y-axis) of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, plant pathogenic, animal pathogenic, mycoparasites and saprotrophic fungi, in response to soil parameters (water content, pH and P content; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m. s. (marginally significant) $p<0.1$. Single graphs of the regressions with the points corresponding to all the samples are reported in Supplementary figure 2.4. All the slopes and r^2 values for statistically significant regressions are reported in Supplementary table 2.3.

The content of C and N and their ratio were generally positively correlated with the richness (Supplementary figure 2.3), except for ECM, but the correlations were significative for few groups only. Animal pathogens showed the strongest effects of these parameters on their richness (Supplementary figure 2.3; Supplementary table 2.3). ERM fungi richness was marginally correlated with these three parameters (Supplementary figure 2.3; Supplementary table 2.3). N content had a marginally significant effect on mycoparasites and saprotrophs, and for this two guilds and lichenized fungi C/N ratio was significant (Supplementary table 2.3). With respect to the abundance, root associated fungi (ECM, ERM and other root associated) were generally correlated with these three parameters, negatively for ECM and positively for the other two guilds (Supplementary figure 2.4; Supplementary table 2.3). The abundance correlation was less strong for animal pathogens (Supplementary figure 2.4; Supplementary table 2.3). In general, even been significant with 95%

confidence, all the regression had low r^2 values and have to be carefully considered for precise predictions.

2.3.3 Fungal community composition

The composition of the total fungal community changed significantly among the three different habitats, as visualized in the NMDS ordination (figure 2.5A), resulted in a two-dimensional final solution with a stress value of 0.153. The MRPP analysis revealed that the difference was statistically supported (A=0.082, p=0.001).

For some of the functional guilds identified in the community, the NMDS analyses resulted in a final 2-dimensional ordination with stress values of 0.168 for animal pathogens, 0.187 for plant pathogens and 0.169 for saprotrophic fungi. For the other guilds, a 3-dimensional solution was necessary, resulting in final stress values of 0.188 for ECM fungi, 0.169 for ERM fungi, 0.132 for root associated fungi, 0.175 for lichenized fungi, and 0.136 for mycoparasites. In all cases, the NMDS ordinations (figure 2.5) showed a strong structuring of fungal communities according to the different habitats, as confirmed by the MRPP analyses, that revealed a significative difference in the distribution among the three different habitats (p=0.001), with the strongest effects for animal and plant pathogens (A=0.154 and A=0.107, respectively). All the other guilds showed less strong diversification among the habitats (A=0.079 for ECM fungi, A=0.086 for ERM fungi, A=0.066 for root-associated fungi, A=0.050 for lichenized fungi, A=0.045 for mycoparasites, and A=0.084 for saprotrophs). pH and C/N ratio resulted to be the strongest two parameters for the community composition for almost all the guilds (Supplementary Table 2.4).

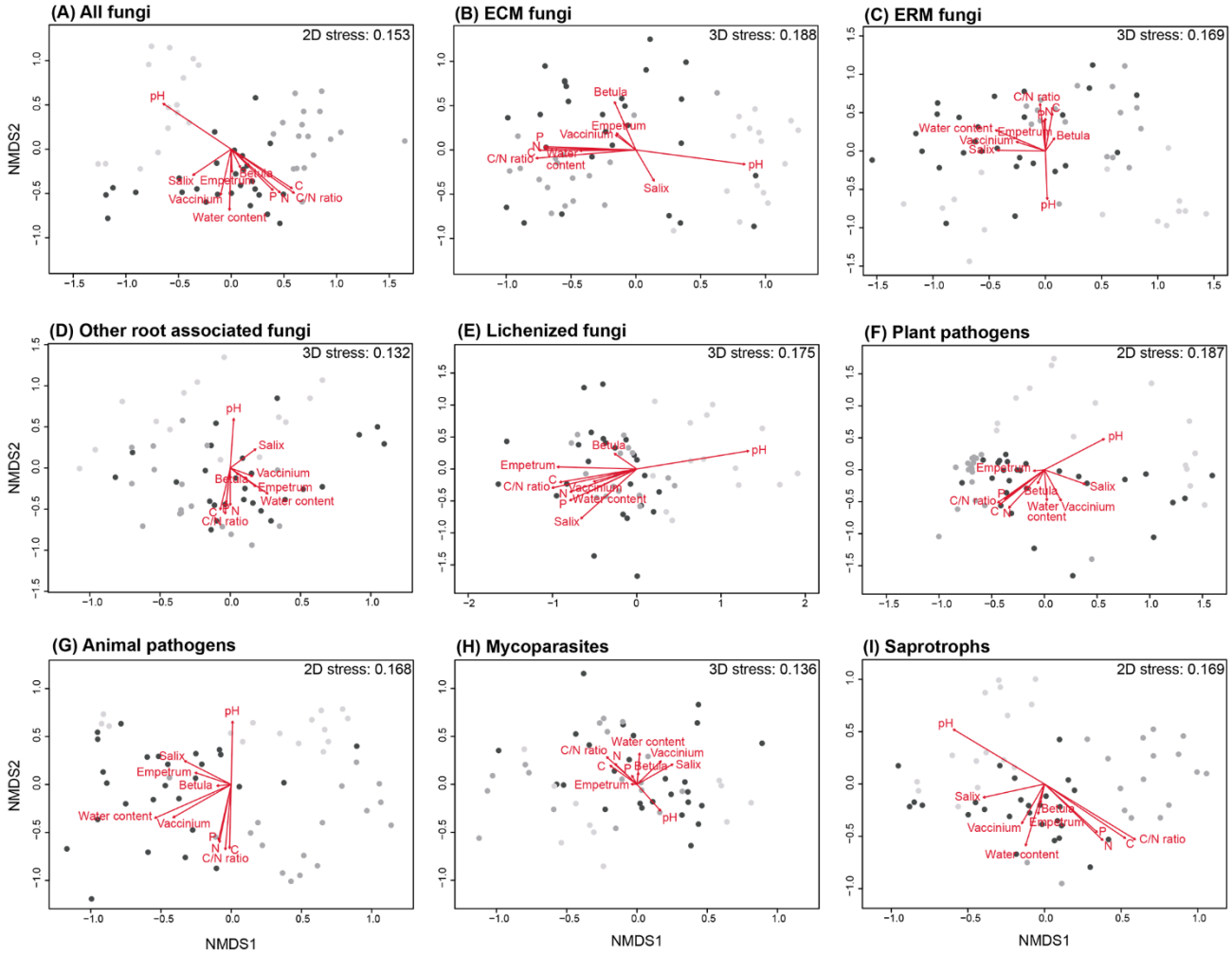


Figure 2.5 Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in composition of fungal communities (Hellinger transformed OTUs abundances) in the habitats studied (light grey, Bare Ground plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots) for the total fungal community (A) and for ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, mycoparasites, animal pathogens, plant pathogens and saprotrophic fungi (B-I). The significance and the strength of the correlation of all the variables in the figure is reported in supplementary table 2.4.

2.3.4 Effects of edaphic parameters and type of coverage on fungal community composition

In order to assess the effect of the soil physico-chemical characteristics on fungal community structure, the differences in community composition (Bray-Curtis distance) were plotted against the differences in the single soil parameters. All regressions were highly significant (Supplementary figure 2.5).

PerMANOVA analysis was used to infer the degree to which edaphic parameters and the presence/absence and type of vegetation coverage could explain the variance in community composition among samples. When considering the available parameters independently, all were

significant in determining the structure of the fungal communities, even at the level of every single functional guild identified (except water content for lichenized fungi; Table 2.1). The type of habitat (BG, BSC or VV) resulted to be the strongest parameter, followed by the pH in all the guilds, except animal pathogens (Table 2.2). When combined, depending on their influence, C and P content were not independent from the other parameters in shaping the total community. The type of habitat explained by itself more than 18% of the variance for the total community and was dominant for all the groups, with the highest percentages for plant and animal pathogens (20.03% and 28.07%, respectively; Table 2.2). The second dominant parameter acting independently for almost all the guilds (except lichenized fungi and mycoparasites) was the pH. Nitrogen content also was an independent driver of total community composition and of many guilds, including ECM fungi and saprotrophs. Finally, water content, even being a determinant parameter for the total community, was significant only for ERM fungi among functional guilds.

Table 2.1 Proportion of variation in fungal community composition, explained by soil variables calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed fungal community matrices. Significant variables were included in the final model for each fungal guild (Table 2.2).

Variable	**All fungi**		**ECM fungi**		**ERM fungi**		**Other root associated fungi**		**Lichenized fungi**	
	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
Habitat	18.145	0.001	16.549	0.001	17.028	0.001	14.083	0.001	11.713	0.001
pH	10.267	0.001	10.259	0.001	8.836	0.001	9.974	0.001	6.063	0.001
Water content	6.325	0.001	5.634	0.001	5.913	0.001	4.845	0.005	2.708	0.093
C	7.705	0.001	8.796	0.001	6.127	0.001	6.683	0.001	4.460	0.003
N	6.508	0.001	7.638	0.001	5.227	0.002	5.456	0.001	3.878	0.006
C/N ratio	9.315	0.001	9.591	0.001	8.198	0.001	7.673	0.001	5.285	0.002
P	5.968	0.001	7.632	0.001	4.683	0.002	5.666	0.003	3.341	0.019

Variable	**Plant pathogens**		**Animal pathogens**		**Mycoparasites**		**Saprotrophs**	
	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
Habitat	20.030	0.001	28.070	0.001	11.041	0.001	18.464	0.001
pH	14.895	0.001	11.731	0.001	6.659	0.001	10.734	0.001
Water content	4.116	0.020	12.803	0.001	4.763	0.006	6.376	0.001
C	9.780	0.001	11.712	0.001	4.601	0.012	8.624	0.001
N	7.910	0.001	10.162	0.001	4.332	0.017	7.126	0.001
C/N ratio	11.346	0.001	13.548	0.001	5.219	0.002	10.315	0.001
P	7.303	0.001	8.271	0.002	3.987	0.025	6.199	0.001

Table 2.2 Proportion of variation in fungal community composition, at level of the total community and the eight functional guilds, explained by soil variables added sequentially (from the first to the last) in a model, depending on their independent influence in the variance, as reported in Table 2.1. Significant values in bold.

All fungi			**ECM fungi**			**ERM fungi**			**Other root associated fungi**			**Lichenized fungi**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Habitat	**18.145**	**0.0001**	Habitat	**16.549**	**0.0001**	Habitat	**17.028**	**0.0001**	Habitat	**14.083**	**0.0001**	Habitat	**11.713**	**0.0001**
pH	**3.697**	**0.0004**	pH	**4.230**	**0.0002**	pH	**3.063**	**0.0055**	pH	**3.547**	**0.0179**	pH	1.781	0.3655
C/N ratio	**2.886**	**0.0017**	C/N ratio	2.015	0.0769	C/N ratio	**2.464**	**0.0319**	C/N ratio	0.927	0.7922	C/N ratio	2.005	0.2446
C	1.355	0.3958	C	1.460	0.3682	C	1.395	0.4009	C	0.614	0.9429	C	0.798	0.9625
N	**3.708**	**0.0001**	N	**2.495**	**0.0158**	Water content	**4.655**	**0.0001**	P	0.782	0.8698	N	**4.126**	**0.0010**
Water content	**2.119**	**0.0302**	P	**2.170**	**0.0448**	N	1.846	0.1547	N	2.748	0.0667	P	2.153	0.1775
P	1.779	0.1001	Water content	2.066	0.0680	P	1.927	0.1217	Water content	0.653	0.9278	Residuals	77.424	
Residuals	66.311		Residuals	69.015		Residuals	67.622		Residuals	76.647				

Plant pathogens			**Animal pathogens**			**Mycoparasites**			**Saprotrophs**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Habitat	**20.030**	**0.0001**	Habitat	**28.070**	**0.0001**	Habitat	**11.041**	**0.0001**	Habitat	**18.464**	**0.0001**
pH	**4.366**	**0.0027**	C/N ratio	**5.213**	**0.0012**	pH	2.717	0.0822	pH	**3.914**	**0.0003**
C/N ratio	2.027	0.1156	Water content	2.292	0.0508	C/N ratio	1.606	0.3841	C/N ratio	**2.926**	**0.0032**
C	2.475	0.0574	pH	**4.840**	**0.0004**	Water content	2.864	0.0645	C	1.639	0.1706
N	**3.681**	**0.0086**	C	1.173	0.3555	C	1.705	0.3514	N	**4.030**	**0.0003**
P	2.225	0.0813	N	1.784	0.1266	N	0.474	0.9611	Water content	2.031	0.0527
Water content	0.964	0.6422	P	1.766	0.1339	P	**4.528**	**0.0044**	P	1.811	0.0994
Residuals	64.232		Residuals	54.862		Residuals	75.066		Residuals	65.186	

2.3.5 Effect of shrub composition on fungal communities

The effect of the relative abundance of shrubs genera in VV plots (Supplementary Table 2.1) was addressed via PerMANOVA analysis. In this case, all the parameters were significant in shaping the total community, but very few of them were determinant for many of the functional guilds (Table 2.3). In particular, for ECM fungi two shrub genera (*Vaccinium* and *Empetrum*) were not significant, and for other root associated fungi only *Salix* was determinant for the observed variance. Combining the variables, it resulted that *Salix* relative abundance was the main driver of VV plots communities (Table 2.4), explaining the highest percentages of variance for all the guilds except ECM fungi, that were surprisingly mainly driven by abiotic parameters (pH and water content). Instead, ERM fungi were mainly shaped by shrub genera abundance, even if *Salix* effect was dominant in respect to *Vaccinium* and *Empetrum* (Table 2.4). For saprotrophic fungi also, the presence of different types of shrub was the main determinant factor for community composition.

2.4 Discussion

This study is the first to characterize fungal communities in a Western Greenland landscape. Data presented here reveal that fungal community composition, as well as richness and relative abundance of functional guilds clearly differ among the three sampled habitats. Such strong structuring at small spatial scales confirms patterns observed in Eastern Greenland (Grau *et al.*, 2017), Svalbard (Blaalid *et al.*, 2014; Mundra et al., 2015), and the North American Arctic (Timling *et al.*, 2014; Geml *et al.*, 2016). However, the observed positive relationship between fungal richness and vegetation complexity differs from previous findings in Eastern Greenland, where, in the corresponding heath tundra, the bare ground habitat had the highest fungal richness, significantly higher than habitats dominated by *Dryas* and *Salix* (Grau *et al.*, 2017). In our study, bare ground plots, showing the lowest richness, had a high percentage (16%) of exclusive OTUs and the highest number of indicator species (Supplementary Table 2.2). In spite of the differences in richness patterns among habitats, the high number of taxa specific to bare soils is a remarkable common feature with the study of Grau *et al.* (2017) and confirms that the harshest habitats in the Arctic appear to harbour a unique set of stress-tolerant fungi that likely are outcompeted in more vegetated habitat types. As also stated by Grau *et al.* (2017), the expected expansion of shrub dominated communities to previously unvegetated areas could lead to the loss of fungal diversity, specific of habitats without vegetation cover.

Table 2.3 Proportion of variation in fungal community composition of vascular vegetation (VV) plots explained by soil variables and shrub genera relative abundances, calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed fungal community matrices. Significant variables (in bold) were included in the final model for each fungal guild (Table 2.4).

Variables	**All fungi**		**ECM fungi**		**ERM fungi**		**Other root associated fungi**		**Lichenized fungi**	
	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
pH	**10.417**	**0.001**	**11.492**	**0.001**	**8.675**	**0.003**	**10.924**	**0.003**	4.526	0.418
Water content	**7.925**	**0.003**	**12.691**	**0.001**	**6.999**	**0.014**	2.451	0.756	2.310	0.920
C	**7.878**	**0.007**	**11.698**	**0.001**	**6.344**	**0.036**	5.265	0.193	2.750	0.833
N	**7.346**	**0.004**	**11.272**	**0.001**	**6.283**	**0.042**	3.983	0.401	2.274	0.917
C/N ratio	**6.838**	**0.008**	**10.272**	**0.001**	5.617	0.082	5.220	0.207	3.619	0.665
P	**7.070**	**0.008**	**11.002**	**0.001**	**6.335**	**0.040**	3.720	0.476	2.557	0.890
Salix	**13.155**	**0.001**	**10.657**	**0.001**	**10.549**	**0.001**	**21.695**	**0.001**	**9.625**	**0.003**
Betula	**6.723**	**0.010**	**7.588**	**0.008**	**7.827**	**0.008**	3.294	0.550	4.610	0.395
Vaccinium	**6.245**	**0.018**	5.581	0.099	**7.093**	**0.015**	1.715	0.865	3.949	0.573
Empetrum	**8.435**	**0.002**	5.837	0.060	**9.910**	**0.001**	6.650	0.092	4.951	0.324

Variables	**Plant pathogens**		**Animal pathogens**		**Mycoparasites**		**Saprotrophs**	
	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
pH	**10.017**	**0.014**	**7.786**	**0.027**	**7.861**	**0.024**	**11.430**	**0.001**
Water content	6.280	0.107	**9.035**	**0.012**	4.485	0.318	**7.627**	**0.008**
C	**9.737**	**0.017**	**8.175**	**0.034**	5.754	0.127	**8.114**	**0.002**
N	**8.341**	**0.036**	6.849	0.071	5.697	0.138	**7.575**	**0.007**
C/N ratio	4.995	0.221	6.795	0.061	4.817	0.255	**7.089**	**0.012**
P	6.044	0.096	6.144	0.079	5.173	0.183	**7.368**	**0.007**
Salix	**16.513**	**0.001**	**10.254**	**0.010**	**9.268**	**0.022**	**13.505**	**0.001**
Betula	4.401	0.291	4.808	0.231	3.835	0.419	**6.468**	**0.025**
Vaccinium	**15.556**	**0.001**	**7.826**	**0.029**	6.010	0.132	**6.642**	**0.021**
Empetrum	**12.262**	**0.002**	7.064	0.051	**7.670**	**0.026**	**9.157**	**0.002**

Table 2.4 Proportion of variation in fungal community composition of vascular vegetation (V) plots, at level of the total community and the eight functional guilds explained by shrub genera abundance and soil variables. Terms were added sequentially (first to the last) in a model, depending on their independent influence in the variance, as reported in Table 2.3. Significant values in bold.

All fungi			**ECM fungi**			**ERM fungi**			**Other root associated fungi**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Salix	**13.155**	**0.0001**	Water content	**12.691**	**0.0001**	*Salix*	**10.549**	**0.0001**	*Salix*	**21.695**	**0.0003**
pH	**6.262**	**0.0007**	C	**6.349**	**0.0031**	*Empetrum*	**9.203**	**0.0001**	pH	1.671	0.8237
Empetrum	**7.244**	**0.0003**	pH	**7.384**	**0.0001**	pH	**6.415**	**0.0011**	Residuals	76.634	
Water content	**7.981**	**0.002**	N	4.108	0.0917	*Betula*	**5.726**	**0.0050**			
C	**4.174**	**0.0354**	P	4.130	0.0864	*Vaccinium*	**6.189**	**0.0021**			
N	**4.266**	**0.0340**	*Salix*	**6.374**	**0.0021**	Water content	**7.324**	**0.0003**			
P	3.121	0.2533	C/N ratio	2.791	0.4924	C	2.955	0.3461			
C/N ratio	2.982	0.3097	*Betula*	**4.731**	**0.0342**	P	4.031	0.0808			
Betula	3.995	0.0548	Residuals	51.442		N	2.099	0.7252			
Vaccinium	3.023	0.2889				Residuals	45.510				
Residuals	43.796										
Plant pathogens			**Animal pathogens**			**Mycoparasites**			**Saprotrophs**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Salix	**16.513**	**0.0001**	*Salix*	**10.254**	**0.0023**	*Salix*	**9.268**	**0.0135**	*Salix*	**13.505**	**0.0001**
Vaccinium	**13.659**	**0.0001**	Water content	**9.049**	**0.0020**	pH	4.086	0.3051	pH	**6.671**	**0.0005**
Empetrum	3.399	0.2546	C	**8.039**	**0.0037**	*Empetrum*	6.560	0.0514	*Empetrum*	**7.920**	**0.0002**
pH	4.385	0.1226	*Vaccinium*	**7.460**	**0.0066**	Residuals	80.086		C	**4.655**	**0.0196**
C	4.182	0.1527	pH	7.417	0.0082				Water content	**6.664**	**0.0006**
N	2.809	0.3981	Residuals	57.781					N	**5.193**	**0.0071**
Residuals	55.053								P	2.559	0.5066
									C/N ratio	3.664	0.1034
									Vaccinium	3.183	0.2203
									Betula	3.311	0.1822
									Residuals	42.676	

Community composition appeared to be mainly shaped by the type of habitat and by differences in abiotic conditions connected to the habitats. In particular, among the edaphic parameters tested, pH differed among habitats and correlated strongly with fungal richness and abundance, and community composition. Similar trends for soil pH values have been reported in primary successional chronosequences at glacier fronts, where young soils (bare soils), containing a relatively low amount of organic matter and clay minerals, had higher pH than older soils covered by biological crusts and then by shrubs (Kwon *et al.*, 2015). Soil pH has been reported as one of the strongest parameters in shaping the fungal communities across the Arctic (Timling *et al.*, 2014). In particular, pH variations, resulting in different plant communities, have been observed to be the major drivers at the transition between the bioclimatic subzones D and E in northern Alaska, where the main differences in fungal communities were observed (Timling *et al.*, 2014). A strong effect of pH on the community structure has been observed also for soil bacteria across different biomes in the same regions (Chu *et al.*, 2010; Siciliano *et al.*, 2014).

The observed increase in P content with increased vegetation complexity in our plots (Figure 2.2) is similar to what was already reported for a glacier foreland (Borchhardt *et al.*, 2019), where microbial P storage increased with distance from the glacier. Despite being abundant in the topsoil of bedrock of glaciated regions due to mineral weathering (Bradley *et al.*, 2014), P is subjected to high rates of release by leaching (Wu *et al.*, 2015). Microbial assimilation and storage of P is fundamental for its availability for plant colonization. The possible increasing in plant coverage could increase fungal richness and this could explain the strong positive relationship of P content and fungal richness that we found. Even being significant for community composition, in most cases P effect was not independent from other parameters. P availability is highly affected by soil pH, the main determinant of our communities. In fact, P is highly soluble at neutral pH values and becomes less available at basic and acidic pH levels. Similar effects for soil P content have been reported also for root associated fungi in the high Arctic, making it difficult to disentangle the effects of pH from those of other edaphic factors (Fujimura and Egger, 2012).

With respect to functional guilds, richness and relative abundance of ECM fungi were higher in the VV plots, likely due to increased abundance of their hosts. However, both richness and relative abundance of ECM fungi were surprisingly high also in BG plots, with no significant difference compared to VV plots, being lower only in BSC samples (figure 2.1 and Supplementary figure 2.2). Root associated fungi have shown to be highly diverse already in recently exposed areas in the Arctic (Blaalid *et al.*, 2012) and ECM fungi in the Arctic bioclimatic subzone A, lacking of woody species and characterized by fine-grained soils, occasionally covered by lichens, bryophytes, cyanobacteria,

and scattered forbs (Timling *et al.*, 2014). In first instance, this situation could be explained by the fact that in the earliest stages of fungal colonization and communities development, stochastic processes are the main driving factors of communities composition (Jumpponen, 2003), favoured by the already well documented high dispersal ability of Arctic fungi (Geml *et al.*, 2012), of which ECM fungi are the main components. Some ECM sequences found in these plots could originate from spores, which could facilitate the expansion of shrubs from the surrounding areas. However, it is remarkable that the composition of ECM fungal communities in the bare grounds was well differentiated from the communities in the vegetated plots, suggesting a negligible effect of 'spore rain'. Therefore, a probable explanation for the high prevalence on ECM fungi in the BG plots is that most of them are likely associated with the roots of shrubs and other hosts growing in adjacent vegetated patches, present in BG samples as small fragments. Furthermore, the compositional differences among habitats likely indicate the influence of edaphic factors on the community composition of ECM fungi, associated with the root system of the same host, at small spatial scales. For example, parts of a shrub root system may grow in soils with different pH, which is expected to shape ECM community composition. Additionally, taking into account only VV plots, we found that pH and water content had a stronger effect on ECM community composition than above shrub composition, confirming the importance of differences in edaphic conditions in shaping ECM fungal community structure at small spatial scales. Finally, the negative relationship between soil N content and ECM fungi richness and abundance has been repeatedly reported (Bödeker *et al.*, 2014), which likely explains at least in part the patterns observed here, i.e. the lower abundance and richness of ECM fungi in BSC plots, characterized by higher N content.

ERM fungi showed an increase in richness with increased vegetation coverage, likely due to the presence of their host species (figure 2.1). Their diversity and, to a lesser extent, their abundance were also high in BSC plots. ERM fungi have evolved recently from saprotrophic fungi (Martino et al., 2018) and, unlike ECM fungi that, during the transition to fully mycorrhizal habit, have lost genes coding for plant cell wall-degrading enzymes (Martin *et al.*, 2016), all ERM fungi, regardless of their taxonomic position, feature a large set of degrading enzymes, specifically those involved in the degradation of hemicelluloses, pectins, glucans and mannans. This suggests that they still are in a transitional evolutionary stage between saprotrophy and mutualism (Martino *et al.*, 2018) and, thus, are not obligate mutualists. Therefore, ERM fungi can occur in BSC plots, where their symbiotic hosts are not present and where they can survive as saprotrophs, thanks to their ability to thrive in more limiting conditions. Among the habitats sampled in our study, ERM fungal community composition

was mainly driven by edaphic variables. They are usually characteristic of acidic soils, with low nutrients availability and high content of recalcitrant compounds (Cairney and Meharg, 2003); the strong negative correlations between the richness and relative abundance of ERM fungi and soil pH confirm that. Additionally, in the VV plots, we found that despite the presence of two Ericaceae genera being significant in shaping the structure of ERM communities, the effect of the *Salix* relative abundance was stronger. This pattern may be explained by the versatile habit of ERM fungi as well, because, besides being able to act as decomposers, ERM fungi can also live as endophytes in root tips of ECM plants (Bergero *et al.*, 2000; Tedersoo *et al.*, 2009; Grelet *et al.*, 2010; Kernaghan and Patriquin, 2011; Vohník *et al.*, 2013), and colonize both co-occurring ECM and non-ECM species (Chambers *et al.*, 2008).

Saprotrophic fungi had a higher richness and relative abundance in VV plots, accounting also for the great part of indicator OTUs with an assigned function (33.6% of the OTUs identified as indicator species for this habitat). Their greater richness and relative abundance in the vegetated plots is likely due, in part, to greater soil moisture (Supplementary figure 2.3) and possibly to an increase in plant litter biomass. Within the saprotrophic guild, the above increase was primarily driven by litter decomposer and wood decaying fungi (mainly basidiomycetes), as opposed to the generalist saprotrophs relying on simple sugars (mainly ascomycetes), as indicated by the relative richness values of the two phyla among the habitats (Supplementary figure 2.1).

The soil C content and C/N ratio were lower in VV than in BSC plots, while N content was lower, but not statistically different (figure 2.2). Lichen thalli, which make up for a large part of the BSCs biomass, are more easily degradable than woody plants, which could explain the higher soil C content in BSCs.

The greater production of woody litter resistant to decomposition, due to shrub encroachment, especially by *Betula* (Hobbie and Chapin, 1996; Weintraub and Schimel, 2005; Wookey *et al.*, 2009), could result in the reduction of C turnover rates (Natali *et al.*, 2012; Sistla *et al.*, 2013).

We found a positive relationship between the richness of pathogenic fungi and the vegetation cover, possibly due to an increase in the abundance of their hosts and to corresponding changes in water availability and pH values, in accordance with Timling *et al.* (2014).

Lichenized fungal richness peaked in BSC plots, where lichens are one of the main components of aboveground biomass, with an evident decrease of species richness in VV plots due to competition with vascular plants (Cornelissen *et al.*, 2001; Jagerbrand *et al.*, 2009). Among the edaphic parameters considered, richness of lichenized fungi was negatively corelated to the soil water content, seemed not

to be related with pH, and only N content was an independent predictor of community composition, suggesting that other parameters have to be considered for describing the distribution of these species. Interestingly, not only the increase in shrub coverage, but also its composition, could lead to a deep modification of the Arctic soil ecosystems, as well as C and N fluxes. Changes in vegetation composition influence rhizosphere microbial communities by altering the quality and quantity of litter inputs (Hobbie, 1992, Bardgett, 2005). For example, leaves of *Betula nana*, a deciduous shrub strongly encroaching in tundra environments and dominant in the vegetation plots examined, are expected to be decomposed faster than other plant species, given their high nitrogen content (Aerts et al., 2006), but the overall associated litter decomposition may be reduced by the production of a greater proportion of more recalcitrant woody litter (Hobbie and Chapin, 1996; Weintraub and Schimel, 2005; Wookey *et al.*, 2009).

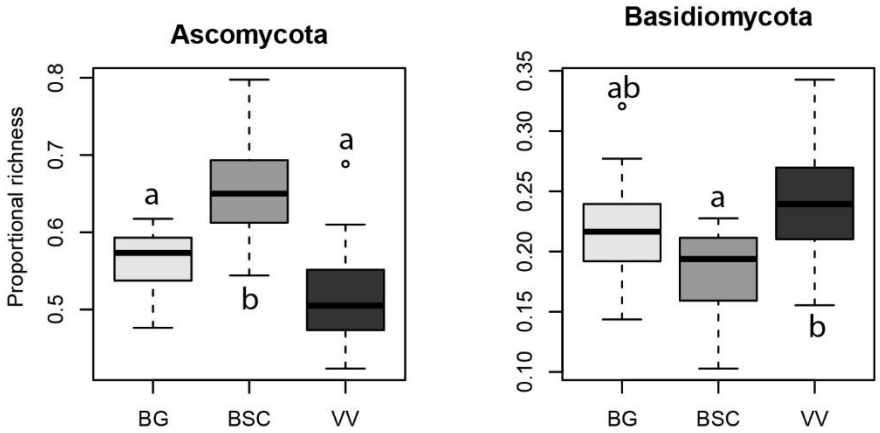
The significant impact of shrub composition on fungal community and on many functional guilds is consistent with well-documented intimate associations between plant species and their mycorrhizal symbionts (Wang and Qiu, 2006) and pathogens (Gilbert and Webb, 2007). Perhaps surprisingly, among the environmental variables considered, the *Salix* percent coverage explained the highest proportion of variance in the composition of animal pathogens. Deciduous shrubs (such as *Betula nana* or *Salix glauca*) have rapid growth and a high leaf turnover, with little investment in defence. Differently, slow-growing evergreen shrubs (*Empetrum nigrum* and *Vaccinium uliginosum*), with slow leaf turnover, invest more in defence (MacLean and Jensen, 1985). Therefore, differences among the preferences of insect species with respect to different shrub species may partly explain the compositional differences in fungal animal pathogens.

2.5 Conclusions

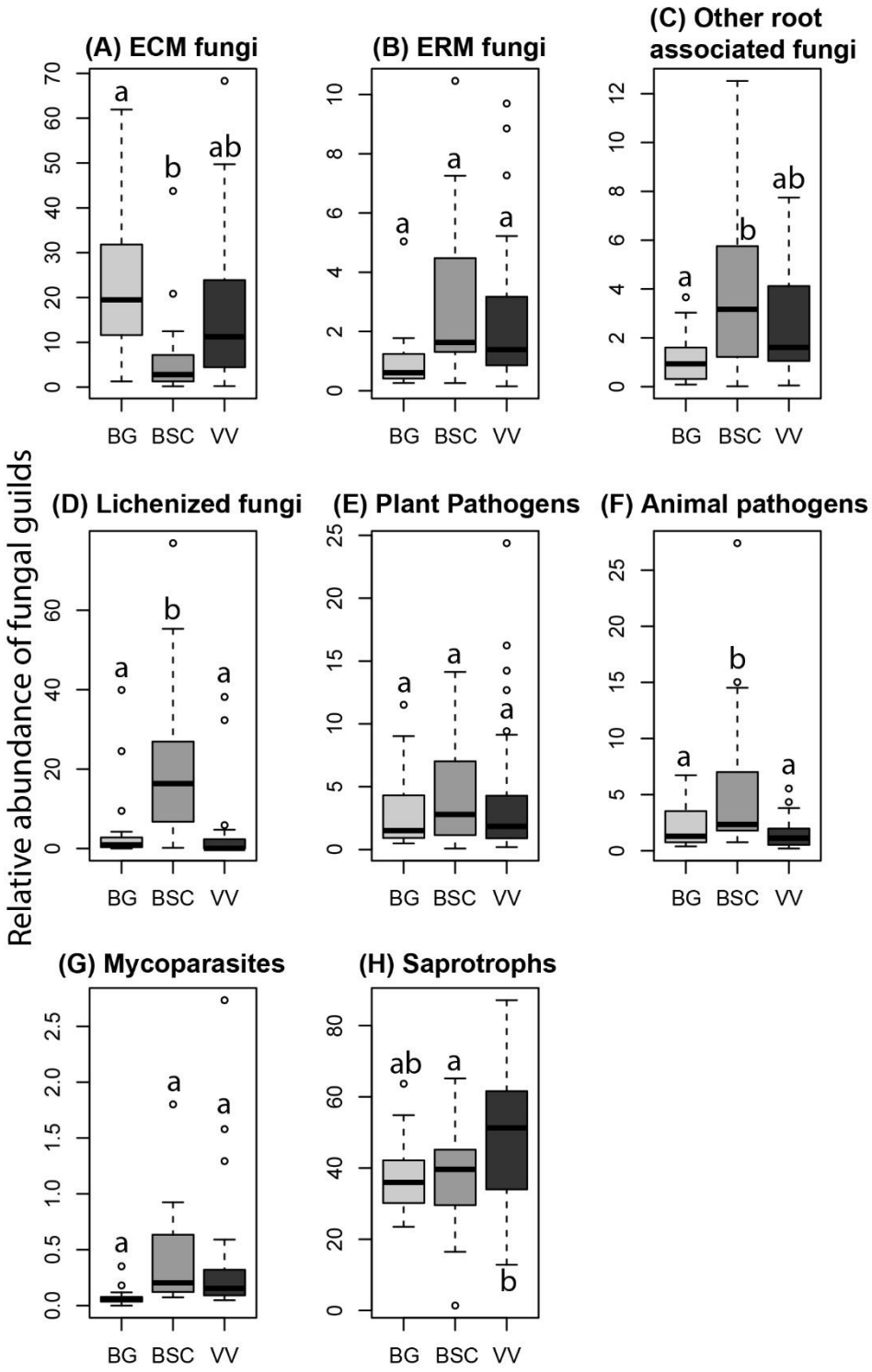
Data presented in this study describe the composition and functionality of fungal communities connected with three different soil environments representing a gradient of vegetation coverage in Western Greenland, never studied before. Overall, our results support the idea that fungal community composition changes in correlation with vegetation coverage among the plots, with concomitant changes in edaphic factors. In particular, we expect a loss of fungal diversity connected with the expansion of shrub vegetation, given the high number of species unique of uncovered plots. We also highlighted some possible interactions among the members of different functional guilds, mainly regarding the degradation of organic matter and nutrient cycling in this nutrient-limited environment.

Moreover, considering the soil communities of plots with vascular vegetation, we were able to discern a strong effect of shrub composition on the spatial distribution of fungal species. These outcomes should be taken into account also in the context of global change and the connected encroachment of shrubs occurring in the Arctic, which could lead to substantial changes in the soil communities, with possible effects on the degradation of the organic matter, as well as C and N fluxes to the atmosphere. In this optic, not only the expansion of vascular vegetation, but also its composition should be considered, having a determinant effect on associated soil fungal communities and organic matter turnover.

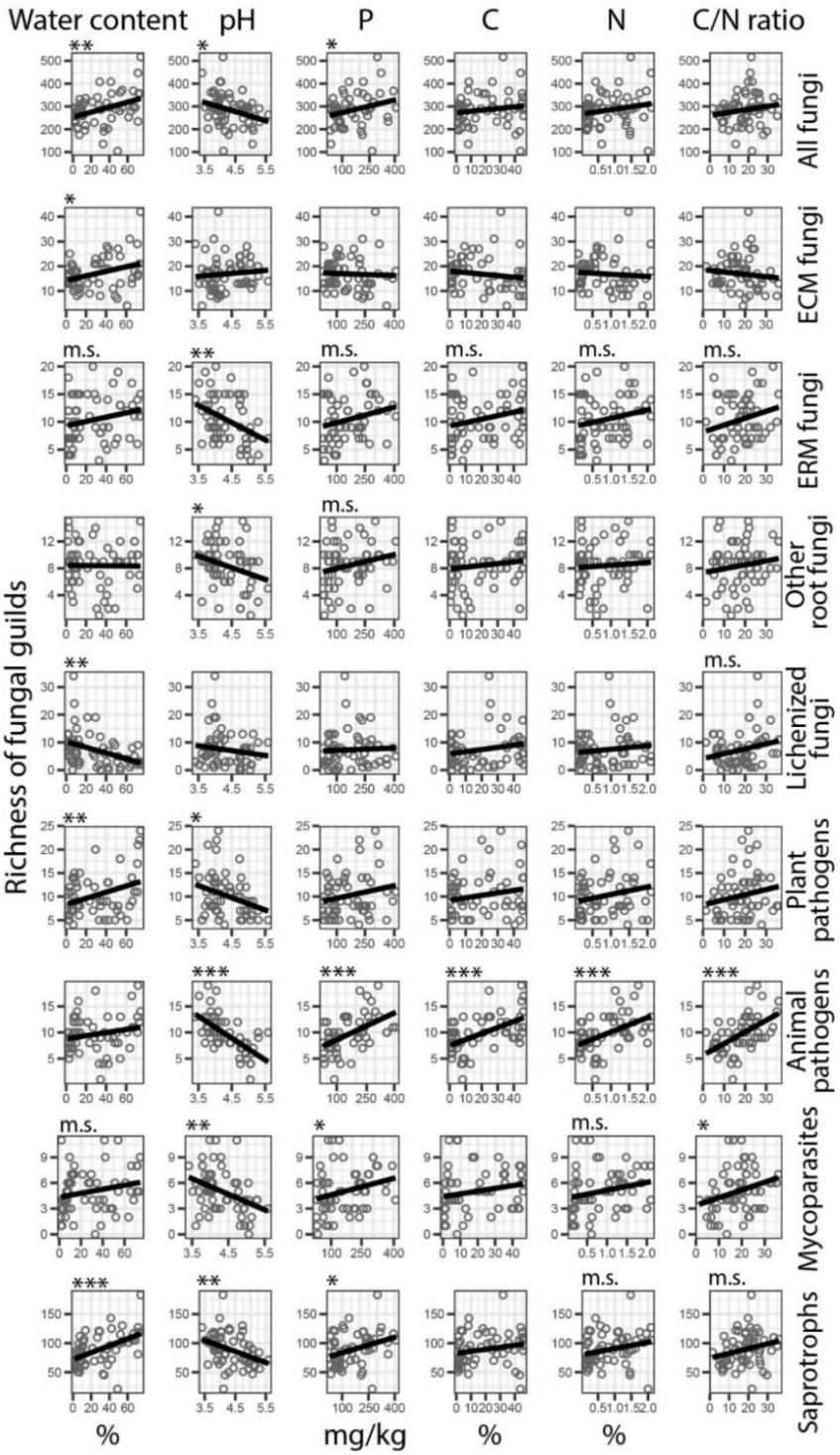
Supplementary Material



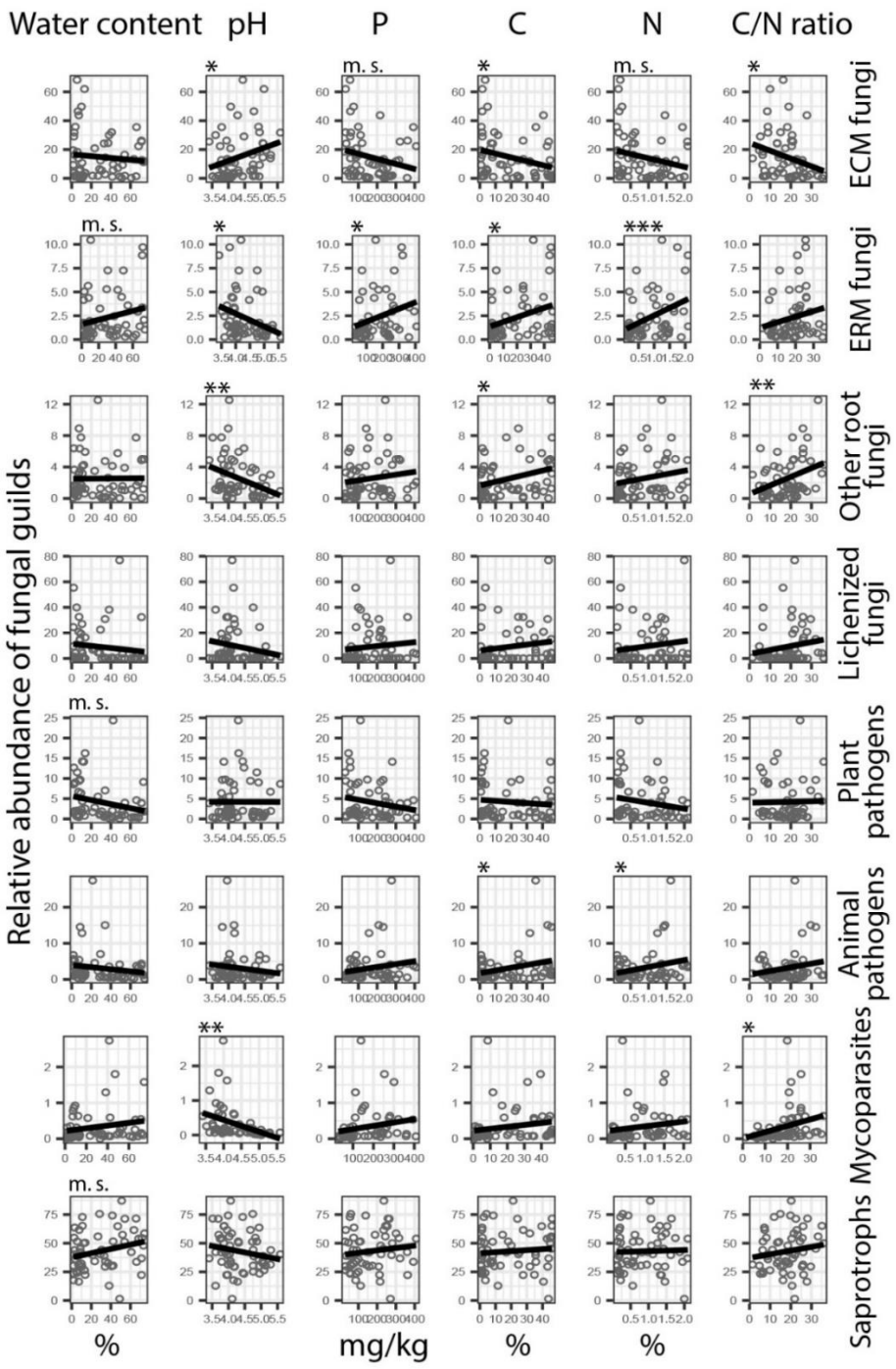
Supplementary Figure 2.1: Proportional richness of Ascomycota and Basidiomycota in each habitat (white, Bare Ground plots; light grey, Biological Soil Crusts plots; dark grey, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc TukeyHSD test (significant for $p<0.05$).



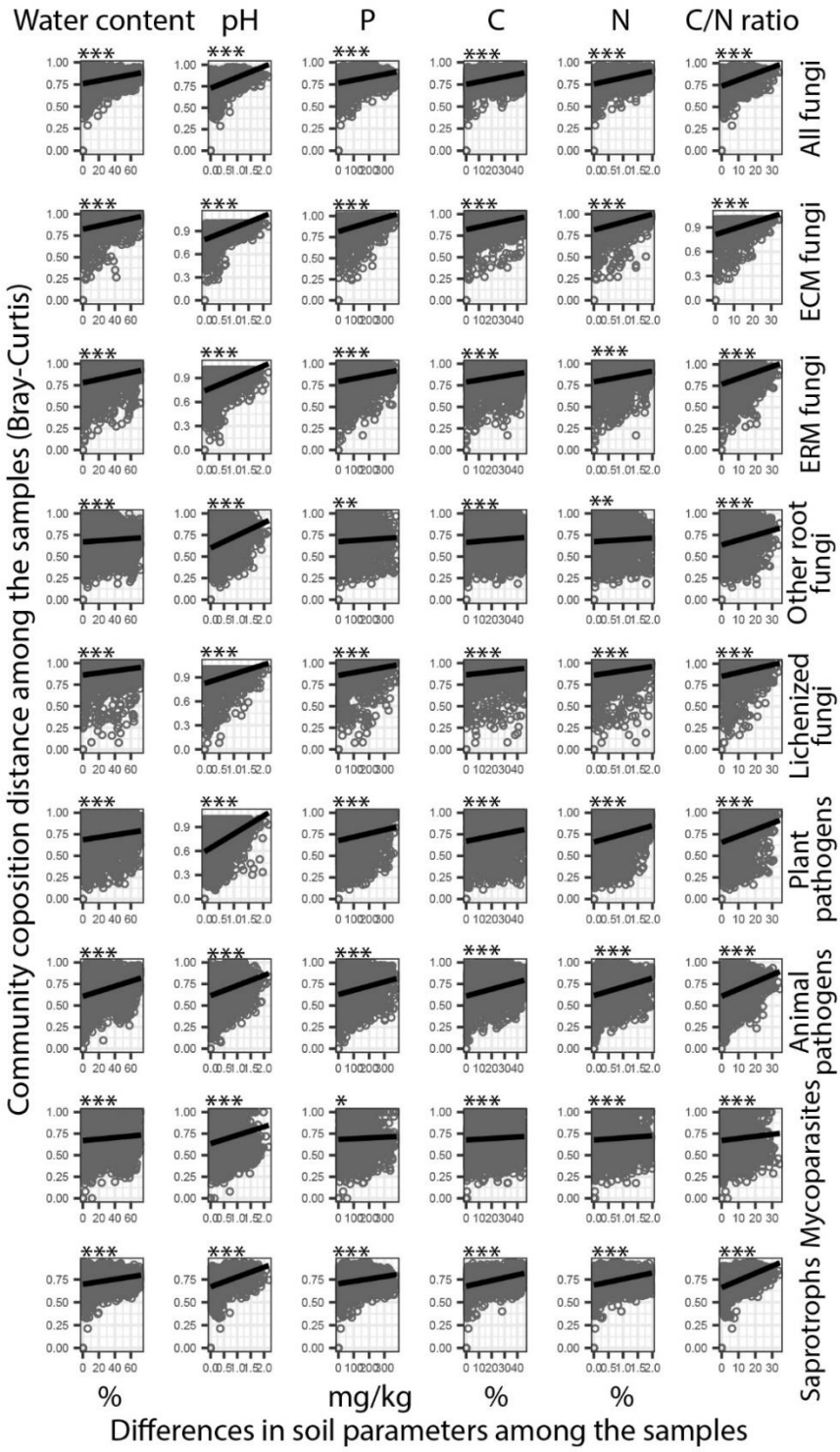
Supplementary Figure 2.2 Relative abundance of fungal functional guilds: ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, plant pathogenic, animal pathogenic, mycoparasites, and saprotrophic fungi in each habitat (light grey, Bare Ground plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc TukeyHSD test (significant for $p<0.05$).



Supplementary Figure 2.3: Scatter plots for the variation of richness (y-axis) of the total fungal community and of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, mycoparasites, animal pathogens, plant pathogens and saprotrophic fungi, in response to different soil parameters (water content, pH, P, C and N content, and C/N ratio; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m. s. (marginally significant) $p<0.1$. All the slopes and r^2 values for statistically significant regressions are reported in supplementary table 2.3.



Supplementary Figure 2.4 Scatter plots for the variation of relative abundance (y-axis) of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, mycoparasites, animal pathogens, plant pathogens and saprotrophic fungi, in response to different soil parameters (water content, pH, P, C and N content, and C/N ratio; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m. s. (marginally significant) $p<0.1$. All the slopes and r^2 values for statistically significant regressions are reported in supplementary table 2.3.



Supplementary Figure 2.5: Scatter plots for the differences in community composition (Bray-Curtis distance; y-axis) of the total fungal community and of ectomycorrhizal (ECM), ericoid mycorrhizal (ERM), other root associated, lichenized, mycoparasites, animal pathogens, plant pathogens and saprotrophic fungi, in response to the differences in soil parameters (water content, pH, P, C and N content, and C/N ratio; x-axis) among the samples. The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

Supplementary Table 2.1: Characteristics of the 20 sampling plots (altitude and coordinates). For plots of vascular vegetation covered soils the percentage of the most abundant shrub genera are reported.

Plots	Altitude (m a.s.l.)	Coordinates	*Salix*	*Betula*	*Vaccinium*	*Empetrum*
Vascular Vegetation plot 1	49	64°07'53"N 51°23'10"W	1	12	5	25
Vascular Vegetation plot 2	23	64°07'55"N 51°23'04"W	0	0	10	60
Vascular Vegetation plot 3	43	64°07'56"N 51°23'02"W	0	90	5	0
Vascular Vegetation plot 4	41	64°07'58"N 51°22'58"W	0	20	35	2
Vascular Vegetation plot 5	34	64°08'04"N 51°22'48"W	0	0	0	60
Vascular Vegetation plot 6	0	64°08'12"N 51°22'36"W	0	62	0	23
Vascular Vegetation plot 7	29	64°08'05"N 51°22'48"W	2	0	15	5
Vascular Vegetation plot 8	68	64°08'18"N 51°22'27"W	0	0	0	90
Vascular Vegetation plot 9	109	64°08'25"N 51°22'18"W	30	0	15	5
Biological Soil Crust plot 1	0	64°08'12"N 51°22'36"W				
Biological Soil Crust plot 2	23	64°07'56"N 51°23'05"W				
Biological Soil Crust plot 3	43	64°07'56"N 51°23'02"W				
Biological Soil Crust plot 4	66	64°07'53"N 51°23'12"W				
Biological Soil Crust plot 5	83	64°07'51"N 51°23'21"W				
Biological Soil Crust plot 6	107	64°07'53"N 51°23'11"W				
Bare Ground plot 1	15	64°14'48"N 51°17'45"W				
Bare Ground plot 2	25	64°10'44"N 51°22'31"W				
Bare Ground plot 3	45	64°07'59"N 51°22'43"W				
Bare Ground plot 4	77	64°07'53"N 51°23'20"W				
Bare Ground plot 5	109	64°08'25"N 51°22'17"W				

Supplementary Table 2.2 Fungal OTUs considered as significant indicators of the three habitats (BG: Bare Ground; BSC: Biological Soil Crusts; VV: Vascular Vegetation) with corresponding p-values, assigned functional guilds, and matching Species Hypotheses, ITS1 rDNA sequence similarity (%) of the most similar matching sequence in the UNITE + INSD dynamic Species Hypotheses database. Only a portion of the table is reported as an example in this version. For the full table see the online version (https://www.frontiersin.org/articles/10.3389/fmicb.2019.02348/full#supplementary-material).

OTU	Habitat	p-value	Match	Functional guild
OTU1285	BG	0,0464	100%|JN885548|SH200713.07FU	Saprotroph
OTU473	BG	0,014	96.8%|JQ346870|SH181489.07FU	Animal Pathogen
OTU114	BG	0,0062	97.6%|FJ554329|SH208595.07FU	Animal Pathogen
OTU577	BG	0,0152	93.9%|KP756480|SH640122.07FU	Arbuscular Mycorrhizal
OTU2355	BG	0,0152	94.9%|KP756480|SH640122.07FU	Arbuscular Mycorrhizal
OTU443	BG	0,0004	96.1%|DQ400126|SH005209.07FU	Arbuscular Mycorrhizal
OTU596	BG	0,0152	96.4%|KP756480|SH640122.07FU	Arbuscular Mycorrhizal
OTU866	BG	0,0046	96.6%|KF206544|SH203297.07FU	Arbuscular Mycorrhizal
OTU599	BG	0,0152	96.9%|KP756480|SH640122.07FU	Arbuscular Mycorrhizal
OTU649	BG	0,001	98.6%|HE858428|SH194637.07FU	Arbuscular Mycorrhizal
OTU316	BG	0,0112	98.9%|FJ554264|SH011124.07FU	Arbuscular Mycorrhizal
OTU307	BG	0,001	100%|DQ102671|SH188476.07FU	Ectomycorrhizal
OTU152	BG	0,0006	100%|JN580877|SH214853.07FU	Ectomycorrhizal
OTU34	BG	0,0002	100%|JQ711860|SH188594.07FU	Ectomycorrhizal
OTU1136	BG	0,0052	93.7%|AF083205|SH014937.07FU	Ectomycorrhizal
OTU115	BG	0,0002	94%|AY534209|SH187320.07FU	Ectomycorrhizal
OTU2397	BG	0,0002	95.8%|KF617237|SH181110.07FU	Ectomycorrhizal
OTU174	BG	0,0002	96.3%|KF617717|SH201237.07FU	Ectomycorrhizal
OTU965	BG	0,0152	96.4%|UDB021463|SH135196.07FU	Ectomycorrhizal
OTU8	BG	0,0006	96.6%|AB354285|SH030455.07FU	Ectomycorrhizal
OTU302	BG	0,014	97.3%|AM882947|SH188901.07FU	Ectomycorrhizal
OTU62	BG	0,0128	97.8%|UDB012704|SH184885.07FU	Ectomycorrhizal
OTU51	BG	0,0002	98.3%|KF617717|SH201237.07FU	Ectomycorrhizal
OTU39	BG	0,0004	98.7%|KF007250|SH210197.07FU	Ectomycorrhizal
OTU1351	BG	0,0128	99.2%|FM992957|SH219260.07FU	Ectomycorrhizal
OTU1325	BG	0,0192	99.2%|GU234030|SH190354.07FU	Ectomycorrhizal

Supplementary Table 2.3 Regression slopes and r^2 for the variation of relative richness and abundance of the total fungal community and eight functional guilds in response to soil physicochemical properties (significant for p<0,05; §marginally significant, p<0.1; n. s. non-significant).

		Water content (%)		pH		P content (mg/kg)		C content (%)		N content (%)		C/N ratio	
		slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2
All fungi	Richness	1.1083	0.1259	-40.19	0.0745	0.1834	0.0557	n. s.		n. s.		n. s.	
ECM fungi	Richness	0.0918	0.0892	n. s.		n. s.		n. s.		n. s.		n. s.	
	Abundance	n. s.		8.272	0.0600	-0.035§	0.0362	-0.2665	0.0611	-5.949§	0.0350	-0.5419	0.0619
ERM fungi	Richness	0.0389§	0.0347	-3.1287	0.1471	0.0094§	0.0394	0.0629§	0.0474	1.5113§	0.0328	0.1254§	0.0457
	Abundance	0.0232§	0.0350	-1.357	0.0702	0.0068	0.0675	0.0491	0.0949	1.5762	0.1376	n. s.	
Other root associated fungi	Richness	n. s.		-1.7431	0.0635	0.0069§	0.0313	n. s.		n. s.		n. s.	
	Abundance	n. s.		-1.7180	0.108	n. s.		0.0477	0.0771	n. s.		0.1105	0.1065
Lichenized fungi	Richness	-0.1034	0.1387	n. s.		n. s.		n. s.		n. s.		0.1776§	0.0362
	Abundance	n. s.		n. s.		n. s.		n. s.		n. s.		n. s.	
Plant pathogens	Richness	0.0654	0.1026	-2.602	0.0752	n. s.		n. s.		n. s.		n. s.	
	Abundance	-0.0497§	0.0425	n. s.		n. s.		n. s.		n. s.		n. s.	
Animal pathogens	Richness	n. s.		-4.1856	0.3929	0.0172	0.2468	0.1157	0.2888	2.7649	0.2171	0.2231	0.2611
	Abundance	n. s.		n. s.		n. s.		0.0760	0.0626	1.9074	0.0502	n. s.	
Mycoparasites	Richness	0.0234§	0.0309	-1.8274	0.1261	0.0064	0.0508	n. s.		0.9206§	0.0302	0.0905	0.0665
	Abundance	n. s.		-0.3437	0.1252	n. s.		n. s.		n. s.		0.0176	0.0719
Saprotrophs	Richness	0.5974	0.2467	-19.081	0.1141	0.0890	0.0920	n. s.		10.970	0.0394	0.8194§	0.0404
	Abundance	0.1862§	0.0481	n. s.		n. s.		n. s.		n. s.		n. s.	

Supplementary Table 2.4 Regression r^2 and significance of each variable fitted in the NMDS analyses (figure 2.5).

Variables	**All fungi**		**ECM fungi**		**ERM fungi**		**Other root associated fungi**		**Lichenized fungi**	
	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*
pH	0.6865	0.001	0.7359	0.001	0.3998	0.001	0.3626	0.001	0.2952	0.001
Water content	0.4767	0.001	0.1867	0.005	0.2726	0.002	0.1716	0.008	0.0796	0.109
C	0.5326	0.001	0.5603	0.001	0.2493	0.001	0.2634	0.001	0.1406	0.019
N	0.4577	0.001	0.4943	0.001	0.1811	0.006	0.2071	0.001	0.1212	0.034
C/N ratio	0.6042	0.001	0.6029	0.001	0.3840	0.001	0.3134	0.001	0.1761	0.013
P	0.3728	0.001	0.5017	0.001	0.1629	0.007	0.1807	0.004	0.1400	0.019
Salix	0.2190	0.002	0.1446	0.008	0.1841	0.007	0.0871	0.078	0.1674	0.008
Betula	0.0508	0.248	0.3246	0.001	0.0355	0.351	0.0100	0.748	0.0207	0.595
Vaccinium	0.2784	0.001	0.0529	0.211	0.0817	0.083	0.0356	0.378	0.0483	0.261
Empetrum	0.0679	0.136	0.0597	0.162	0.1141	0.031	0.0833	0.100	0.1428	0.020

Variables	**Plant pathogens**		**Animal pathogens**		**Mycoparasites**		**Saprotrophs**	
	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*
pH	0.5607	0.001	0.4356	0.001	0.1049	0.046	0.6276	0.001
Water content	0.2351	0.002	0.4195	0.001	0.1059	0.051	0.3656	0.001
C	0.4804	0.001	0.4460	0.001	0.0755	0.106	0.5499	0.001
N	0.4713	0.001	0.3734	0.001	0.0708	0.119	0.4351	0.001
C/N ratio	0.4630	0.001	0.4641	0.001	0.1290	0.021	0.6324	0.001
P	0.3553	0.001	0.3439	0.001	0.0100	0.745	0.3322	0.001
Salix	0.2009	0.006	0.1719	0.001	0.1100	0.060	0.1767	0.001
Betula	0.0506	0.233	0.0107	0.716	0.0148	0.660	0.0325	0.355
Vaccinium	0.2631	0.001	0.2909	0.001	0.0884	0.083	0.1653	0.004
Empetrum	0.0113	0.738	0.0818	0.095	0.0016	0.947	0.0842	0.081

Chapter 3

Expansion of shrubs could result in local loss of soil bacterial richness in Western Greenland

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Abstract

Climate warming in Greenland is facilitating the expansion of shrubs across wide areas of tundra. Given the close association between plants and soil microorganisms and the important role of soil bacteria in ecosystem functioning, it is of utmost importance to characterize microbial communities of arctic soil habitats and assess the influence of plant edaphic factors on their composition. We used 16S rRNA gene amplicons to explore the bacterial assemblages of three different soil habitats representative of a plant coverage gradient: bare ground, biological soil crusts dominated by mosses and lichens, and vascular vegetation dominated by shrubs. We investigated how bacterial richness and community composition were affected by the vegetation coverage, and soil pH, moisture, and carbon (C), nitrogen (N) and phosphorus (P) contents. Bacterial richness did not correlate with plant coverage complexity, while community structure varied between habitats. Edaphic variables affected both the taxonomic richness and community composition. The high number of Amplicon Sequence Variants (ASVs) indicators of bare ground plots suggests a risk of local bacterial diversity loss due to expansion of vascular vegetation.

Keywords: Arctic**,** environmental filtering, climate change, edaphic parameters, metabarcoding, 16S.

3.1 Introduction

In many Arctic environments, rising temperatures are causing an increased melting of glacial ice and an expansion of shrubs (Sturm *et al.*, 2001; Tape *et al.*, 2006) at the expense of vegetation dominated by bryophytes and lichens (Normand *et al.*, 2013; Cahoon *et al.*, 2016; Vowles and Björk, 2019). These trends are particularly apparent in Greenland, one of the regions in the Arctic most affected by climate change (Howat and Eddy, 2011; Bevis *et al.*, 2019).

Soil microbial communities and vegetation are linked by a variety of direct and indirect interaction: plants provide photosynthetically fixed carbon and low molecular weight root exudates that are used as energy sources by soil microorganisms, whose community composition is in turn shaped by their ability to metabolize different compounds and to resist different antimicrobial metabolites present in the exudates (Marschner *et al.*, 2004; Berg and Smalla, 2009; Berendsen *et al.*, 2012; Schulz-Bohm *et al.*, 2018). On the other hand, rhizosphere microorganisms can strongly influence plant growth and health. Arctic tundra is one of the most nitrogen limited environments on Earth and the input from biological nitrogen fixation, mainly contributed by Cyanobacteria, is critical to the development of these ecosystems (Solheim *et al.*, 2006). Additionally, the mobilization of nitrogen from organic matter is an important source for plant growth, and is at least partly influenced by bacterial activity (Chen *et al.*, 2014; Leff *et al.*, 2015). Furthermore, plant growth promoting bacteria may improve resource acquisition, modulate plant hormones secretion or act antagonistically against pathogens (Glick, 2012; Berendsen *et al.*, 2012). This close association between plants and microorganisms have led to the concept of plants as 'superorganisms' that partly rely on their microbiome interactions for specific functions and traits (Mendes *et al.*, 2013).

The reported decline of mosses and lichens due to climate change and their replacement by vascular plants, particularly shrubs (Normand *et al.*, 2013; Cahoon *et al.*, 2016; Vowles and Björk, 2019), can be expected to affect soil bacteria. Changes in the composition and structure of soil bacterial communities in the Arctic have been recently documented in simulations of long-term warming (Deslippe *et al.*, 2012), higher nutrient availability (Koyama *et al.*, 2014; Männistö *et al.*, 2016), and altered precipitation regimes (Ricketts *et al.*, 2016), as well as in natural successions in glacier forelands (Kwon *et al.*, 2015; Kim *et al.*, 2017) and along permafrost thaw gradients (Deng *et al.*, 2015; Frank-Fahle *et al.*, 2014). These responses of bacterial communities to climate changes, and their impact on ecosystem functionality are of utmost scientific relevance. Despite this importance, our understanding of the warming-driven changes in Arctic soil habitats remains uncomplete. For example, the potential effects of the shrub expansion on the structure and functioning of associated bacterial communities are challenging to accurately forecast, since the effects of

vegetation type on soil communities in Arctic tundra are not well known (Krab *et al.*, 2019). Differences have been observed across Alaskan tundra vegetation types (Wallenstein *et al.*, 2007), suggesting that plant communities influence bacterial communities via the quantity and quality of the litter supply, and by modifying the soil physical environment. In soils of Eastern Greenland (Ganzert *et al.*, 2014) abiotic parameters, related to different habitats, shaped microbial communities. In a Canadian low Arctic tundra system Denaturing Gradient Gel Electrophoresis analyses revealed that vegetation coverage plays a key role in shifting bacterial communities (Chu *et al.*, 2011). A more recent study in the Canadian Arctic, using 16S pyrosequencing, suggested that soil responses to warming would be vegetation-specific, likely due to the differences in the structure of microbial communities associated with different plants (Shi *et al.*, 2015). However, other works in Finnish (Männistö *et al.*, 2007) and Canadian tundra ecosystems (Buckeridge *et al.*, 2010), did not show any differences in bacterial communities among vegetation types.

Given the wide metabolic capabilities of bacteria, a deeper knowledge of the relationships between biotic and abiotic factors shaping soil bacterial community structure and function may be useful to predict the effects of global change on the vast and highly vulnerable Arctic soil carbon stocks (Crowther *et al.*, 2016). Shrub encroachment, especially of deciduous species, such as Betula nana, may produce more labile compounds from leaf litter, increasing the turnover of soil C (Weintraub and Schimel, 2005; Wookey *et al.*, 2009). The impact of warming will depend on how efficiently plant-derived carbon is incorporated into microbial biomass or converted to carbon dioxide and released to the atmosphere (Cotrufo *et al.*, 2013). In addition, plant litter and root exudates can facilitate the activity of microorganisms with enhanced decomposition abilities for old stocks of organic matter, in a process known as priming (Fontaine *et al.*, 2003; Kuzyakov, 2002; Walker *et al.*, 2015).

In our study, we selected three different habitats in Western Greenland, representing a gradient of vegetation complexity: bare ground (BG), biological soil crusts (BSCs) dominated by mosses and lichens, and vascular vegetation (VV) dominated by shrubs, e.g. *Empetrum*, *Vaccinium*, *Betula*, and *Salix*. The main aims were: i) to characterize the diversity and composition of soil bacterial communities in this Arctic region; ii) determine whether the diversity and community composition of soil bacteria are related to edaphic parameters; iii) identify the abiotic parameters most closely associated with variation in bacterial communities. Additionally, focusing only on the vascular vegetation plots, we aimed to test for correlations between relative cover of different shrub genera and the composition of soil bacterial communities, to assess the impact of shrub expansion on these patterns.

3.2 Materials and methods

3.2.1 Sampling

Sampling was carried out in July 27-31, 2017 in the area of Kobbefjord, Nuuk, West Greenland (64°08' N, 51°23' W). The climate of the area is classified as low Arctic (Jonasson *et al.*, 2000). The mean annual air temperature in the years 2008-2010 was 0.7 °C, with the mean air temperature of the warmest month, July, 10.7 °C. Over the same period annual precipitation ranged between 838 and 1,127 mm, with an average of 25–50 % of the total annual precipitation falling as snow during the winter period (Søndergaard *et al.*, 2012). Samples were collected in the area close to the NERO line, a permanent vegetation transect established in 2007 to monitor changes in vegetation species composition (Bay *et al.*, 2008).

In total, 20 plots (2m2), representing three habitat types, were sampled: 5 in bare ground (BG), 6 in biological soil crusts (BSC) and 9 in soils covered with vascular vegetation (VV). Exact coordinates, elevation, and shrub genera composition for each plot are listed in Supplementary Table 3.1. In each plot, three replicates soil samples (up to 10 cm depth) were collected aseptically, after removing the top of the soil, plant litter in vascular vegetated plots and the superficial coverage of mosses and lichens in BSC plots. Samples were transported in sterile bags and stored at -20 °C at University of Tuscia, Italy, until further processing.

3.2.2 Edaphic parameters

Gravimetric oil water content was measured on 5 grams subsamples dried at 105 °C (Reynolds, 1970). Measurements were repeated until no variation in weight was observed. pH was measured in a 1:2.5 suspension of dried soil in deionized water, with a HI9321 pH meter (Hanna Instruments Woonsocket, Rhode Island, United States). For each sample, soil moisture and pH were measured in independent triplicates. Phosphorus (P), carbon (C) and nitrogen (N) content were analysed at Eger Innovations Nonprofit Kft. (Eszterházy Károly University, Eger, Hungary). P content was measured by Microwave Plasma Atomic Emission Spectrometry (MP-AES) and C and N content by CNS elemental analyser.

3.2.3 DNA extraction, amplification and sequencing

For each sample, DNA was extracted from 0.5 g of soil using DNEasy Powersoil kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The V4 hypervariable region of 16S rRNA gene was amplified using 515F (Parada *et al.*, 2016) and 806R (Apprill *et al.*, 2015) primers; libraries were prepared following the protocol of Minich *et al.* (2018). The equimolar

pool of uniquely barcoded amplicons was paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform at the Vincent J. Coates Genomics Sequencing Laboratory at University of California, Berkeley.

3.2.4 Bioinformatic analyses

Bcl files were converted to Fastq files, demultiplexed and primer removed using bcl2fastq (v 2.18). Dual-matched 8-bp indexes were used to eliminate the occurrence of "barcode bleed" (or tag-switching) between samples.

16S demultiplexed sequences were processed with the QIIME2 (Quantitative Insights Into Microbial Ecology, v. 2018.11; Bolyen *et al.*, 2018) platform. 3,179,253 starting sequences were denoised, trimmed to length 160 bp, merged and clustered in Amplicon Sequence Variants (ASVs), using DADA2 (Callahan *et al.*, 2016), which includes phiX reads removal and chimera detection. We obtained 12,143 quality filtered ASVs, each with at least two reads in the total dataset. Taxonomy was assigned with the q2-feature-classifier within the database Greengenes v. 13_8 (99% OTUs from 515F/806R region of sequences). Chloroplasts, mitochondrial, chimeric and low identity ASVs (less than 80% identity to other prokaryotic 16S rRNA sequences) were removed, retaining 10,980 ASVs. The dataset was normalized for subsequent analyses, rarefying the number of reads per sample to the lowest reads obtained (14,251 reads) using the rrarefy function in the vegan package v. 2.5-2 (Oksanen *et al.*, 2018) in R v. 3.5.2 (R Core Team, 2018), retaining a total of 10,578 ASVs. Sequences of ASVs were submitted to NCBI gene bank (BioProject PRJNA550020).

3.2.5 Statistical analyses

Unless otherwise specified, all statistical analyses were carried out with the vegan package v. 2.5-2 (Oksanen *et al.*, 2018) in R v. 3.5.2 (R Core Team, 2018). Total bacterial richness (including all the ASVs retrieved), as well as relative richness (proportion of ASVs in a sample belonging to each group) and relative abundance (proportion of total reads in each sample assigned to each group) of most abundant phyla and classes among the three habitats were compared using ANOVA and Tukey's HSD test. Linear regression analyses were used to examine relationships between edaphic factors (pH, soil moisture, C, N and P content, and C/N ratio) and bacterial phyla and classes relative richness and abundances

We performed Non-Metric Multidimensional Scaling (NMDS) of the weighted Bray-Curtis distances of Hellinger transformed matrix of the bacterial community. We used the envfit R function to project edaphic variables (pH, soil moisture, C, N and P content, and C/N ratio) and the relative

abundance values of the shrub genera (*Betula*, *Empetrum*, *Salix*, and *Vaccinium*) or of different taxonomic groups onto the NMDS ordinations. In addition, we tested whether bacterial communities were statistically different among habitat types using the multi response permutation procedure (MRPP). We determined preferences of unique ASVs for each habitat using indicator species analyses on the Hellinger transformed matrix of the bacterial community (Dufrêne and Legendre, 1997) in PC-ORD v. 6.0 (McCune *et al.*, 2002).

Permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001) was carried out on Hellinger transformed Bray-Curtis distance matrix to determine the effect of each soil physicochemical parameter on the observed variance of the total community and of dominant phyla. Significant variables obtained from this analysis, were considered in a model to determine the combined effect of soil parameters on the variance of the community. The same approach was used taking into account only VV plots (27 samples in total) in order to assess the effect of the relative abundance of the four dominant shrub genera (*Salix*, *Betula*, *Vaccinium* and *Empetrum*), in combination with edaphic parameters, on the variance of the total community and of the different phyla.

3.3 Results

3.3.1 Bacterial richness and abundance patterns

The filtered and rarefied dataset of the 16S rRNA gene amplicons contained 10,578 bacterial ASVs. The proportions of ASVs found exclusively in VV plots was the highest (41.6%) compared to BSC and BG plots (12.1 and 18.9%, respectively), whereas the BG samples showed the highest number of indicator ASVs (407 ASVs, compared with 86 and 114 for VV and BSC samples, respectively). Only 759 ASVs (7.2%) out of 10,578 were present in all the habitats.

The total richness of the bacterial communities was higher in VV and BG samples compared to BSC, with the two former not significantly different from each other (Figure 3.1a). We selected 10 most abundant phyla of 35 identified (encompassing both Bacteria and Archaea) and 12 most abundant classes of 103 retrieved for further analyses. The 10 phyla selected represented more than 80% of total reads identified and more than 95% in many samples. The 12 classes were representative of more than 65% of reads identified at this level in all the samples.

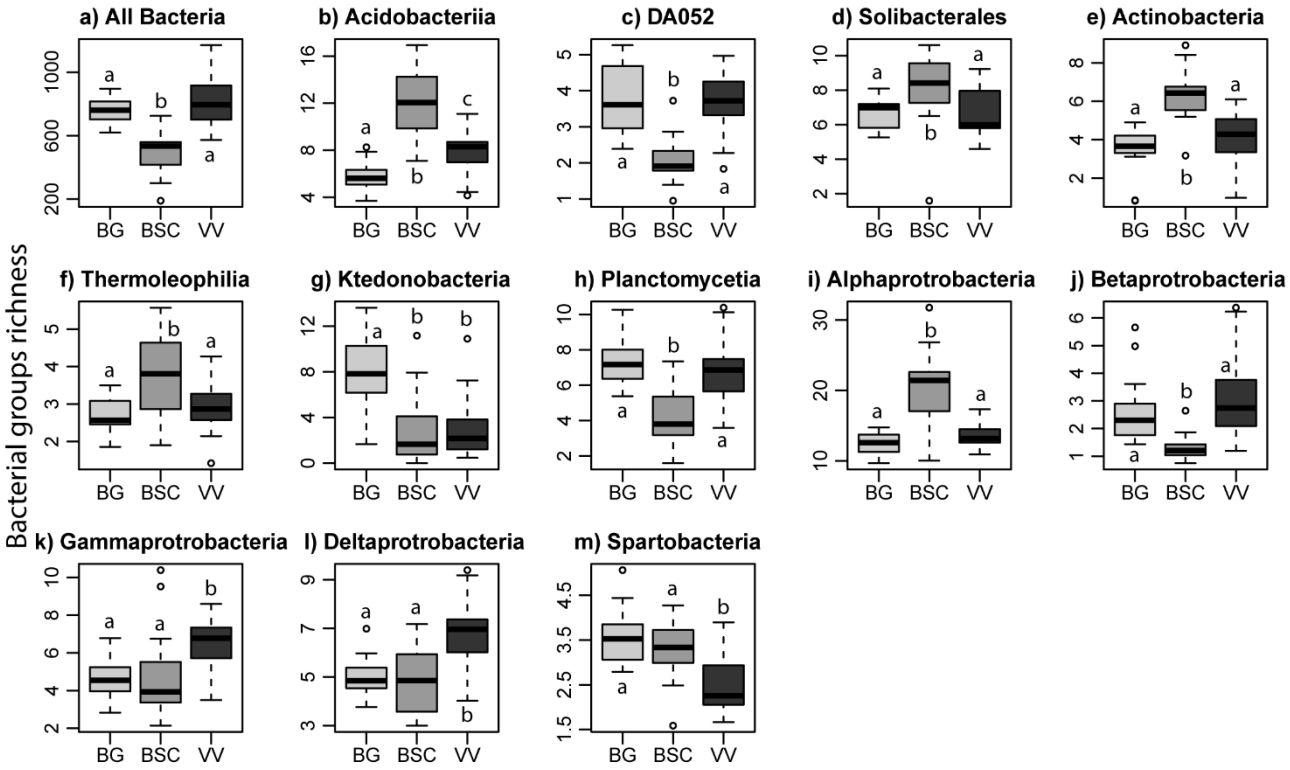


Figure 3.1. Richness of the total bacterial community and relative richness of the 12 dominant bacterial classes in each habitat (light grey, Bare Grounds plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc Tukey's HSD test ($p<0.05$).

Among the most representative classes and phyla studied, those that had the highest richness in BG plots, were Ktedonobacteria (Chloroflexi), Spartobacteria (no statistical significance with BSC; Verrucomicrobia), and the phylum Cyanobacteria (Figure 3.1; Supplementary Figure 3.1).

Classes with the highest richness in BSC plots were Acidobacteriia and Solibacterales (Acidobacteria), while DA052 within the same phylum showed the lowest richness and abundance in this habitat. Classes Actinobacteria and Thermoleophilia (Actinobacteria) had higher richness and abundance in BSC plots, compared to BG and VV. The same was for the class Alphaproteobacteria and the phylum Armatimonadetes, that had higher richness for this habitat type. Instead, Planctomycetia (Planctomycetes), the class Betaproteobacteria, and the phylum Chlamydiae showed lower richness in BSC plots (Figure 3.1; Supplementary Figure 3.1).

Gammaproteobacteria and Deltaproteobacteria (Proteobacteria), as well as Bacteroidetes had the highest richness in VV plots (Figure 3.1; Supplementary Figure 3.1). The same general trends were observed in the relative abundances of individual taxonomical groups (Supplementary Figures 3.2 and 3.3).

3.3.2 Effect of environmental parameters on richness and abundance of different groups

Soil moisture, P and N content of soil samples showed an increase from BG plots to vegetated plots (BSC + VV), while pH decreased. C content and the C/N ratio also increased from BG plots to the vegetated plots, but with higher values in BSC compared to VV plots (Figure 3.2). C content and the C/N ratio were the main predictors of total bacterial richness, with a negative correlation (slope=-4.06 and r2=0.105 for C content; slope=-10,01 and r^2=0.160 for C/N ratio), while N content was only marginally significant (slope=-72.89 and r^2=0.035), and both soil moisture and pH were positively correlated (slope=2.14 and r^2=0.052 for soil moisture; slope=101.38 and r^2=0.058 for pH; Supplementary Figure 3.4).

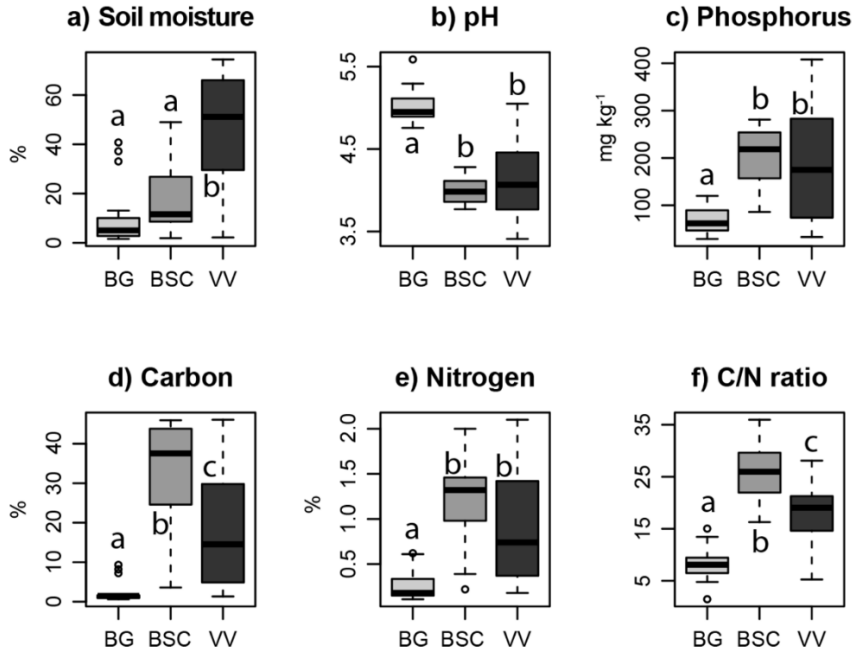


Figure 3.2 Differences in soil parameters across the three habitats (light grey, Bare Ground plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots). Letters indicate significant differences in one-way ANOVA post-hoc Tukey's HSD test ($p<0.05$).

Richness and abundance of the dominant phyla and classes were significantly related to both C and N content and C/N ratio. Correlations were positive for both richness and abundance of Acidobacteria, Actinobacteria (N content not significant for richness), Armatimonadetes and Bacteroidetes and with the richness of Proteobacteria and Verrucomicrobia. Conversely, these three parameters were negatively correlated with both the richness and abundance of Chloroflexi, with the richness of Cyanobacteria and with the abundance of Planctomycetes and Verrucomicrobia (Figure

3.3, Supplementary Figures 3.5). pH significantly influenced phyla richnesses and abundances. There were negative correlations with both the richness and the abundance of Acidobacteria, Actinobacteria, Armatimonadetes and Bacteroidetes and with the richness of Proteobacteria and Verrucomicrobia; and positive correlations with both richness and abundance of Chloroflexi, with the richness of Cyanobacteria and with the abundance of Verrucomicrobia (Figure 3.3, Supplementary Figures 3.5).

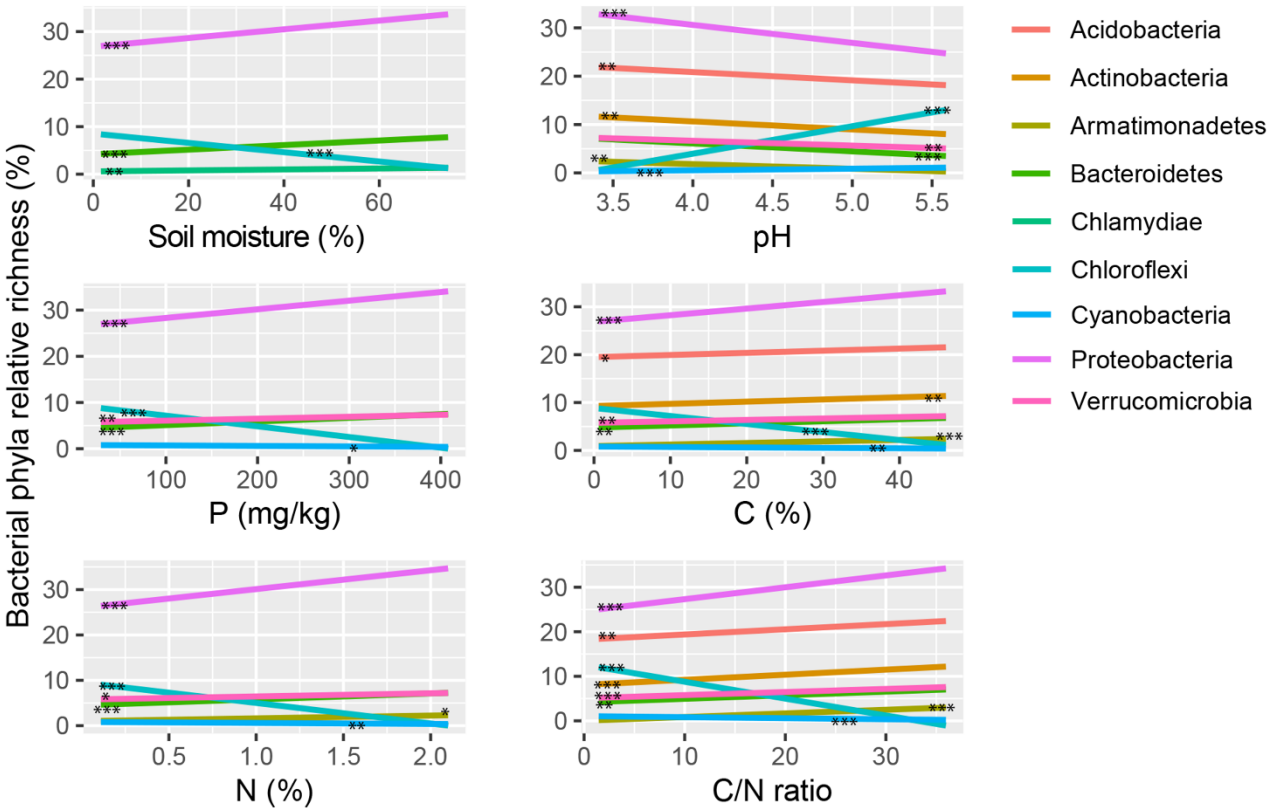


Figure 3.3 Summaries of linear regression models for the variation of richness of 9 dominant bacterial phyla in relation to soil parameters: soil moisture, pH, P content, C and N content and C/N ratio. The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Only significant regressions are reported. See Supplementary Figure 3.4 for individual plots with data points shown.

Soil moisture and P content had a significant effect on a smaller number of the dominant taxonomic groups. Soil moisture was positively correlated with the richness and abundance of Bacteroidetes, Chlamydiae and Proteobacteria and negatively with Chloroflexi (Figure 3.3, Supplementary Figures 3.5). P content was positively correlated with both richness and abundance of Bacteroidetes, the richness of Proteobacteria and Verruomicrobia and the abundance of Actinobacteria. It was also negatively correlated with both richness and abundance of Chloroflexi, with the richness of Cyanobacteria and with the abundance of Verrucomicrobia (Figure 3.3,

Supplementary Figures 3.5). The trends were similar for the classes analysed within these phyla (see Supplementary Table 3.2 for all statistical details).

3.3.3 Community composition

Bacterial communities structure was well differentiated between the habitat types (Figure 3.4a, b; MRPP p=0.001, A=0.132). The same differentiation was also apparent when analysing the phyla-level composition (Supplementary Figure 3.6). Among variables fitted to the ordinations, all soil parameters, relative abundances of the four main shrub genera (see Supplementary Table 3.1) and of the different phyla, were significant, except for the relative abundances of *Betula*, *Empetrum* and Cyanobacteria (Supplementary Table 3.3).

When the effects of the habitat type (BG, BSC or VV) and single edaphic parameters were tested on both the total community composition and the phyla-level composition, all the variables were significant, with the habitat type always explaining the highest proportion of variation (26% for the total community; Supplementary Table 3.4). Conversely, when the variables were combined additionally in a model, only the type of habitat, the pH and the C/N ratio had an independent effect on the observed variance for both the total community and all the phyla considered, with the only exception of Armatimonadetes (Table 3.1). Both for the total communities and many phyla, N content resulted to be an independent parameter, whereas for other phyla (Bacteroidetes, Chlamydiae, Chloroflexi and Cyanobacteria) soil moisture was determinant. C and P content were never explaining an additional variance than the other parameters when combined additionally to them (Table 3.1).

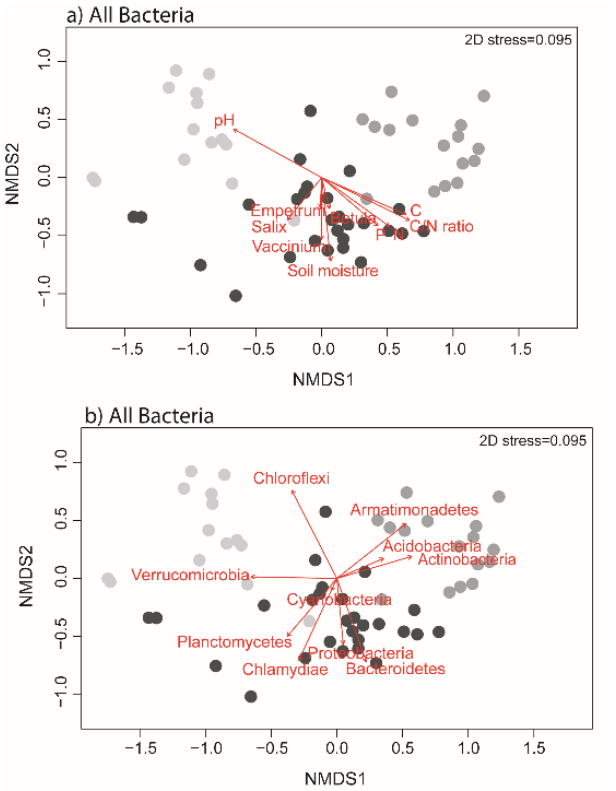


Figure 3.4. a) and **b)** Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in composition of bacterial communities (Hellinger transformed ASVs abundances) in the habitats studied (light grey, Bare Ground plots; dark grey, Biological Soil Crusts plots; black, Vascular Vegetation plots) for the total bacteria communities. Arrows represent projections of a) edaphic variables (pH, soil moisture, carbon, nitrogen and phosphorus content, and C/N ratio) and the relative abundance values of the shrub genera (*Betula*, *Empetrum*, *Salix*, and *Vaccinium*) ; and b) relative abundances of dominant phyla.

3.3.4 Effect of shrub coverage on bacterial community composition

PerMANOVA analysis was used to test for the effect of shrub community composition on soil communities. When the abundance of different shrubs and the soil parameters were tested independently, *Salix* coverage explained the highest variance in community composition for many groups (Supplementary Table 3.5), and *Betula* coverage, never significant, was the main determinant only for Cyanobacteria community composition (Table 3.2). pH was the second strongest predictor, explaining the highest variance in community composition for many groups (Table 3.2). Finally, C, N and soil moisture, and *Empetrum* abundance had a significant independent effect on the variance of total community and, to a different extent, on the phyla studied (Table 3.2).

Table 3.1 Proportion of variation in bacterial community composition explained by habitat (categorical) and soil variables (continuous), based on permutational multivariate analyses of variance. Variables with significant results in individual analyses (Supplementary Table 3.4) were added sequentially in reverse order on explained variance to a combined model. Variables that remained significant in the combined model are in bold.

All Bacteria			**Acidobacteria**			**Actinobacteria**			**Armatimonadetes**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
habitat	**26.73**	**0.0001**	habitat	**33.18**	**0.0001**	habitat	**25.25**	**0.0001**	habitat	**14.18**	**0.0001**
pH	**4.40**	**0.0001**	pH	**4.94**	**0.0001**	pH	**4.28**	**0.0009**	pH	**3.29**	**0.0004**
C/N ratio	**3.22**	**0.0027**	C/N ratio	**3.32**	**0.0030**	C/N ratio	**4.22**	**0.0006**	C/N ratio	1.72	0.2851
C	1.44	0.2206	C	1.25	0.2634	C	1.80	0.1010	Soil moisture	1.64	0.3552
N	**2.96**	**0.0068**	N	**2.79**	**0.0109**	N	**3.58**	**0.0019**	C	1.31	0.7322
P	1.33	0.2886	P	1.20	0.2934	Soil moisture	1.59	0.1640	N	2.00	0.1165
Soil moisture	1.67	0.1225	Soil moisture	1.58	0.1255	P	1.34	0.2904	P	1.40	0.6362
Residuals	58.24		Residuals	51.75		Residuals	57.94		Residuals	74.46	
Bacteroidetes			**Chlamydiae**			**Chloroflexi**			**Cyanobacteria**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
habitat	**21.73**	**0.0001**	habitat	**6.75**	**0.0001**	habitat	**20.07**	**0.0001**	habitat	**9.27**	**0.0001**
pH	**4.25**	**0.0001**	Soil moisture	**2.95**	**0.0006**	pH	**3.50**	**0.0016**	C/N ratio	**2.82**	**0.0114**
C/N ratio	**2.46**	**0.0199**	N	2.01	0.1504	C/N ratio	**3.43**	**0.0015**	pH	**2.50**	**0.0370**
Soil moisture	**3.16**	**0.0032**	pH	**2.44**	**0.0100**	C	1.54	0.2857	C	1.55	0.5352
C	1.54	0.2281	P	1.60	0.7579	Soil moisture	**3.15**	**0.0034**	Soil moisture	**3.13**	**0.0027**
N	1.85	0.1051	C	2.04	0.1321	N	1.79	0.1519	N	**4.23**	**0.0002**
P	1.49	0.2678	C/N ratio	**2.39**	**0.0152**	P	1.33	0.4641	P	1.51	0.5799
Residuals	63.53		Residuals	79.82		Residuals	65.20		Residuals	75.00	

Planctomycetes			**Proteobacteria**			**Verrucomicrobia**			
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	
habitat	**20.57**	**0.0001**	habitat	**25.65**	**0.0001**	habitat	**29.16**	**0.0001**	
C/N ratio	**3.59**	**0.0010**	pH	**4.16**	**0.0003**	pH	**5.33**	**0.0002**	
pH	**3.34**	**0.0027**	C/N ratio	**3.32**	**0.0027**	C/N ratio	**2.97**	**0.0003**	
C	1.50	0.2898	C	1.28	0.3321	C	1.73	0.0774	
N	**2.31**	**0.0338**	N	**3.38**	**0.0013**	N	**2.76**	**0.0103**	
P	1.16	0.6371	Soil moisture	1.80	0.0912	P	1.53	0.1374	
Soil moisture	1.34	0.4134	P	1.43	0.2318	Soil moisture	1.51	0.1771	
Residuals	66.19		Residuals	58.99		Residuals	55.00		

Table 3.2 Proportion of variation in bacterial community composition in the vascular vegetation plots explained by soil variables and relative abundance of shrub genera, based on permutational multivariate analyses of variance. Variables with significant results in individual analyses (Supplementary Table 3.6) were added sequentially in reverse order on explained variance to a combined model. Variables that remained significant in the combined model are in bold

All Bacteria			**Acidobacteria**			**Actinobacteria**			**Armatimonadetes**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Salix	**16.47**	**0.0001**	*Salix*	**21.03**	**0.0001**	*Salix*	**20.61**	**0.0001**	*Salix*	**9.42**	**0.0001**
pH	**7.19**	**0.0013**	pH	**7.18**	**0.0039**	pH	**7.67**	**0.0005**	pH	4.56	0.2019
C	**6.07**	**0.0073**	C	**6.42**	**0.0103**	*Vaccinium*	**9.16**	**0.0006**	Residuals	86.02	
Empetrum	**4.87**	**0.0307**	N	3.37	0.2002	*Empetrum*	**4.51**	**0.0399**			
C/N ratio	3.30	0.2331	C/N ratio	3.90	0.1180	C	3.26	0.1665			
N	**4.61**	**0.0442**	P	1.96	0.7156	N	1.90	0.6643			
Vaccinium	2.49	0.5848	*Vaccinium*	3.19	0.2379	Soil moisture	**7.24**	**0.0009**			
Soil moisture	**4.64**	**0.0426**	*Empetrum*	3.37	0.1984	C/N ratio	2.20	0.5044			
P	2.84	0.4011	Soil moisture	**4.89**	**0.0428**	P	2.33	0.4502			
Residuals	47.52		Residuals	44.69		*Betula*	2.56	0.3713			
						Residuals	38.55				
Bacteroidetes			**Chlamydiae**			**Chloroflexi**			**Cyanobacteria**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
Salix	**17.13**	**0.0001**	*Empetrum*	**7.80**	**0.0001**	*Salix*	**12.34**	**0.0001**	*Betula*	**7.12**	**0.0091**
pH	5.23	0.0566	*Vaccinium*	**5.85**	**0.0034**	*Vaccinium*	**9.34**	**0.0003**	*Salix*	**6.26**	**0.154**
Empetrum	4.99	0.0713	*Salix*	**5.61**	**0.0045**	*Empetrum*	**5.50**	**0.0167**	Residuals	86.63	
C	4.59	0.1047	Soil moisture	4.52	0.0635	pH	4.15	0.1127			
Residuals	68.05		N	3.53	0.4173	C	4.11	0.1166			
			Residuals	72.68		Soil moisture	**6.74**	**0.0020**			
						N	1.89	0.9105			
						P	3.27	0.3373			
						C/N ratio	1.95	0.9000			
						Residuals	50.70				

Planctomycetes			**Proteobacteria**			**Verrucomicrobia**		
Variable	Variance (%)	p	Variable	Variance (%)	p	Variable	Variance (%)	p
Salix	**12.66**	**0.0001**	*Salix*	**15.49**	**0.0001**	pH	**15.56**	**0.0001**
pH	**6.48**	**0.0046**	pH	**7.24**	**0.0007**	*Salix*	**8.91**	**0.0002**
Vaccinium	**7.52**	**0.0019**	C	**5.57**	**0.0113**	C	**7.66**	**0.0005**
C	2.95	0.5087	*Empetrum*	**5.31**	**0.0148**	N	2.67	0.4706
C/N ratio	3.84	0.1747	C/N ratio	3.68	0.1367	Soil moisture	**6.71**	**0.0029**
Empetrum	3.94	0.1549	*Vaccinium*	3.73	0.1319	P	3.10	0.2894
N	3.54	0.2572	Soil moisture	**5.81**	**0.0082**	C/N ratio	2.55	0.5301
P	3.02	0.4711	N	2.58	0.5444	*Empetrum*	4.45	0.0503
Residuals	56.05		P	2.80	0.4284	*Vaccinium*	1.77	0.8979
			Residuals	47.80		Residuals	46.61	

3.4 Discussion

Our study reports the differences in richness and community composition along a gradient of vegetational complexity in Western Greenland. Our findings show that the structure of soil bacterial communities is strongly influenced by vegetation complexity and by the identity of dominant shrub genera. Our results partly confirm previously observed patterns, but also offers new insights. For example, Wallenstein *et al.* (2007) found that bacterial communities in Alaskan acidic tundra soils were dominated by Acidobacteria in tussock tundra, while Proteobacteria dominated shrub tundra. In our study, Acidobacteria clearly preferred BSC soils that had the lowest pH, but they were much less diverse in vascular vegetation plots with similar pH that were dominated by dwarf shrubs (Supplementary Figure 3.1). For Proteobacteria, our data showed that phylum-level patterns can mask potentially important class-level differences. Out of the four Proteobacteria classes, three showed highest richness in shrub-dominated plots, while Alphaproteobacteria showed highest richness in BSC soils (Figure 3.1), a pattern identical to that of Acidobacteria. Acidobacteria are generally considered k-strategists, with lower growth rates, but high efficiency in converting nutrients to biomass and high tolerance to toxic compounds. This results in a greater ability to compete in oligotrophic environments, which accords with their preference for BSC plots (Kielak *et al.*, 2016). Instead, Proteobacteria are generally considered copiotrophic organisms. In the Arctic tundra, organisms of this phylum have been reported to be more abundant after fertilization experiments (Koyama *et al.*, 2014), likely due to increased organic matter input by vascular plants (Ramirez *et al.*, 2010).

The highest number of indicator ASVs recorded in BG plots suggests that these habitats harbour a unique pool of bacteria, adapted to thrive in these conditions. Similar patterns have been reported for fungi in Eastern Greenland (Grau *et al.*, 2017). With the metabarcoding approach it is not possible to estimate the proportion of sequences derived from relic DNA, often considered to be the majority of the total DNA in low biomass soils (Carini *et al.*, 2016). Although we cannot rule out the possibility of some of the observed patterns to be due to such relict sequences, the sequences found exclusively in BG plots in the dataset often belonged to stress-tolerant taxa, such as those of the phylum Chloroflexi. If these taxa are typical of bare-ground habitats, the recent expansion of shrubs into these habitats would likely result in the local extinction of these stress-tolerant microbes, since they could be expected to be outcompeted in fully vegetated habitats.

The highest richness observed for BG and VV plots relative to BSC (Figure 3.1) differs from results found on a primary successional gradient of an Arctic glacier foreland, where richness positively correlated with vegetation complexity (Kwon *et al.*, 2015). Our results also differs from

previous comparisons of vegetated and non-vegetated soils, where lower diversity was observed in the former (Tam *et al.*, 2001; Kumar *et al.*, 2016). In this study, plant coverage is among the most crucial environmental factors influencing bacterial community composition.

In our study, a significant proportion of bacterial community variance was also explained by edaphic parameters (e.g. pH, C/N ratio), that were different among habitat types. Of these, soil pH was the best predictor of community composition and had a key role in predicting the richness and relative abundance of many taxonomic groups (Table 3.1). In agreement with other studies on arctic communities (Chu *et al.*, 2010; 2011; Männistö *et al.*, 2007), the effect of pH was significant in all habitats and its influence on community composition remained significant even when habitat type was accounted for. Indeed, the important role of pH on bacterial communities has been observed on a global scale (Lauber *et al.*, 2009), even if the direct mechanism by which it regulates microbial communities composition and functionality remains largely unknown (Malard and Pearce, 2018). Lauber *et al.* (2009) suggested an indirect pH effect on the availability of different cations fundamental for life. A strong correlation between the relative abundance of genes encoding several metabolic and transport pathways and pH increase has been documented, suggesting a possible greater metabolic activity of bacterial cells in higher-nutrient and alkaline conditions (Bahram *et al.*, 2018).

The C/N ratio, an indicator of substrate quality, was also an important parameter in determining changes in microbial communities (Table 3.1). N availability is an important determining factor for soil life forms (Chen *et al.*, 2014; Leff *et al.*, 2015). In general, oligotrophic species (k-strategists) dominate under N-limiting conditions, such as polar regions, while under abundant N concentrations copiotrophic species (r-strategists), able to utilize more labile C sources, prevail (Fontaine *et al.*, 2003; Chen *et al.*, 2014). We found a higher richness and abundance of Actinobacteria and Armatimonadetes, and a lower richness and abundance of Planctomycetes in BSC plots than in BG and VV ones, with a higher C/N ratio (Supplementary figure 3.1). Among these phyla, Armatimonadetes, although poorly studied, are generally considered oligotrophic (Lee *et al.*, 2014) and Actinobacteria, usually associated with plant roots, have been found in many desert soils (Anandan *et al.*, 2016), while Planctomycetes are usually more abundant in bulk soils than in the rhizosphere (Derakshani *et al.*, 2001). A higher relative abundance of Bacteroidetes and partially Proteobacteria, generally copiotrophic, were found in VV plots in respect to BG and BSC ones, with a more balanced C/N ratio (Supplementary Figure 3.3 and Figure 3.2).

Across the three habitats, the phylum Chloroflexi showed a clear preference for the bare ground habitat (Figure 3.4b, Supplementary Figures 3.1 and 3.3). This phylum mostly includes

oligotrophic organisms, apparently with greater stress-tolerance and/or lesser competitive capabilities than most other phyla (Costello and Schmidt, 2006). In particular, the dominant Ktedonobacteria class showed a higher richness and abundance in the BG plots (Figure 3.1 and Supplementary Figure 3.2) and was the class level grouping most influenced by edaphic parameters (Supplementary Table 3.2). This group is known to include organisms well adapted to extremely oligotrophic conditions and has been found dominant in volcanic soils of the Atacama Desert (Lynch *et al.*, 2012) and in cinder deposits of the Kilauea volcano in Hawaii (King and King, 2014) as well. Additionally, it has been recently proved that organisms belonging to this class are optimal CO and H_2 oxidizers and therefore considered pioneer organisms, allowing to fix atmospheric gasses in nutrient limiting environments (Islam *et al.*, 2019).

Members of Verrucomicrobia, although still poorly studied, have been recorded in soils from many different biomes, even in Antarctica (Bergmann *et al.*, 2011), and reported to be more abundant in plant rhizospheres compared to bulk soils in temperate environments (Jesus *et al.*, 2010; Rocha *et al.*, 2010). Their close relationship with plants is also indicated by the fact that plant extracts must be added to culturing media in order to successfully isolate members of the Spartobacteria class (Sangwan *et al.*, 2004). Although the richness of Verrucomicrobia did not differ significantly among the habitats studied, members of this phylum, including Spartobacteria, were significantly more abundant in BG plots (Supplementary figure 3.2), possibly due to their greater tolerance to low nutrient conditions. In fact, this phylum includes many slow-growing organisms that are highly sensitive to changes in soil properties and, therefore, good indicators for changes in chemical factors linked to fertility (Navarrete *et al.*, 2015).

Members of the phylum Cyanobacteria, despite the lack of a clear abundance pattern, had a higher richness in BG plots (Supplementary Figure 3.1). Filamentous cyanobacteria, in particular, have proved to be key organisms in early stages of soil development (Budel *et al.*, 2016), due to their involvement in N fixation, moisture retention, soil surface stabilization and accumulation of organic matter in nutrient-limited environments, such as Antarctic ice-free regions (Cary *et al.*, 2010). For this reason, we propose that BSC samples, characterized by abundant mosses and lichens, had a lower diversity of Cyanobacteria than BG plots because the latter reflects the earlier stages of microbial colonization.

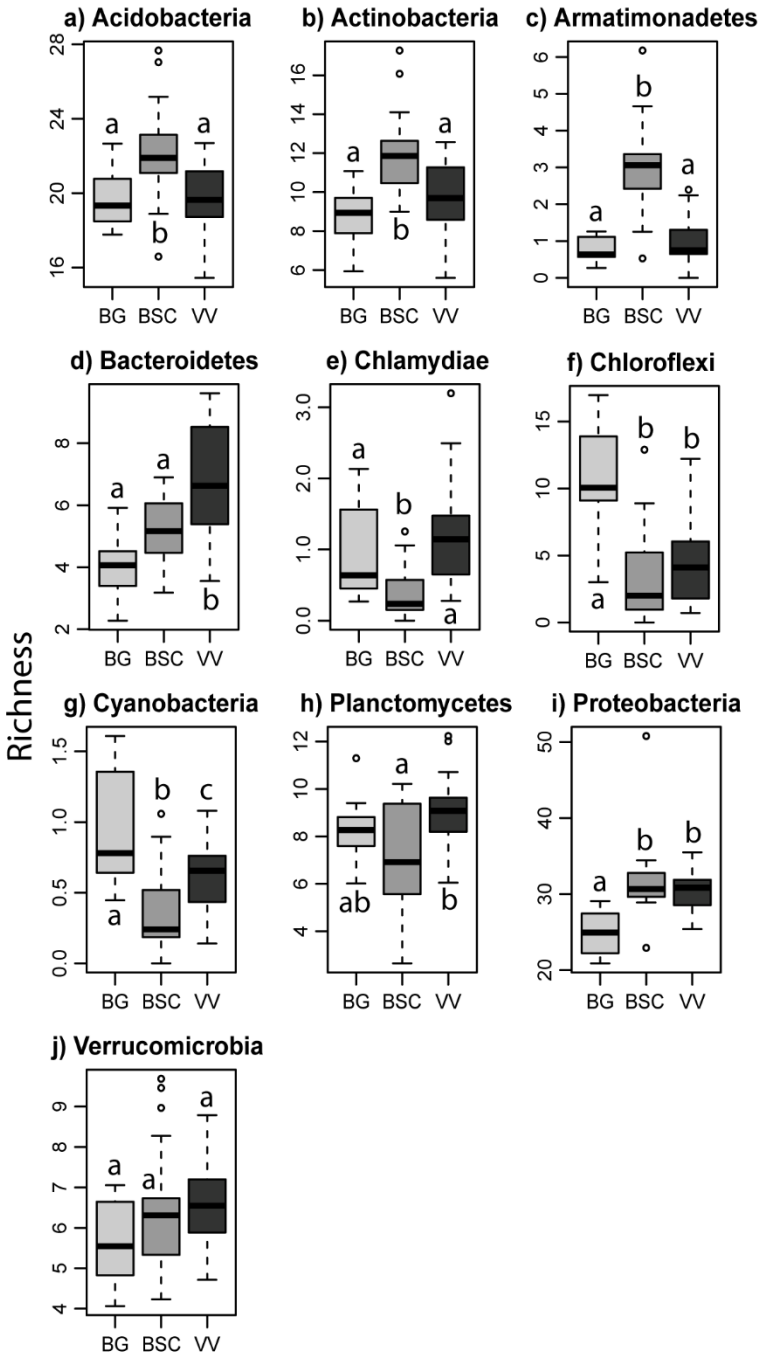
3.5 Conclusions

This study provides a detailed picture of the landscape-level compositional dynamics of soil bacterial communities in Western Greenland, where information about soil microbiota is limited. It

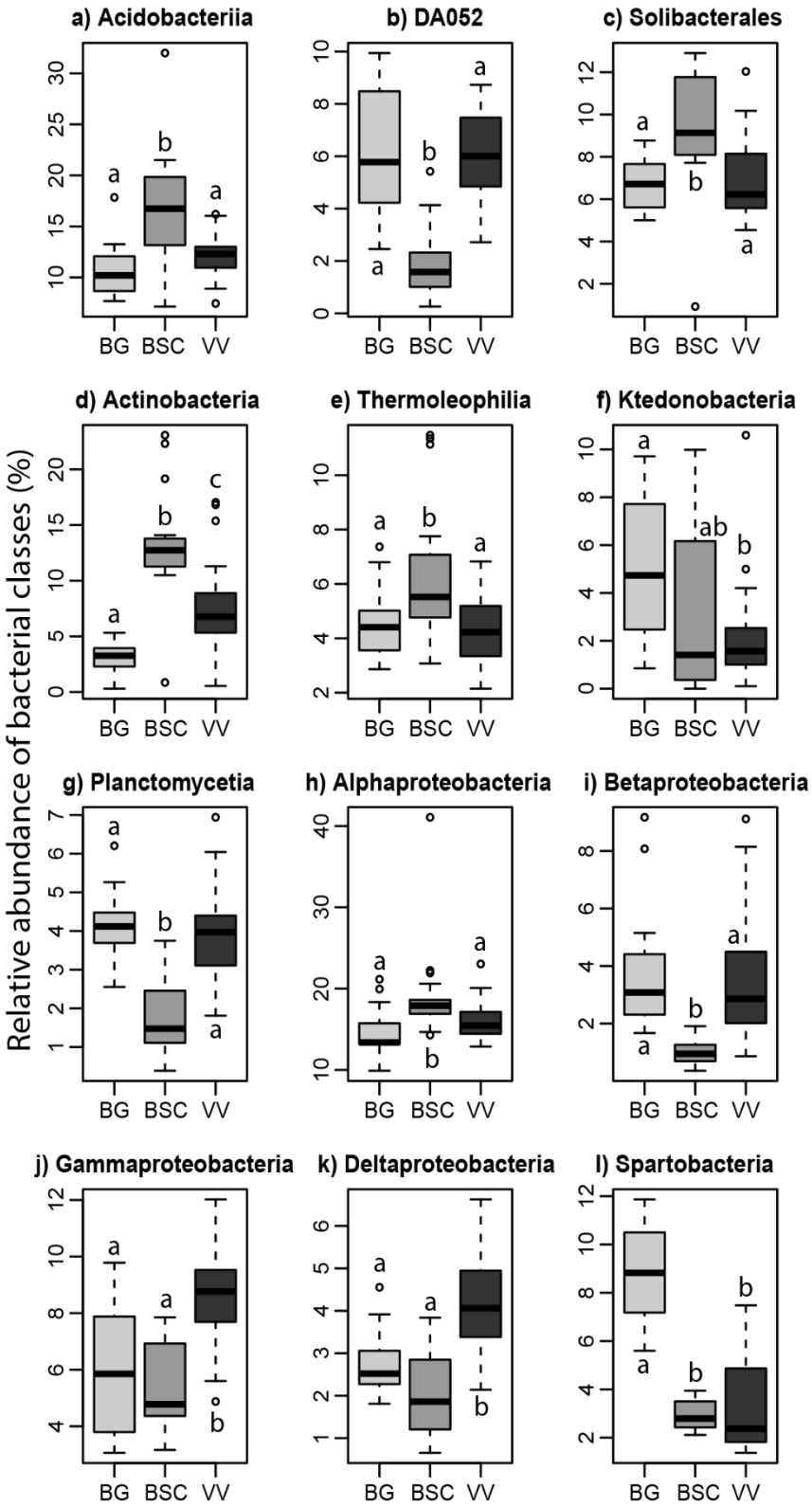
represents, to our knowledge, one of the first metabarcoding assessments of Arctic soil bacterial communities underlying different vegetation types. Bacterial richness did not correlate with increasing vegetation complexity, but there is evidence suggestive of a possible local loss of species connected to the expansion of shrubs at the expense of other soil habitats. Additionally, we found that community composition was strongly differentiated between the habitats and was strongly shaped by the vegetation composition in well vegetated plots.

The present report provides a status of the bacterial community composition, serving as a baseline for long-term monitoring, close to the vegetation transect 'NERO line' established to monitor future changes in the species composition of the plant communities.

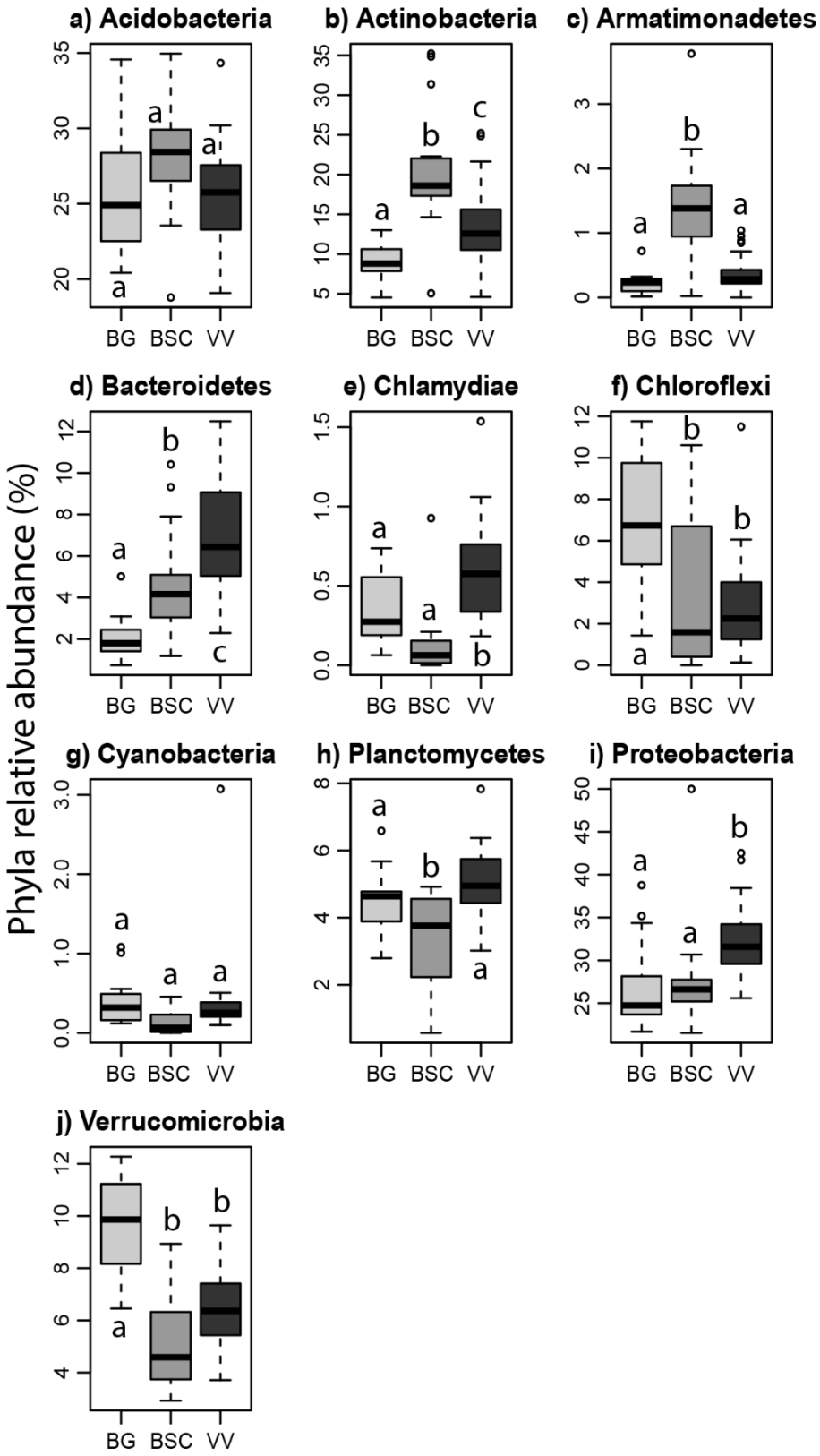
Supplementary Material



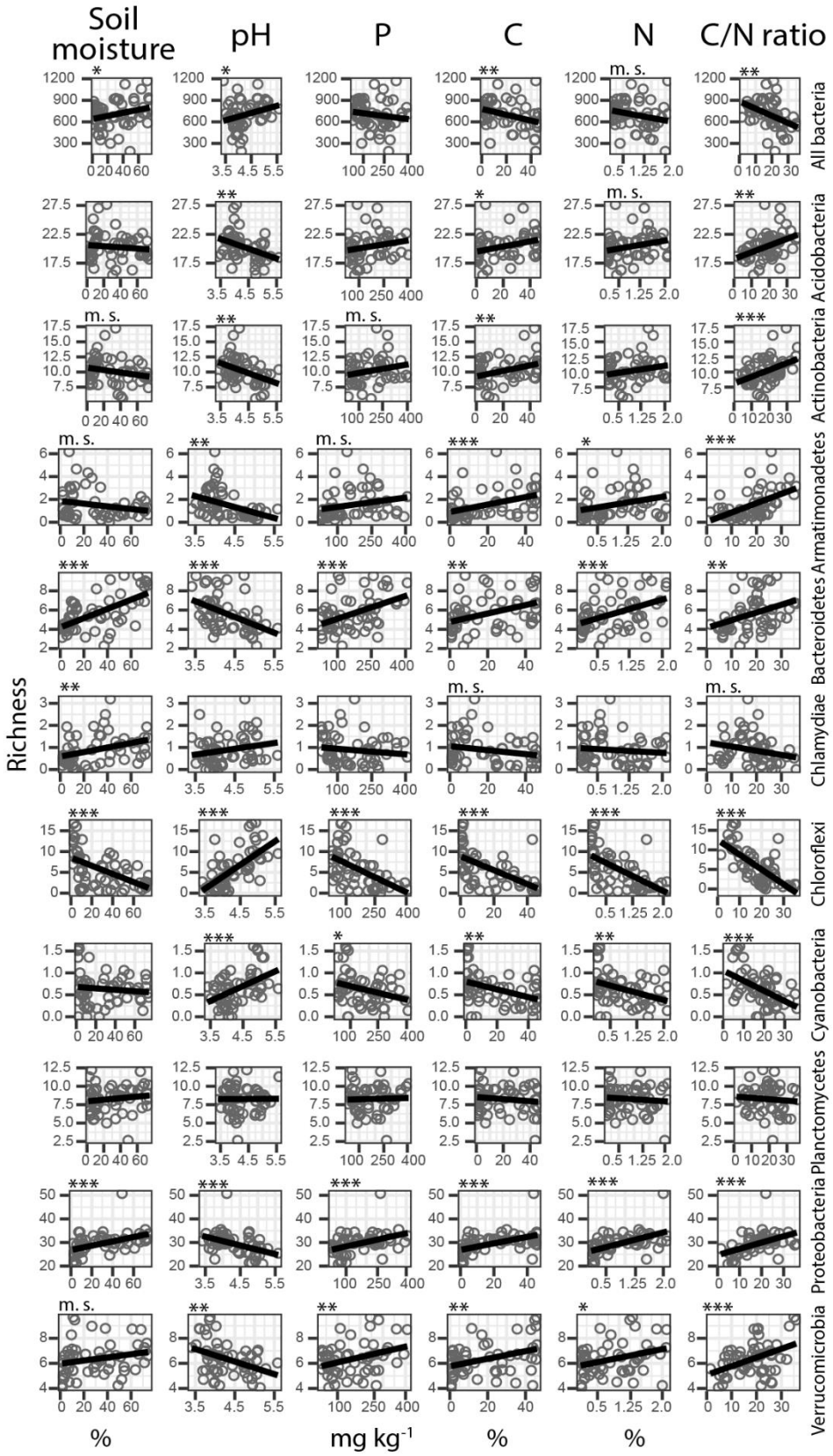
Supplementary Figure 3.1 Relative richness of the 10 dominant bacterial phyla in each habitat (light grey, Bare Grounds; dark grey, Biological Soil Crusts; black, Vascular Vegetation). Letters indicate significant differences in one-way ANOVA post-hoc Tukey's HSD test (significant for $p<0.05$).



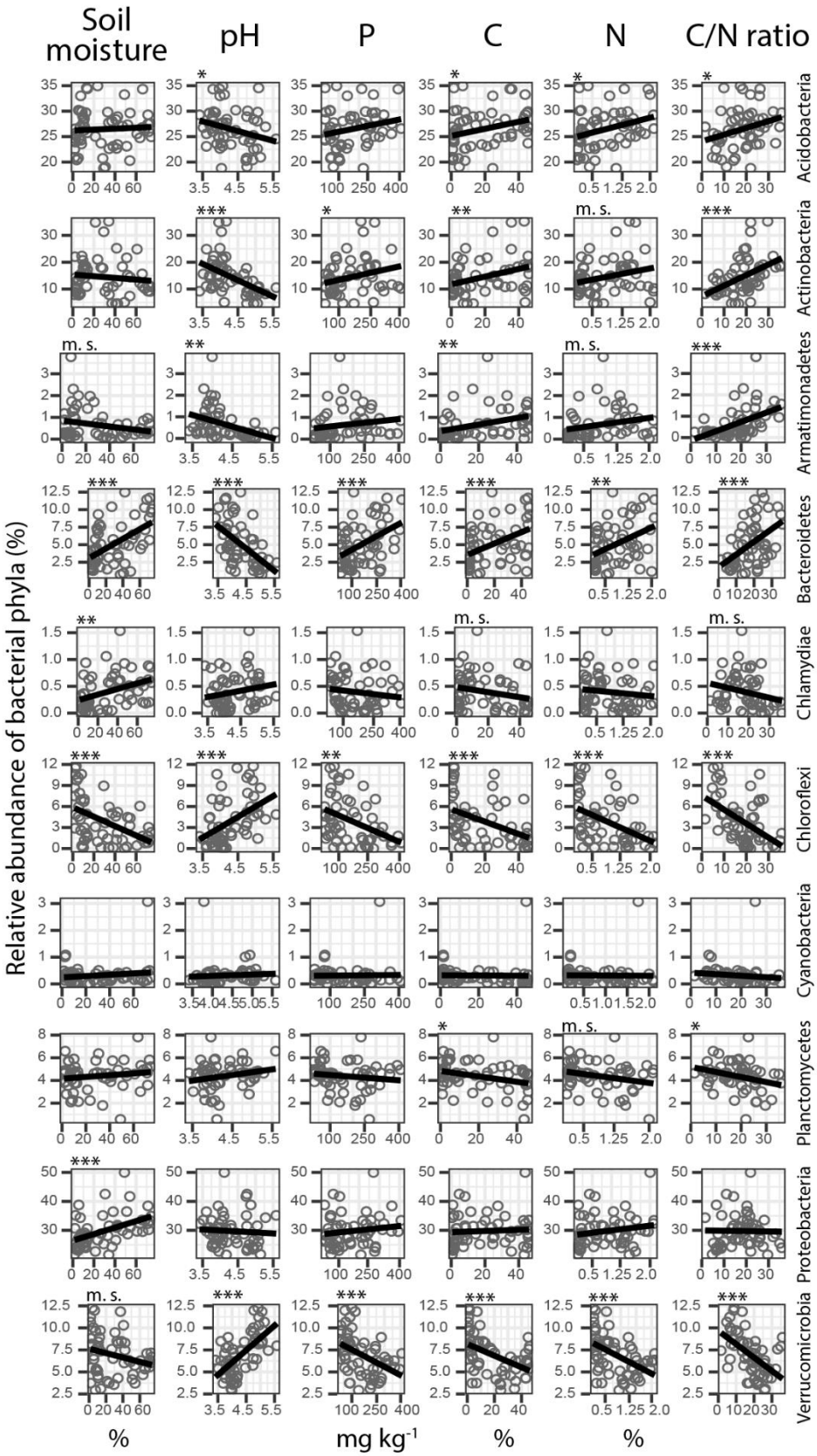
Supplementary Figure 3.2. Relative abundance of the 12 dominant bacterial classes in each habitat (light grey, Bare Grounds; dark grey, Biological Soil Crusts; black, Vascular Vegetation). Letters indicate significant differences in one-way ANOVA post-hoc Tukey's HSD test (significant for $p<0.05$).



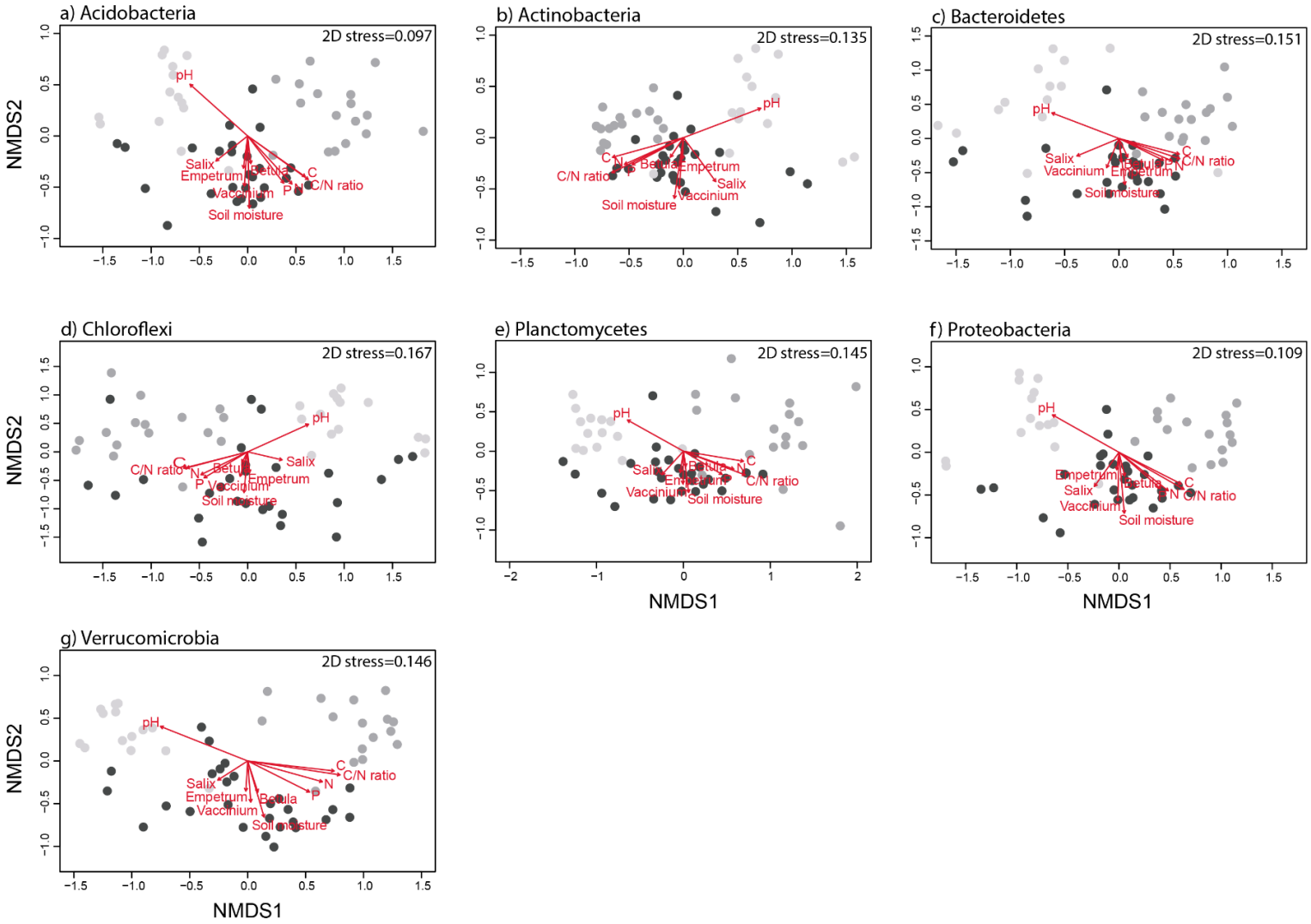
Supplementary Figure 3.3 Relative abundance of the 10 dominant bacterial phyla in each habitat (light grey, Bare Grounds; dark grey, Biological Soil Crusts; black, Vascular Vegetation). Letters indicate significant differences in one-way ANOVA post-hoc Tukey's HSD test (significant for $p<0.05$).



Supplementary Figure 3.4 Scatter plots for the variation of richness (y-axis) of the total bacterial community and of relative richness of 10 dominant phyla in response to different soil parameters (soil moisture, pH, P, C and N content and C/N ratio; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m.s. (marginally significant) $p<0.1$.



Supplementary Figure 3.5 Scatter plots for the variation of relative abundance (y-axis) of 10 dominant phyla in response to different soil parameters (soil moisture, pH, P, C and N content and C/N ratio; x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, * $p<0.05$, m.s. (marginally significant) $p<0.1$.



Supplementary Figure 3.6 Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in composition of 7 bacterial phyla (Hellinger transformed ASVs abundances) in the habitats studied (light grey, Bare Ground; dark grey, Biological Soil Crusts; black, Vascular Vegetation) for the total bacteria communities. Edaphic variables (pH, soil moisture, C, N and P content, and C/N ratio) and the relative abundance values of the shrub genera (*Betula*, *Empetrum*, *Salix*, and *Vaccinium*) have been fitted in the ordinations.

Supplementary Table 3.1: Characteristics of the 20 sampling plots (altitude, coordinates and pH in single samples). For plots of vascular vegetation covered soils the percentage of the most abundant shrub genera are reported

Plots	Altitude (m a.s.l.)	Geographic coordinates	pH			*Salix*	*Betula*	*Vaccinium*	*Empetrum*
Vascular Vegetation plot 1	49	64°07'53"N 51°23'10"W	4.30	4.10	4.04	1	12	5	25
Vascular Vegetation plot 2	23	64°07'55"N 51°23'04"W	3.69	3.96	3.70	0	0	10	60
Vascular Vegetation plot 3	43	64°07'56"N 51°23'02"W	3.41	3.61	3.79	0	90	5	0
Vascular Vegetation plot 4	41	64°07'58"N 51°22'58"W	3.52	3.75	3.79	0	20	35	2
Vascular Vegetation plot 5	34	64°08'04"N 51°22'48"W	4.07	3.99	3.61	0	0	0	60
Vascular Vegetation plot 6	0	64°08'12"N 51°22'36"W	4.07	4.44	4.48	0	62	0	23
Vascular Vegetation plot 7	29	64°08'05"N 51°22'48"W	4.76	3.94	4.74	2	0	15	5
Vascular Vegetation plot 8	68	64°08'18"N 51°22'27"W	4.31	4.43	4.67	0	0	0	90
Vascular Vegetation plot 9	109	64°08'25"N 51°22'18"W	4.73	5.05	4.75	30	0	15	5
Biological Soil Crust plot 1	0	64°08'12"N 51°22'36"W	3.86	4.02	3.87				
Biological Soil Crust plot 2	23	64°67'56"N 51°23'05"W	3.96	4.17	4.28				
Biological Soil Crust plot 3	43	64°07'56"N 51°23'02"W	4.19	3.81	4.11				
Biological Soil Crust plot 4	66	64°07'53"N 51°23'12"W	4.16	3.86	3.79				
Biological Soil Crust plot 5	83	64°07'51"N 51°23'21"W	4.03	3.97	4.07				
Biological Soil Crust plot 6	107	64°07'53"N 51°23'11"W	3.77	3.91	3.99				
Bare Ground plot 1	15	64°14'48"N 51°17'45"W	4.95	4.76	5.14				
Bare Ground plot 2	25	64°10'44"N 51°22'31"W	4.82	4.94	5.29				
Bare Ground plot 3	45	64°07'59"N 51°22'43"W	4.87	5.59	5.28				
Bare Ground plot 4	77	64°07'53"N 51°23'20"W	5.09	4.92	4.78				
Bare Ground plot 5	109	64°08'25"N 51°22'17"W	4.95	5.06	5.05				

Supplementary Table 3.2 Regression slopes and r^2 for the variation of relative richness and abundance of 13 dominant classes in response to soil physicochemical characteristics (significant for p<0,05; §marginally significant, p<0.1; n. s. non-significant).

		Soil moisture (%)		pH		P content (mg kg^{-1})		C content (%)		N content (%)		C/N ratio	
		slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2	slope	r^2
Acidobacteriia	Richness	n. s.		-3.391	0.3227	0.0114	0.1369	0.1031	0.299	2.3898	0.2105	0.2282	0.3524
	Abundance	n. s.		-3.261	0.1385	0.0129	0.0813	0.1159	0.1809	2.8395	0.1421	0.2297	0.1683
DA052	Richness	0.0149	0.0971	n. s.		n. s.		-0.0164§	0.0491	n. s.		-0.0391	0.0730
	Abundance	0.0428	0.1402	n. s.		n. s.		-0.0318	0.0245	n. s.		-0.0990	0.1368
Solibacteres	Richness	-0.0249	0.1249	-0.7141§	0.0386	n. s.		n. s.		n. s.		n. s.	
	Abundance	-0.0350	0.1084	-1.2498	0.0594	n. s.		n. s.		n. s.		0.0787	0.0557
Actinobacteria	Richness	-0.0173§	0.0427	-1.5948	0.2293	0.0044	0.0594	0.0434	0.1667	0.7915	0.0643	0.1023	0.2263
	Abundance	n. s.		-5.762	0.307	0.0176	0.1043	0.1433	0.1844	3.070	0.1066	0.3705	0.3045
Thermoleophilia	Richness	n. s.		-0.3770§	0.04111	n. s.		0.0118§	0.0403	n. s.		0.0287	0.0642
	Abundance	-0.0295	0.1121	n. s.		n. s.		n. s.		n. s.		n. s.	
Ktedonobacteria	Richness	-0.0869	0.3038	3.8960	0.2927	-0.0173	0.2274	-0.1245	0.3012	-3.4805	0.3166	-0.2815	0.3714
	Abundance	-0.0586	0.229	1.6522	0.0764	-0.0087	0.0873	-0.0589	0.1029	-1.7415	0.1236	-0.1306	0.1236
Planctomycetia	Richness	n. s.		1.4772	0.1463	-0.0058	0.0854	-0.0609	0.2632	-1.4291	0.1895	-0.1196	0.2406
	Abundance	n. s.		1.3011	0.2243	-0.0049	0.1207	-0.0477	0.3093	-1.1072	0.2188	-0.0976	0.3092
Alphaproteobacteria	Richness	n. s.		-3.095	0.1178	0.0105§	0.0452	0.1097	0.1537	2.6498	0.1163	0.2337	0.1679
	Abundance	n. s.		n. s.		n. s.		n. s.		n. s.		n. s.	
Betaproteobacteria	Richness	0.0247	0.1562	1.0359	0.1289	n. s.		-0.0228	0.0536	n. s.		-0.0484	0.0592
	Abundance	0.0230§	0.0433	0.2243	0.2606	-0.0061	0.0655	-0.0497	0.1202	-0.9310§	0.0472	-0.1085	0.1393
Gammaproteobacteria	Richness	0.0391	0.2616	-0.9517	0.0617	0.0047	0.0596	0.0253§	0.0386	0.8251	0.0629	n. s.	
	Abundance	0.0507	0.2862	-1.276	0.0748	0.0060	0.0650	n. s.		0.8072§	0.0321	n. s.	
Deltaproteobacteria	Richness	0.0401	0.3819	n. s.		0.0051	0.1062	0.0225§	0.0432	0.8630	0.102	n. s.	
	Abundance	0.0320	0.292	n. s.		n. s.		n. s.		n. s.		n. s.	
Spartobacteria	Richness	-0.0255	0.6181	0.6948	0.2084	-0.0038	0.2501	-0.0191	0.1546	-0.6709	0.2668	-0.0312	0.0915
	Abundance	-0.0654	0.2718	4.5543	0.6567	-0.0176	0.3858	-0.1152	0.4166	-3.2009	0.4318	-0.2549	0.4895

Supplementary Table 3.3 Regression r^2 and significance of each variable fitted in the NMDS analysis of the total community (Figure 4 a and b). Significant values in bold.

Variables	**Community composition**	
	r^2	p value
pH	**0.6272**	**0.001**
Soil moisture	**0.5208**	**0.001**
P	**0.3557**	**0.001**
C	**0.5137**	**0.001**
N	**0.4399**	**0.001**
C/N ratio	**0.5821**	**0.001**
Salix	**0.2012**	**0.001**
Betula	0.0751	0.127
Vaccinium	**0.2841**	**0.002**
Empetrum	0.0975	0.137
Acidobacteria	**0.1573**	**0.010**
Actinobacteria	**0.3608**	**0.001**
Armatimonadetes	**0.5010**	**0.001**
Bacteroidetes	**0.5678**	**0.001**
Chlamydiae	**0.5837**	**0.001**
Chloroflexi	**0.6936**	**0.001**
Cyanobacteria	0.0128	0.753
Planctomycetes	**0.3907**	**0.001**
Proteobacteria	**0.3244**	**0.001**
Verrucomicrobia	**0.5743**	**0.001**

Supplementary Table 3.4 Proportion of variation in bacterial community composition, explained by soil variables calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed community matrices. Significant variables were included in the final model for each group (Table 3.1).

Variable	All Bacteria		Acidobacteria		Actinobacteria		Armatimonadetes		Bacteroidetes		Chlamydiae	
	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p
habitat	26.73	0.001	33.18	0.001	25.25	0.001	14.18	0.001	21.73	0.001	6.75	0.001
Soil moisture	8.52	0.001	9.30	0.001	8.53	0.001	4.93	0.001	8.66	0.001	4.09	0.001
pH	15.46	0.001	18.07	0.001	15.72	0.001	6.82	0.001	11.45	0.001	2.58	0.009
P	8.81	0.001	10.12	0.001	8.47	0.001	3.62	0.002	6.05	0.001	2.55	0.014
C	12.29	0.001	15.09	0.001	12.45	0.001	4.65	0.001	7.56	0.001	2.55	0.019
N	10.07	0.001	11.93	0.001	10.06	0.001	3.81	0.001	6.47	0.001	2.75	0.002
C/N ratio	15.02	0.001	18.07	0.001	14.96	0.001	6.05	0.001	9.62	0.001	2.42	0.022

Variable	Chloroflexi		Cyanobacteria		Planctomycetes		Proteobacteria		Verrucomicrobia	
	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p
habitat	20.07	0.001	9.27	0.001	20.57	0.001	25.65	0.001	29.16	0.001
Soil moisture	7.81	0.001	3.45	0.004	5.58	0.001	8.66	0.001	9.79	0.001
pH	11.00	0.001	4.57	0.001	12.82	0.001	14.63	0.001	17.96	0.001
P	7.29	0.001	2.77	0.042	7.52	0.001	8.49	0.001	11.46	0.001
C	9.21	0.001	4.25	0.001	10.97	0.001	11.68	0.001	15.25	0.001
N	7.56	0.001	3.33	0.005	8.69	0.001	9.80	0.001	13.11	0.001
C/N ratio	10.69	0.001	5.52	0.001	13.02	0.001	14.62	0.001	17.75	0.001

Supplementary Table 3.5 Proportion of variation in bacterial community composition of VV plots explained by soil variables and shrub genera relative abundance calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed community matrices. Significant variables (in bold) were included in the final model for each group (Table 3.2).

Variable	**All Bacteria**		**Acidobacteria**		**Actinobacteria**		**Armatimonadetes**		**Bacteroidetes**		**Chlamydiae**	
	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p
Soil moisture	**7.73**	**0.013**	**7.59**	**0.028**	**8.37**	**0.009**	3.69	0.598	6.14	0.071	**5.60**	**0.023**
pH	**13.54**	**0.001**	**16.89**	**0.001**	**13.75**	**0.001**	**8.12**	**0.001**	**12.91**	**0.002**	4.06	0.350
P	**7.66**	**0.011**	**8.75**	**0.009**	**7.31**	**0.015**	4.00	0.462	5.83	0.069	4.53	0.153
C	**9.27**	**0.002**	**10.92**	**0.004**	**9.87**	**0.002**	4.40	0.310	**6.50**	**0.049**	5.09	0.050
N	**8.05**	**0.005**	**9.13**	**0.010**	**8.48**	**0.014**	4.17	0.386	5.84	0.079	**5.29**	**0.023**
C/N ratio	**8.15**	**0.006**	**8.78**	**0.012**	**7.77**	**0.021**	4.68	0.251	5.68	0.105	4.13	0.310
Salix	**16.47**	**0.001**	**21.03**	**0.001**	**20.61**	**0.001**	**9.42**	**0.001**	**17.14**	**0.001**	**6.34**	**0.003**
Betula	5.74	0.061	6.01	0.069	**6.86**	**0.048**	5.55	0.081	5.13	0.148	4.38	0.213
Vaccinium	**7.97**	**0.004**	**8.34**	**0.017**	**11.77**	**0.003**	3.12	0.796	5.41	0.108	**6.96**	**0.002**
Empetrum	**8.19**	**0.006**	**7.64**	**0.028**	**11.12**	**0.004**	3.86	0.474	**6.88**	**0.032**	**7.80**	**0.002**

Variable	**Chloroflexi**		**Cyanobacteria**		**Planctomycetes**		**Proteobacteria**		**Verrucomicrobia**	
	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p	Variance (%)	p
Soil moisture	**7.47**	**0.006**	4.40	0.247	5.48	0.063	**7.85**	**0.004**	**11.31**	**0.001**
pH	**8.13**	**0.004**	4.49	0.255	**11.66**	**0.001**	**12.84**	**0.001**	**15.56**	**0.001**
P	**6.93**	**0.018**	3.69	0.476	**5.96**	**0.039**	**7.44**	**0.007**	**10.86**	**0.001**
C	**7.70**	**0.006**	4.56	0.219	**7.22**	**0.011**	**9.00**	**0.003**	**12.45**	**0.001**
N	**6.99**	**0.017**	4.31	0.282	**6.13**	**0.029**	**7.82**	**0.004**	**11.32**	**0.001**
C/N ratio	**6.37**	**0.030**	3.65	0.540	**6.92**	**0.020**	**8.49**	**0.005**	**10.15**	**0.001**
Salix	**12.33**	**0.001**	**5.93**	**0.025**	**12.66**	**0.001**	**15.49**	**0.001**	**14.06**	**0.001**
Betula	4.68	0.184	**7.12**	**0.017**	5.17	0.096	5.68	0.054	5.59	0.090
Vaccinium	**9.39**	**0.001**	5.56	0.066	**7.83**	**0.004**	**8.02**	**0.007**	**7.25**	**0.020**
Empetrum	**8.70**	**0.003**	4.19	0.323	**6.29**	**0.034**	**8.64**	**0.003**	**8.74**	**0.002**

Chapter 4

Altitude and fungal diversity influence the structure of Antarctic cryptoendolithic Bacteria communities

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Running title: Antarctic cryptoendolithic communities

Abstract

Endolithic growth within rocks is a critical adaptation of microbes living in harsh environments where exposure to extreme temperature, radiation, and desiccation limits the predominant life-forms, such as in the ice-free regions of Continental Antarctica. The microbial diversity of the endolithic communities in these areas has been sparsely examined. In this work, diversity and composition of bacterial assemblages in the cryptoendolithic lichen-dominated communities of Victoria Land (Continental Antarctica) were explored using a high-throughput metabarcoding approach, targeting the V4 region of 16S rDNA. Rocks were collected in 12 different localities (from 14 different sites), along a gradient ranging from 1,000 to 3,300 m a.s.l. and at a sea distance ranging from 29 to 96 km. The results indicate Actinobacteria and Proteobacteria are the dominant taxa in all samples and defined a 'core' group of bacterial taxa across all sites. The structure of bacteria communities is correlated with the fungal counterpart and among the environmental parameters considered, altitude was found to influence bacterial biodiversity, while distance from sea had no evident influence.

Keywords: Environmental factors, Adaptation, Antarctica, Cryptoendolithic lichen-dominated communities, Stress-tolerance, 16S Metabarcoding

4.1 Introduction

Assessment of microbial diversity in extreme environments can provide perspective on the ecological function and strategies for adaptation to resource limited and harsh ecosystems. Most prior investigations on microbial diversity in terrestrial Antarctic soils have focused on Bacteria and Archaea. Studies of soils in sites in East Antarctica including Bratina Island and Windmill Islands (Smith *et al.*, 2006; Chong *et al.*, 2009), West Antarctica South Shetland Archipelago (Ganzert *et al*., 2011), and Luther Vale, located near the north border of Victoria Land (Niederberger *et al*., 2008), revealed a high estimate of bacterial diversity. Victoria Land spans southward from the west side of the Ross Sea from 70°30'S to 78°00'S and westward from the coastline to the edge of the polar plateau (USGS, 2014). It is divided into two regions: Northern Victoria Land, encompassing Terra Nova Bay, Edmonson Point and Cape Hallett, and Southern Victoria Land, including the widest ice-free area of the continent, the McMurdo Dry Valleys, and nearby coastal regions. Dry Valleys soils are highly oligotrophic and support relatively low biomass (Smith et al., 2006; Pointing *et al*., 2009; Rao *et al*., 2011; Lee *et* al., 2012). In this area, soil communities are dominated by Actinobacteria and other cosmopolitan taxa (Aislabie *et al.*, 2006; Smith *et al*., 2006; Niederberger *et al.*, 2008; 2012; Stomeo *et al*., 2012), while cyanobacteria-dominated biofilms are predominant in hypolithic communities (de los Rìos *et al.*, 2014a; Chan *et al*., 2012; Wei *et al*., 2016).

Rocks in the ice-free areas of the McMurdo Dry Valleys from mountain peaks that rise above the Polar Plateau, along the entire Victoria Land, are the primary substratum for life, supporting the highest permanent biomass in these regions of Continental Antarctica (Cowan and Tow, 2004; Cary *et al*., 2010; Cowan *et al*., 2014). The extreme stress conditions experienced by organisms living in these environments include low temperatures, wide thermal fluctuations, high radiation exposure, low relative humidity, and scarce liquid water availability, that have restricted life forms to almost exclusively specialized microbes (Nienow and Friedmann, 1993; Vincent, 2000; Zucconi *et al*., 2016). The narrow window of permissive temperature, light and humidity regimes that can support life promotes the settlement of highly adapted, extremotolerant and extremophilic microorganisms, mostly dwelling inside rocks (endoliths) where the environmental condition are buffered from the extreme ranges of the exposed surfaces (Friedmann, 1982; McKay and Friedmann, 1985; Nienow and Friedmann, 1993; Cary *et al*., 2010; Cowan *et al*., 2014). Among endoliths (Golubic *et al.*, 1981; Nienow and Friedmann, 1993; de los Ríos *et al*., 2014b), cryptoendolithic communities are among the

most widespread in the McMurdo Dry Valleys. They are complex assemblages of microorganisms, including bacteria, cyanobacteria, Chlorophyta and both free-living and lichen-forming fungi (Friedmann, 1982; de la Torre *et al.*, 2003).

Despite the increasing interest in investigating the extremotolerant and extremophilic microbes adapted to endolithic lifestyles in the Antarctic desert, investigation of microbial diversity of these peculiar ecosystems has been limited owing to the difficulty of collecting samples. Available data are patchy and based on a small number of rock samples from different locations or on few samples from a single site (de La Torre *et al*., 2003; Pointing *et al*., 2009; Yung *et al*., 2014; Archer *et al*., 2017) and the relative importance of environmental gradients in shaping these communities remains unexplored. With appreciation of the rapidity of global climate change, it is urgent to develop a baseline knowledge of Antarctic terrestrial ecosystems in order to allow future comparisons and to identify possible changes on these ecosystem (Hogg and Wall, 2011; NAS 2011). In this study we investigated diversity and community composition of bacterial assemblages associated to cryptoendolithic lichen-dominated communities in 42 samples collected over a wide area along Victoria Land (Continental Antarctica), exposed to different degrees of environmental pressures due to variation in altitude and sea distance. The fungal community makeup of these same samples was recently described by Coleine *et al*. (2018a).

The present work supplies a high-resolution inventory of microbial diversity and test if environmental constraints shape and structure diversity in these communities. These observations are important for development of tools to evaluate how communities respond to changes in global temperature especial in polar regions where the change is expected to be most pronounced (Selbmann *et al*., 2017). A metabarcoding approach was used to measure and describe microbial diversity in a relatively unbiased manner (Ji *et al*., 2013). The V4 region of 16S rDNA was amplified from DNA extracted from rocks collected in 14 sites during the XXVI Italian Antarctic Expedition (2010-2011), along 14 sites along a latitudinal transect ranging from 73°29’26”S (Stewart Heights, Northern Victoria Land) to 76°54'36’’S (Battleship Promontory, McMurdo Dry Valleys, Southern Victoria Land) from 1,000 (Battleship Promontory) to 3,300 m a.s.l. (Shafer Peak site 2) and from 29 km (Thern Promontory) to 96 km (Ricker Hills) distance from sea.

The primary aims of this study were to i) assess bacterial diversity, and community composition in the cryptoendolithic niches in Victoria Land; ii) identify ‘core’ group of

bacterial members among samples analyzed; iii) to determine if and how differences in bacterial community structure are correlated with the fungal community or with altitude and distance from the sea.
A detailed methodological information is reported in Appendix 4.1.

4.2 Results and discussion

In this study, 16S rDNA gene amplicon sequencing was used to profile bacterial composition and to test the effects on bacterial assemblages of environmental parameters (altitude and sea distance) and fungal diversity, already investigated in Coleine et al. (2018a), on bacterial assemblage.16S rDNA gene metabarcoding produced a total of 864,425 quality-filtered reads, ranging from 32,481 up to 75,174 reads per sample (Table 4.1). Sequences were grouped into 712 Operational Taxonomic Units (OTUs) and singletons and rare taxa (<5 reads) were removed (152 out of 712 OTUs; Appendix 4.1 in Supporting Material), generating 560 quality filtered OTUs. The species accumulation curve did not reach saturation; however, all rarefaction curves captured the dominant bacterial OTUs for each sample (see Supplementary figures 4.1 and 4.2).

Across the dataset 14 bacterial phyla were detected with abundance varying considerably among sampling sites. Actinobacteria (20-50% of total reads) and Proteobacteria (10-30%) predominated, followed by Acidobacteria (3-13%), Firmicutes (2-15%), Armatimonadetes (1-13%) Cyanobacteria (3-10%), Bacteroidetes (4-7%) and Planctomycetes (0.1-9%). Taxa belonging to *Deinococcus-Thermus*, Fusobacteria and Verrucomicrobia were detected as only a small fraction (1-7%, 1-3% and 1-4%, respectively) and only from a few sites. Unclassified OTUs were present in all sites (5-30%) and these OTUs had no detectable similar sequence using BLASTN preventing taxonomy assignment at even the Phylum level (figure 4.1).

Table 4.1 List of sampling sites following an altitudinal gradient, with altitudes (m a.s.l.) and sea distances (km) reported. Diversity metrics for 16S rRNA gene sequencing for each site. Data are reported as follows: number of reads, species richness index (S), Shannon's index (H') and Simpson's Index of Dominance (1-D).

Sites	Altitude	Sea distance	16S-reads	S	H'	1-D
Battleship Promontory	1000	33.5	66,567	287	3.12	0.89
Trio Nunatak site 1	1000	82	64,152	57	2.59	0.88
Ricker Hills	1115	96	64,744	69	2.89	0.91
Mt Billing	1300	44	59,433	156	2.15	0.70
Trio Nunatak site 2	1400	84.5	32,481	101	2.24	0.82
Thern Promontory	1500	29	54,279	57	1.36	0.65
Bobby Rocks	1680	91	61,406	73	2.64	0.88
Mt Bowen	1874	39.5	58,002	157	2.64	0.86
Richard Nunatak	2000	71.6	70,826	86	2.55	0.87
Stewart Heights	2670	74	75,174	218	4.24	0.98
Timber Peak	2800	49.5	64,023	201	1.89	0.70
Mt New Zealand	2888	47	61,739	144	2.79	0.89
Shafer Peak site 1	3100	59	66,326	50	1.1	0.61
Shafer Peak site 2	3300	48	65,273	156	3.46	0.91

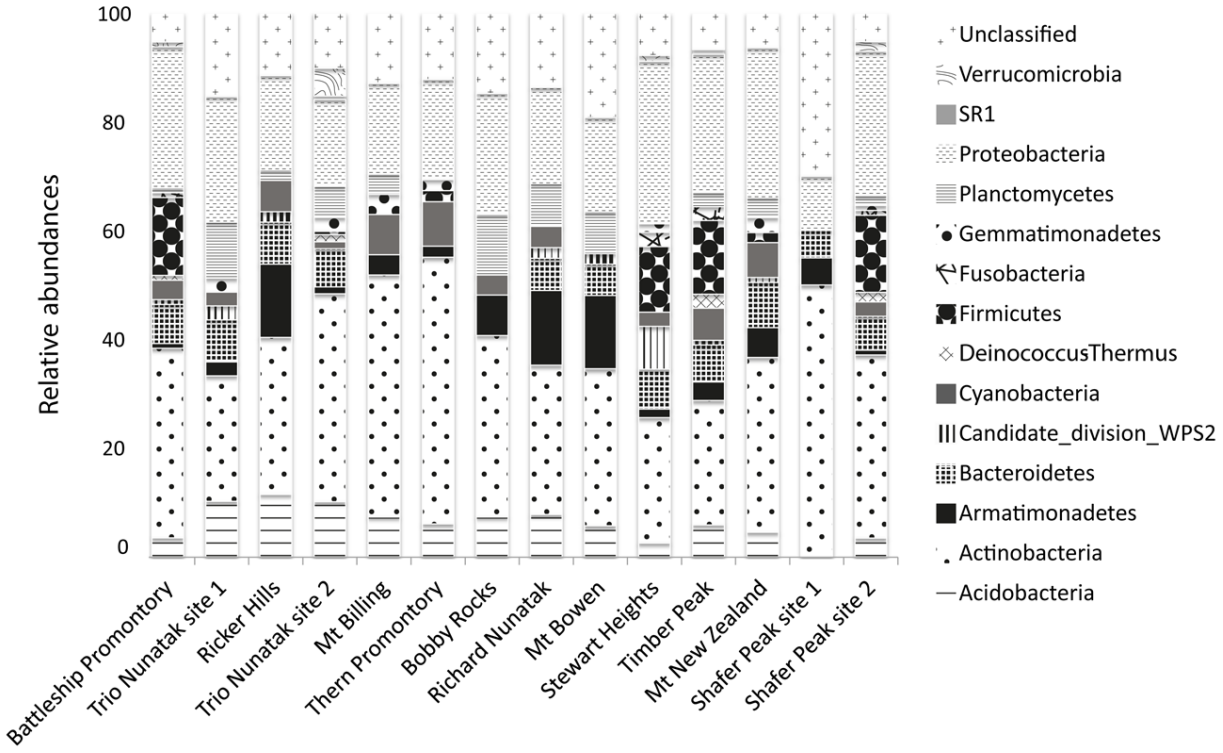


Figure 4.1 Relative abundances of the dominant bacterial OTUs in the cryptoendolithic communities in Victoria Land, Antarctica. Abundances based upon sequence taxonomy classified at the rank of Phylum.

At the order rank, the communities were dominated by Actinomycetales (18-38%) (Actinobacteria), Rhodospirillales (3-18%) (Proteobacteria), Armatimonadales (3-12%) (Armatimonadetes) and Rubrobacterales (1-12%) (Actinobacteria); other orders, such as Bacillales (Firmicutes) were the rarest members, present only in some sites (Supplementary figure 4.3).

A bacterial 'core' community (i.e. OTUs present in at least 75% of the samples) composed of 48 (out of 560) OTUs, less than 10% of total reads, was identified (Table 4.2), highlighting a very strong variability among sites analyzed. Most 'core' members belonged to the Phyla Actinobacteria (17) and Proteobacteria (12). Few taxa were assigned to Armatimonadetes, Acidobacteria, Bacteroidetes or Planctomycetes. Only a single phylotype of Cyanobacteria (unidentified) was recovered among the sample sites. Comparison of the genera present in the sample revealed *Acidisoma* (Proteobacteria), *Granulicella* (Acidobacteria) and *Mucilaginibacter* (Bacteroidetes) as 'core' community members (Table 4.2).

Table 4.2 Taxonomic identity of 48 core Operational Taxonomic Units (OTU) identified**.**

Taxonomic assignment		
OTU id	**Phylum (confidence >1)**	**Identification (confidence >0.97)**
OTU1	Cyanobacteria	-
OTU4	Actinobacteria	Suborder Corynebacterineae
OTU7	Bacteroidetes	Family Sphingobacteriaceae
OTU8	Proteobacteria	Genus *Acidisoma*
OTU10	Proteobacteria	Order Rhizobiales
OTU11	Armatimonadetes	-
OTU12	Proteobacteria	Family Acetobacteraceae
OTU16	Proteobacteria	Family Acetobacteraceae
OTU17	Acidobacteria	Genus *Granulicella*
OTU18	Actinobacteria	Sub-order Frankineae
OTU20	Acidobacteria	Genus *Granulicella*
OTU21	Proteobacteria	Family Acetobacteraceae
OTU23	Actinobacteria	Order Actinomycetales
OTU24	Proteobacteria	Family Acetobacteraceae
OTU25	Proteobacteria	Family Acetobacteraceae
OTU27	Unclassified	-
OTU31	Proteobacteria	Family Acetobacteraceae
OTU32	Unclassified	-
OTU36	Actinobacteria	Suborder Corynebacterineae
OTU37	Armatimonadetes	-
OTU39	Actinobacteria	Family Conexibacteraceae
OTU40	Actinobacteria	Suborder Micrococcineae
OTU43	Acidobacteria	Subgroup Acidobacteria Gp1
OTU49	Bacteroidetes	Genus *Mucilaginibacter*
OTU58	Planctomycetes	Family Planctomycetaceae
OTU70	Bacteroidetes	-
OTU80	Proteobacteria	Family Acetobacteraceae
OTU81	Actinobacteria	Phylum Actinobacteria
OTU87	Armatimonades	-
OTU98	Actinobacteria	Order Actinomycetales
OTU115	Actinobacteria	Suborder Pseudonocardineae
OTU129	Unclassified	-
OTU150	Actinobacteria	Family Micromonosporaceae
OTU164	Actinobacteria	Family Conexibacteraceae

OTU 170	Planctomycetes	Family Planctomycetaceae
OTU191	Proteobacteria	Order Rhizobiales
OTU195	Actinobacteria	Suborder Micrococcineae
OTU217	Actinobacteria	-
OTU233	Actinobacteria	Family Conexibacteraceae
OTU245	Unclassified	-
OTU249	Proteobacteria	Family Acetobacteraceae
OTU281	Unclassified	-
OTU322	Actinobacteria	Order Actinomycetales
OTU334	Unclassified	-
OTU412	Actinobacteria	Sub-order Frankineae
OTU587	Actinobacteria	Order Actinomycetales
OTU614	Acidobacteria	Subgroup Acidobacteria Gp1
OTU621	Proteobacteria	Family Comamonadaceae

A graphical representation of the distribution of ‘core’ specimens was performed to identify association among the shared phylotypes and sampled locations. The 48 most informative taxa were present in almost all sites. The visited localities were further hierarchically clustered by ‘core’ OTUs abundance to identify patterns of similarities in community composition, but they did not exhibit a remarkable clustering of locations by geography (figure 4.2).

Previous works have recovered Actinobacteria and Proteobacteria from other Antarctic ecosystems including soil biotopes (Saul *et al*., 2005; Aislabie *et al*., 2006), cryoconite holes (Christner *et al*., 2003) and cryptoendolithic communities (e.g. Hirsch *et al*., 1988, 2004; de la Torre *et al*., 2003). Actinobacteria and Proteobacteria were reported as the predominant Phyla in these studies, suggesting that these heterotrophic bacteria may have important roles in these communities. These same Phyla were also dominant in other cold climate rock-inhabiting microbial communities from the Arctic (Choe *et al*., 2018), supporting the idea that highly stress-tolerant microbial communities may harbor similar microorganisms, even if found on different continents (Büdel, 1999; Fajardo-Cavazos and Nicholson, 2006). Their success in these environments may be due to a capacity to withstand multiple stress conditions as part of a global meta-community and unique adaptations to the lithic habitat (Walker and Pace, 2007).

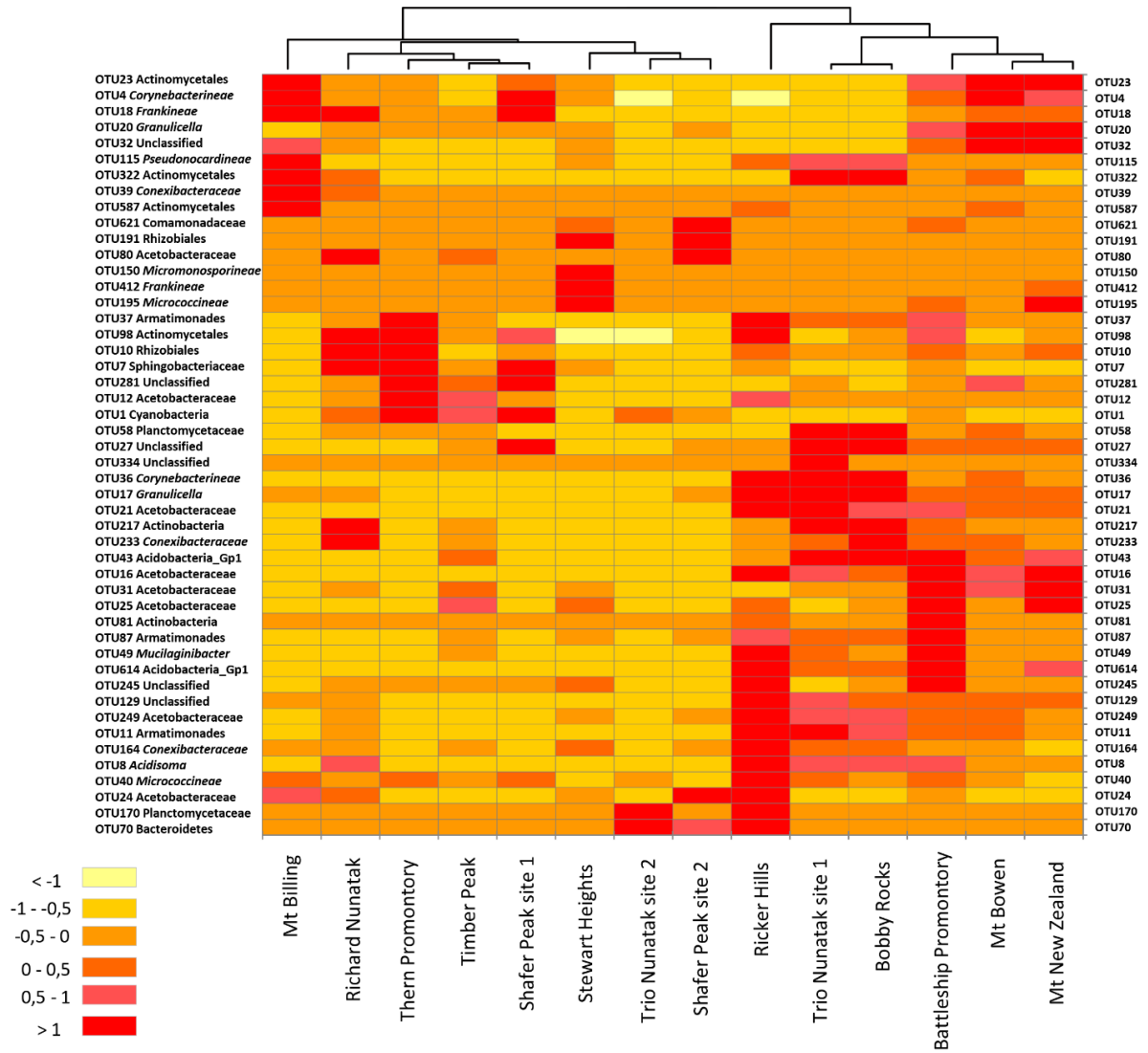


Figure 4.2 Heat map of the 'core' taxa relative abundance and UPGMA hierarchical clustering of sites. Values are scaled (log-transformed) by OTUs relative abundances across all sites. Abundances are indicated by the color intensity: dark and light red indicate higher relative abundances; orange and pale-orange indicate lower relative abundances. Yellow indicates a frequency < -1. Both the 'core' OTUs and sites were clustered using a Bray-Curtis index.

Among the 'core' members, taxa belonging to *Acidisoma* sp. (Rhodospirillales, Proteobacteria) was never observed in ice-free areas of Victoria Land, but have been frequently detected in cold regions such as Alps (Nakai *et al*., 2013). *Granulicella* species (Acidobacteria) were also uniquely found in these ice-free areas which encompasses several cold-adapted species described from Arctic tundra soils (Männistö *et al*., 2012).

The sub-order Frankineae (Actinobacteria, G+) was previously reported from Antarctic soil communities (Learn-Han *et al*., 2012). This group together with Rhizobiales (Proteobacteria, G-) include nitrogen-fixing bacteria typically associated with plants. The family Rhizobiaceae (Rhizobiales) includes nitrogen-fixing bacteria, while the sub-order Frankineae encompasses 4 families, including the nitrogen-fixing genus *Frankia*. Since the used approach in this study, the identification of Rhizobiales OTUs can be resolved confidently only at order or sub-order level, and, even if it is well known that N-fixing bacteria are frequent in lichen microbiomes, we cannot conclude with certainty that nitrogen fixing bacteria belonging to these groups are actually present in the assemblages analyzed.

We found Cyanobacteria at low abundance in this survey, even though they are dominant members in other Antarctic endolithic ecosystems such as endolithic cyanobacteria-dominated communities (Friedmann and Ocampo-Friedmann, 1988; de los Ríos et al., 2004; 2007; Büdel et al., 2008). Cyanobacterial taxa were rarely isolated in cryptoendolithic lichen-dominated communities in McMurdo Dry Valleys (Friedmann and Ocampo, 1976; Friedmann *et al*., 1988);

Deinococcus-like organisms, well known for their ability to withstand the high solar irradiation of the South Pole, especially large amounts of UV, and also to survive to ionizing radiation, limiting damage to their DNA (Mattimore and Battista, 1996; Battista *et al.*, 1999), have been detected in endolithic communities (de la Torre *et al*., 2003; Hirsch *et al*., 1988; Siebert and Hirsch, 1988) and a lichen thallus of *Umbilicaria decussata* from Kay Island, Antarctica (Selbmann *et al*., 2010), but represented a very small fraction of recovered OTUs in our study.

Biodiversity analysis of species richness (from 50 to 287) and Shannon's index (ranging from 1.1 to 4.24) (Table 4.1) confirmed that Antarctic microbial endolithic communities harbor relatively low bacterial diversity (see also Archer *et al*., 2017; Selbmann *et al*., 2017; Coleine *et al.*, 2018), compared with temperate microbial biotopes, which typically have values of Shannon's index between 6 and 7 (Dunbar *et al*., 2000). The β diversity across the 14 sampled sites was measured with a Jaccard index and the contribution of altitude and sea distance in shaping the bacterial communities was estimated using an MRM analysis. A distance matrix was computed to investigate the contribution of altitude and sea distance in shaping the bacterial assemblages. There were not strong relationships between community composition and environmental variables. Sampled sites at similar altitude and distance from sea did not show a

high degree of homogeneity in community composition in the two sites at Trio Nunatak, showing only 18%; for example, the two visited sites at Trio Nunatak showed only 18% of similarity; similar trend was obtained for Shafer Peak site 1 and 2 (25.4% of similarity) (data not shown).

The Pareto Lorenz curves inferred from community composition indicated a high degree of specialization of these communities. The average F0 value was 89% (figure 4.3), indicating the dominance of a very few but highly specialized species while other members occur at very low frequency (Marzorati et al., 2008). This is also supported by inferred Simpson's dominance indices (1-D), which ranged from 0.61 (Shafer Peak site 1) to Stewart Heights (0.98), with a mean value of 0.83 (Table 4.1), indicating a high degree of specialization of these ecosystems and suggesting a potential scant resiliency and recovery capacity after disturbance.

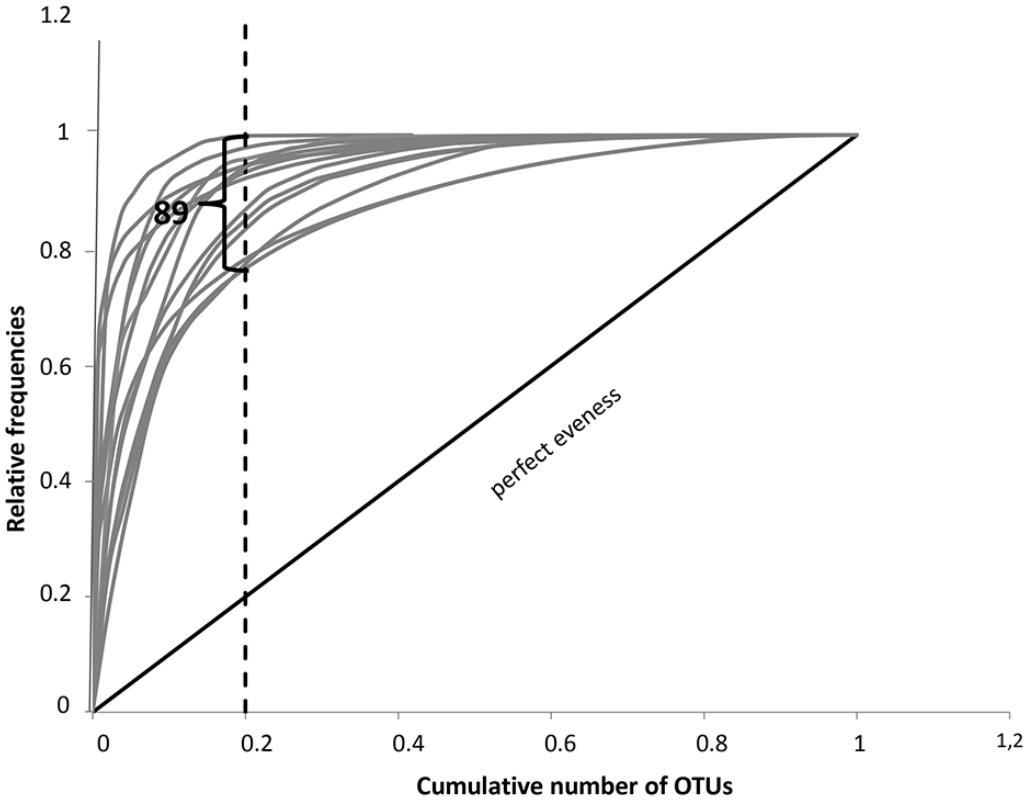


Figure 4.3 Pareto-Lorenz distribution curves based on the number of OTUs and their frequencies. The dashed vertical line at the 0.2 x-axis level is plotted to evaluate the range of the Pareto values. Each line represents a sampling site.

Additionally, bivariate analysis on the distance matrices and Spearman's correlation analysis indicated that the differences in community structure among the samples were not correlated with the differences in the environmental parameters (figure 4.4 and supplementary figure 4.4) as estimated richness from Shannon's and Simpson's indices were similar among the samples ($p>0.05$), even though differences in altitude and sea distances were significant ($p<0.05$; data not shown).

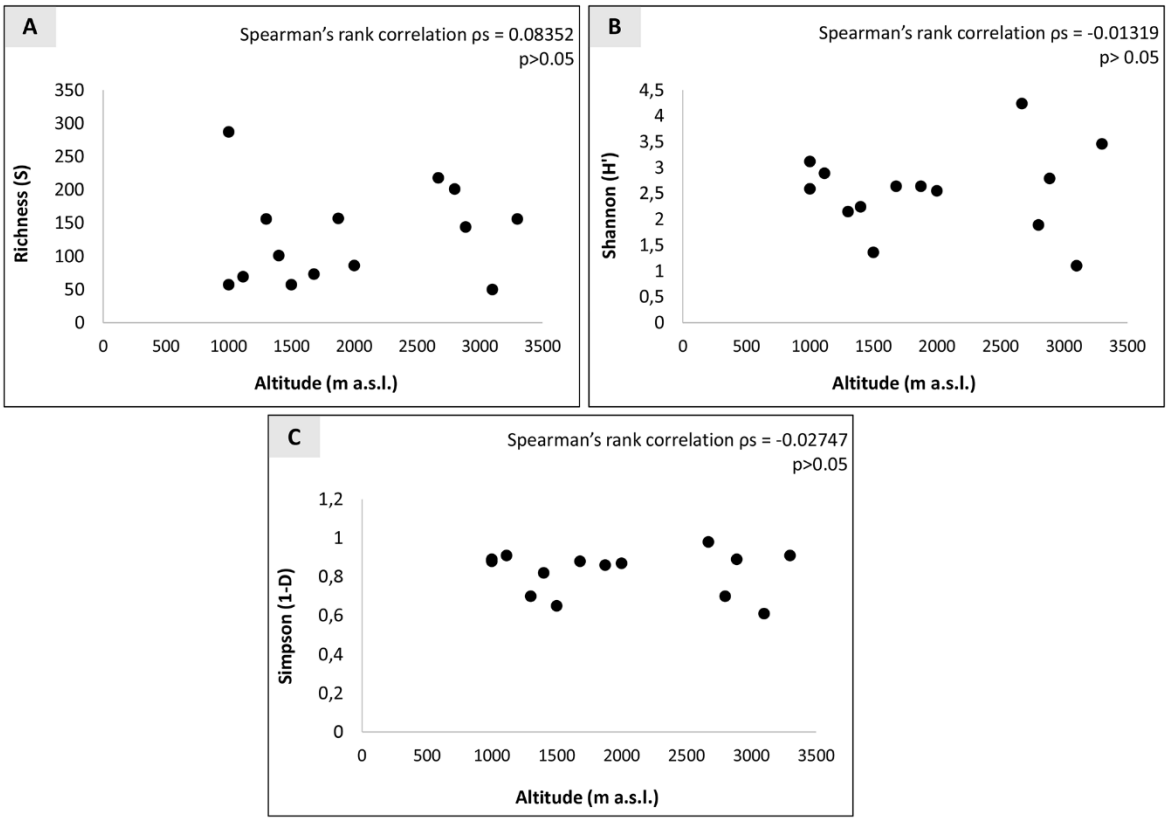


Figure 4.4 Spearman's correlation ranks of biodiversity indices (Richness, Shannon's diversity and Simpson's dominance indices) correlated to the altitudinal gradient.

Nevertheless, PERMANOVA analysis showed a clear influence ($p<0.05$) of altitude on bacterial community composition (incidence and reads abundance data), explaining more than 41% of observed variance, while the parameter sea distance did not show any influence ($p>0.05$).

It has been reported that lichen species and individual thallus traits may influence associated bacterial diversity (Bates *et al*., 2012; Cardinale *et al*., 2012); therefore, we also

tested the effects of fungal community composition (reported in Coleine *et al*., 2018a) in our samples, where lichen species represent 91% of fungal community, on bacterial counterpart. The results clearly indicate a significant correlation between fungal and bacterial biodiversity (figure 4.5a); this relationship was highlighted by regression of pairwise comparisons of Bray-Curtis distances in community composition and Mantel test ($p<0.01$) (figure 4.5b). Actinobacteria and Proteobacteria resulted the predominant phyla in communities herein analyzed; the same findings were obtained in previous study based on culture-dependent approaches (Selbmann *et al.*, 2010).

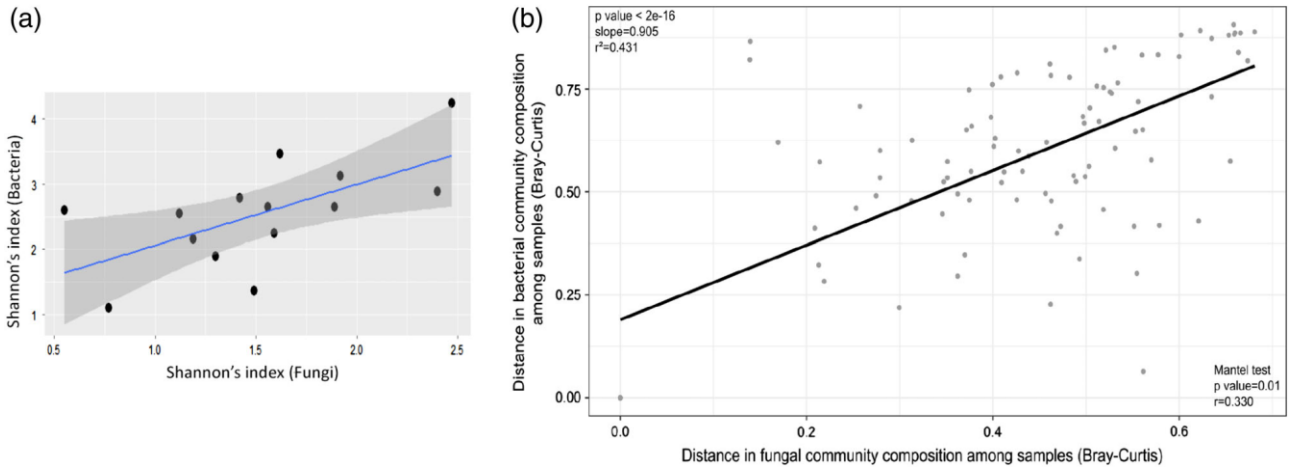


Figure 4.5 Linear regression correlation between bacterial and fungal biodiversity (a) and fungal and bacterial composition distances (b; Bray-Curtis distances, usign Hellinger transformed OTUs tables). Correlation was also tested with Mantel test ($p<0.01$).

These results indicated that the establishment and development of bacteria in the cryptoendolithic lichen-dominated microbial communities of Victoria Land are not influenced by sea distance, but altitude-induced environmental conditions were found to be important factors. This suggests these the combination of UV or temperature have more of an impact than proximity to sea for these cryptoendolithic communities in Victoria Land. Additionally, the positive correlation found between fungi and bacteria community diversity suggests that, as fungal assemblage changes, the bacterial community structure changes as well.

While this study improved our understanding of bacterial endolithic communities and their interactions with environmental factors, we hypothesize that additional microclimate environmental parameters (e.g. water availability, average rock temperature and sun exposure) may be more important determinants of community diversity and structure. Indeed, sun exposure, which likely influences temperature and water availability as well as generating visible difference in texture and weathering properties (McKay and Friedmann, 1985), has been implicated in shaping composition and distribution of functional groups of fungi in Antarctic endolithic communities (Coleine *et al*., 2018b). Further analysis of functional roles and capabilities of endolithic bacteria can clarify relationships between physical-chemical parameters and the possible functional redundancy in bacterial assemblages associated to these communities. In contrast to previous studies which were based on only 2-4 samples, this work sampled endolithic communities along 14 sites in Victoria Land (42 rock samples) and provides the first detailed picture of the bacterial biodiversity of Antarctic cryptoendolithic lichen-dominated communities.

Sequencing, bioinformatics, and molecular ecological analysis of this study was performed in same manner as previous study (Coleine et al., 2018a) and description of the materials and methods is available in Supporting Information Appendix 4.1. The amplicon sequence data and metadata have been deposited in the NCBI Sequence Read Archive database under BioProject accession number PRJNA379160.

Supplementary Material

Appendix 4.1: Material and methods

Sampling area Sampling sites were located in Victoria Land, Antarctica. Sandstones were collected during the XXVI Italian Antarctic Expedition (2010-2011), along a latitudinal transect ranging from 73°29'26"S (Stewart Heights, Northern Victoria Land) to 76°54'36''S (Battleship Promontory, McMurdo Dry Valleys, Southern Victoria Land) from 1,000 (Battleship Promontory) to 3,300 m a.s.l. (Shafer Peak site 2) and from 29 km (Thern Promontory) to 96 km (Ricker Hills) distance from sea. In each site, three rocks colonized by cryptoendolithic lichen dominated communities were sampled. Epilithic thalli were not evident on the rock surface.
Rock samples were the same to those previously analyzed for fungi community composition in Coleine *et al*. (2018), as reported in Table 4.1 and supplementary figure 4.5.

DNA extraction and sequencing Metagenomic DNA was extracted from 0.3 g of powdered rocks using MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. V4 region was amplified using the new developed barcoded primers F515/R806 (515F: GTGYCAGCMGCCGCGGTAA; R806: GGACTACNVGGGTWTCTAAT), modified from the original primers F515/R806 (Caporaso *et al.,* 2010). Reactions were carried out with a total volume of 25 µl, containing 1 µl of each primer, 12.5 µl of Taq DNA Polymerase (Thermo Fischer Scientific Inc., Waltham, MA, USA), 9.5 µl of nuclease-free water (Sigma–Aldrich, UK), and 5 ng of DNA. The PCR solution was subjected to an initial denaturation at 93°C for 3 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 1 min, extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min in an automated thermal cycler (BioRad, Hercules, CA).
Individual sample amplicons were purified, normalized and quantified with the Qubit dsDNA HS Assay Kit (Life Technologies, USA) followed by pooling samples at equimolar concentrations and further concentration quantification with Qubit. Sequencing was performed on Illumina MiSeq platform to generate 2x300 bp reads at the Institute for Integrative Genome Biology, University of California, Riverside.
Technical duplicates for each rock were sequenced and all replicates datasets were merged to increase the amount of sequence information.
The three rocks collected in each site were sequenced and analyzed separately.
Raw sequence data were submitted to the GenBank databases under BioProject accession number PRJNA379160.

Sequencing data analysis V4 datasets were processed according to Coleine *et al*. (2018) with the AMPtk: Amplicon ToolKit for NGS data (formally UFITS) v. 0.9.3 (Palmer *et al*., 2018) and QIIME (Caporaso *et al*., 2010) v. 1.9.1. Briefly, barcodes and primers were removed from

the amplicons sequencing data, reads were demultiplexed with split_libraries.py script implemented in QIIME and then quality trimming, PhiX screening and chimera removal were performed using AMPtk using default parameters. Singletons and rare OTUs (<5 reads) were additionally trimmed off as recommended by Lindahl *et al*. (2013). The cleaned individual sample sequence files were merged into a single file clustered to identify molecular Operational Taxonomic Units (OTUs) with a 97% identity threshold using the VSEARCH (v 2.3.2) (Rognes *et al*., 2016) algorithm. Taxonomy was assigned by using the hybrid SINTAX/UTAX database (Edgar, 2010).

Additionally, we calculated and mapped the OTU 'core', defined as the OTUs present in at least 75% of the samples. PRIMER-E software v7 (PRIMER-E Ltd. Plymouth, UK) was utilized to map the relative abundance of the community 'core' members on a heat-map (Clarke and Gorley, 2015) using log-transformed taxonomic counts and UPGMA clustering approach was implemented to highlight similarities in the bacterial community composition among the sampled sites, calculating Bray-Curtis index.

Species rarefaction curves and species accumulation plots were generated to examine if sampling effort was capable to capture whole bacterial community using 'rarecurve' and 'specaccum' functions in the package 'vegan' (v. 2.3-4; Oksanen *et al*., 2013) in R 3.2.0 (R-Development-Core-Team 2015).

Biodiversity and statistical analysis Species richness (S), Shannon's index (H') (Shannon and Weaver, 1949; Ludwig, 1988) and Simpson's index of Dominance (1-D) (Simpson, 1949) were considered to examine diversity, evenness and composition of bacterial communities.

In order to test for a correlation between the environmental variables (altitude: m a.s.l. and sea distance: km) and biodiversity indices considered (S, H' and 1-D), Spearman's rank correlation coefficients were calculated (Spearman, 1904) according to Coleine *et al*. (2018).

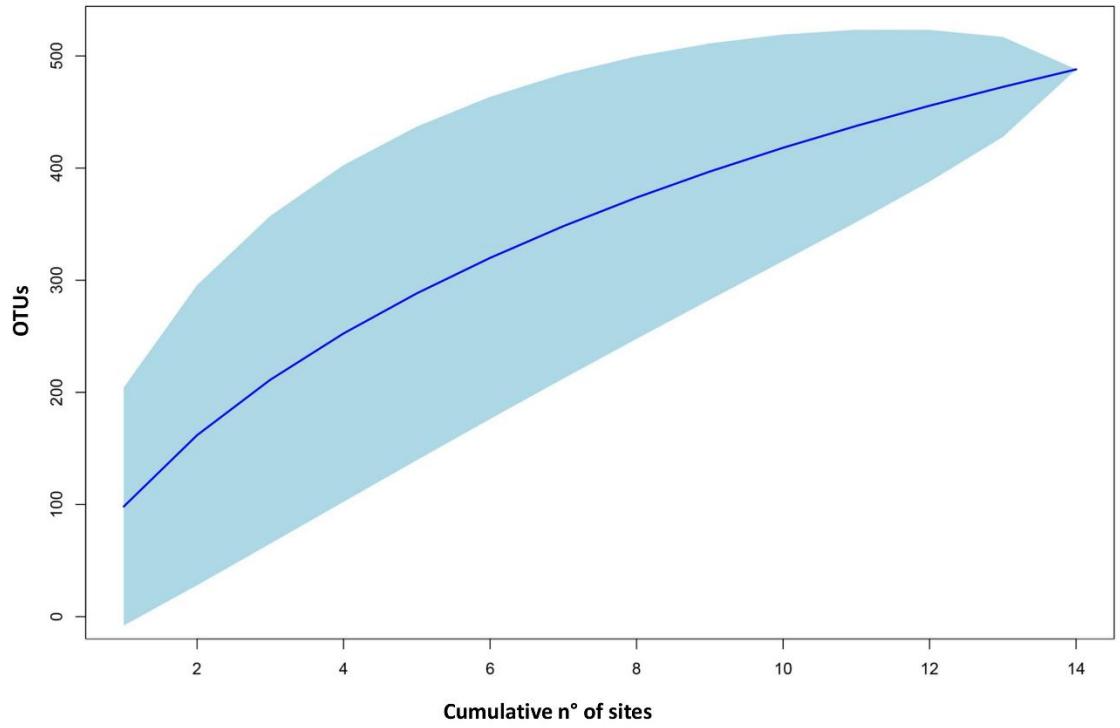
Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was carried out on Hellinger transformed Bray-Curtis distance matrix with 9999 permutations, with the 'adonis' function in the vegan package in R in order to determine the effect of environmental parameters and fungal richness on the observed variance of the total community.

To analyzed the effect of fungal community composition (Coleine *et al.,* 2018a) on bacterial assemblage we tested a linear regression of the pairwise comparisons of Bray-Curtis distances calculated on Hellinger transformed fungal and bacterial OTU tables (based on all the OTUs) in vegan. Additionally, a Mantel test of Bray-Curtis distances of square-rooted matrices in PC-ORD v. 6.0 (McCune and Mefford, 2002) was additionally implemented. Fungal (Coleine *et al.,* 2018a) and bacterial diversity indices were correlated using MICtools (Albanese *et al*., 2018) software. MICtools allows the identification of relationships of various degrees of complexity reporting the Pearson correlation coefficient, the Spearman rank coefficient and the Maximal Information Coefficient (MIC) (Reshef *et al*., 2011).

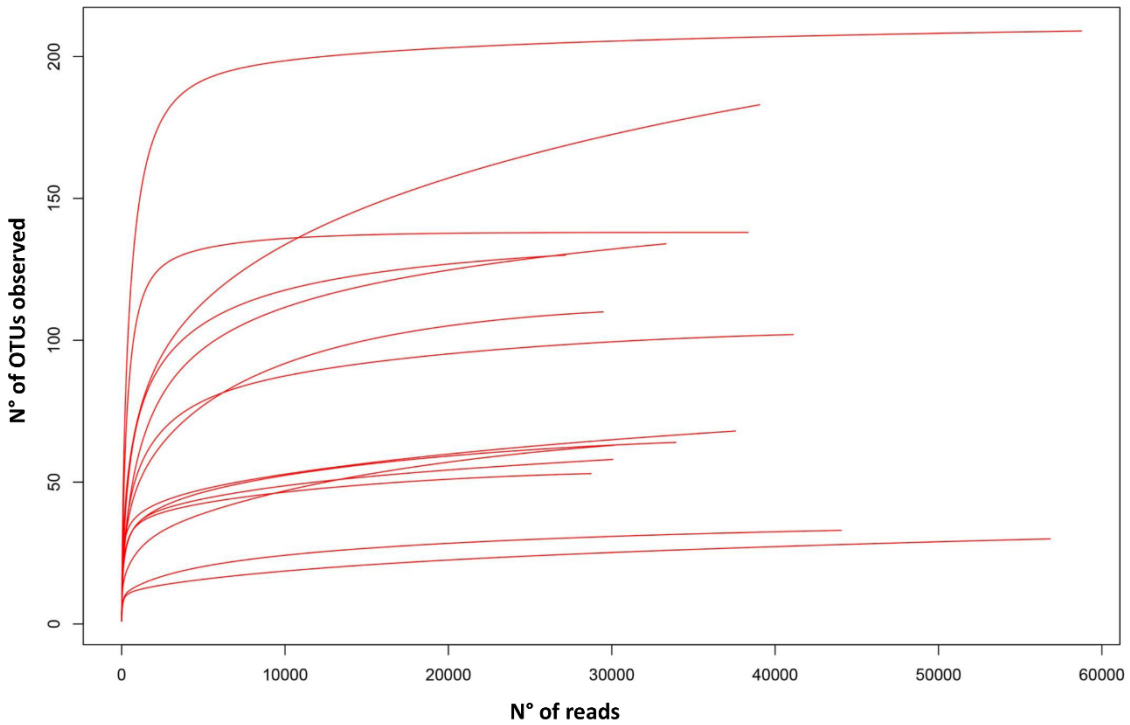
We also measured the β diversity between the community composition (considering presence/absence data) and sampled locations. Multiple linear regression on distance Matrices (MRM) (Legendre *et al*., 2005; Lichstein, 2007; Ptacnik *et al.,* 2010) was computed by using 'MRM' function implemented in ecodist package (Goslee and Urban, 2007) in R (R Development Core Team, 2010) v. 3.4.2. Environmental distances matrices (quantified by

means of the Euclidean distance pairwise) between sampling sites were regressed against the species composition dissimilarity (calculated using incidence-based Jaccard index). To evaluate the significance of the associations, we performed 10,000 permutations on the original dataset, and *P*-values for MRM models were obtained by comparing each observed regression coefficient in the distribution of 10,000 permuted values.
All statistical tests were considered significant at $p<0.05$.

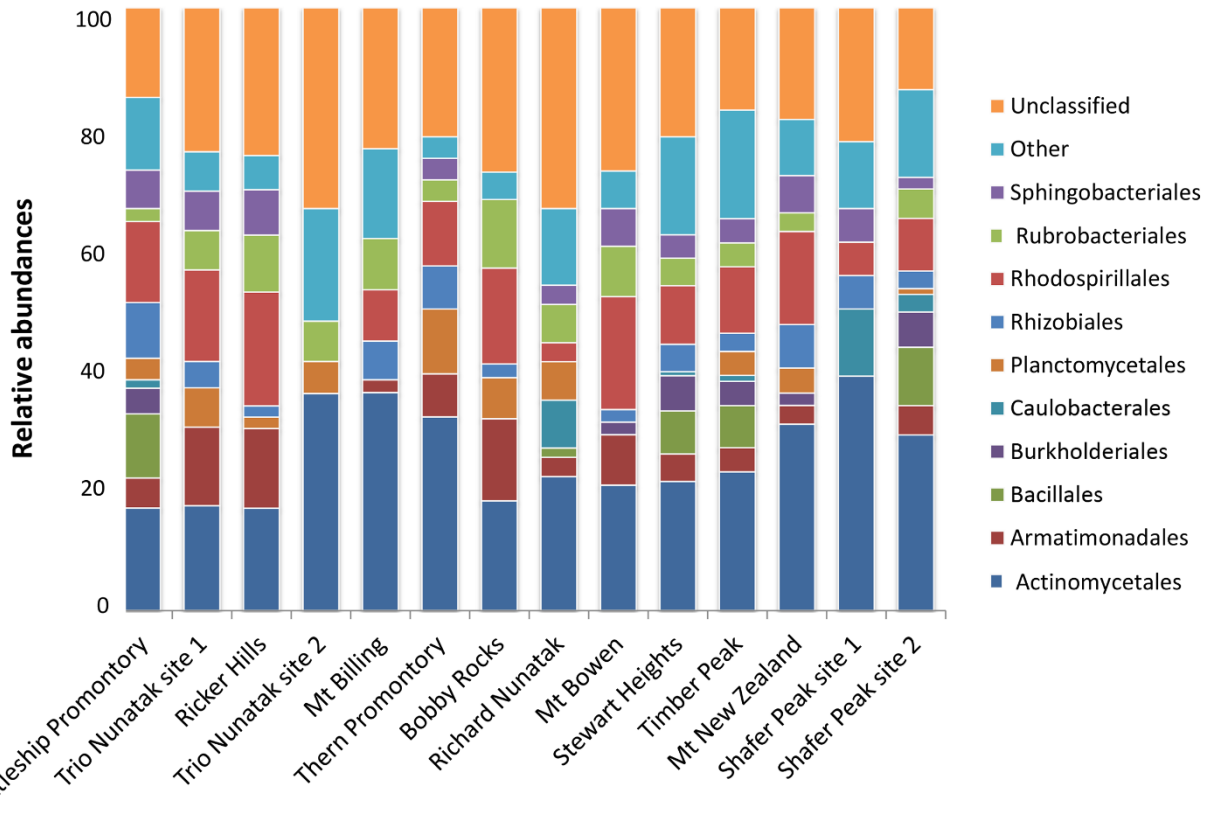
Pareto Lorenz curves The evenness of biodiversity distribution in endolithic communities was graphically represented with Lorenz distribution curves (Lorenz, 1905), based on amplicons profiles. For each site, the cumulative normalized number of the OTUs was used as x-axis, and their respective cumulative normalized frequencies represented the y-axis. Curves were also evaluated based on the Pareto principle, where the cumulative y axis value (F_o value) corresponding to the 20% level on the x-axis is evaluated (Possemiers *et al.*, 2004; Al-Mutairi, 2009). An increasing deviation of Lorenz distribution curve from the theoretical perfect evenness line (i.e. the 45° diagonal), means that a lower evenness can be observed in the structure of the studied ecosystems (Marzorati *et al*., 2008); for example, high values of F_o represent a highly specialized communities with very few species dominant and others present at low abundance.



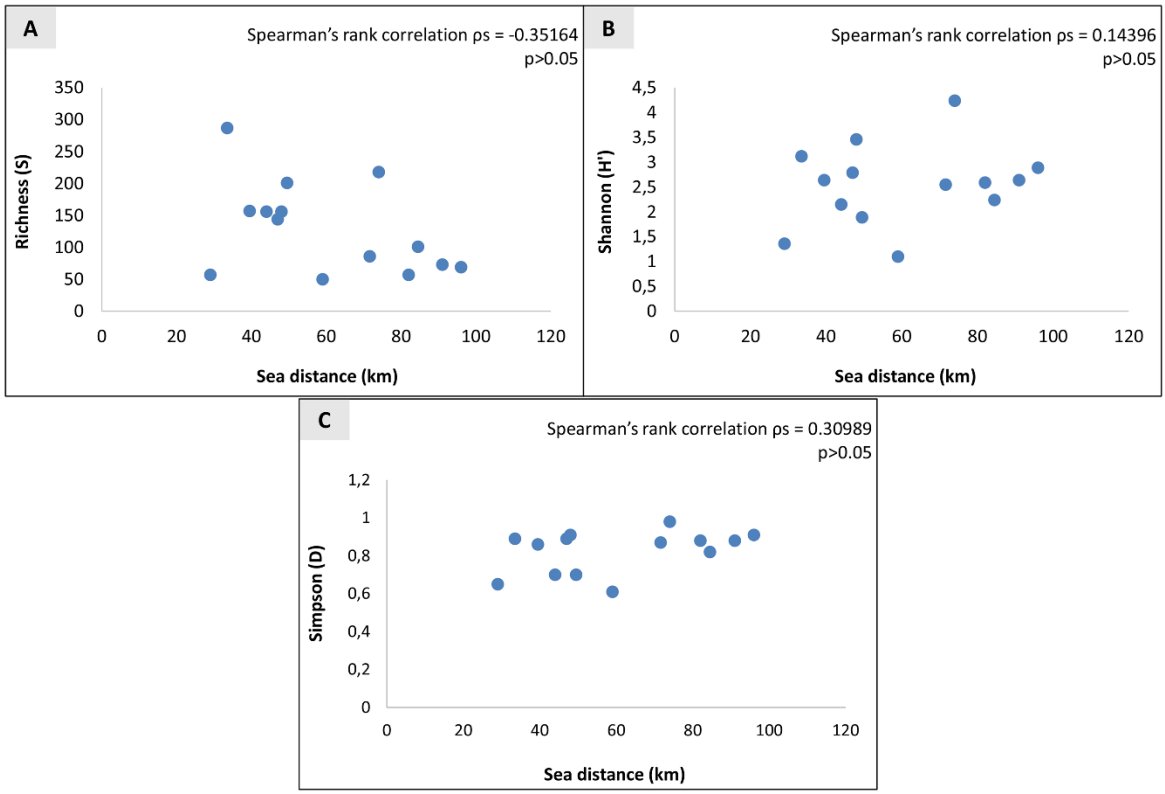
Supplementary figure 4.1 Species accumulation curve, calculated using 'specaccum' function implemented in the R library vegan, represents the number of OTUs found at increasing number of sites. Shaded area corresponds to the deviation standard.



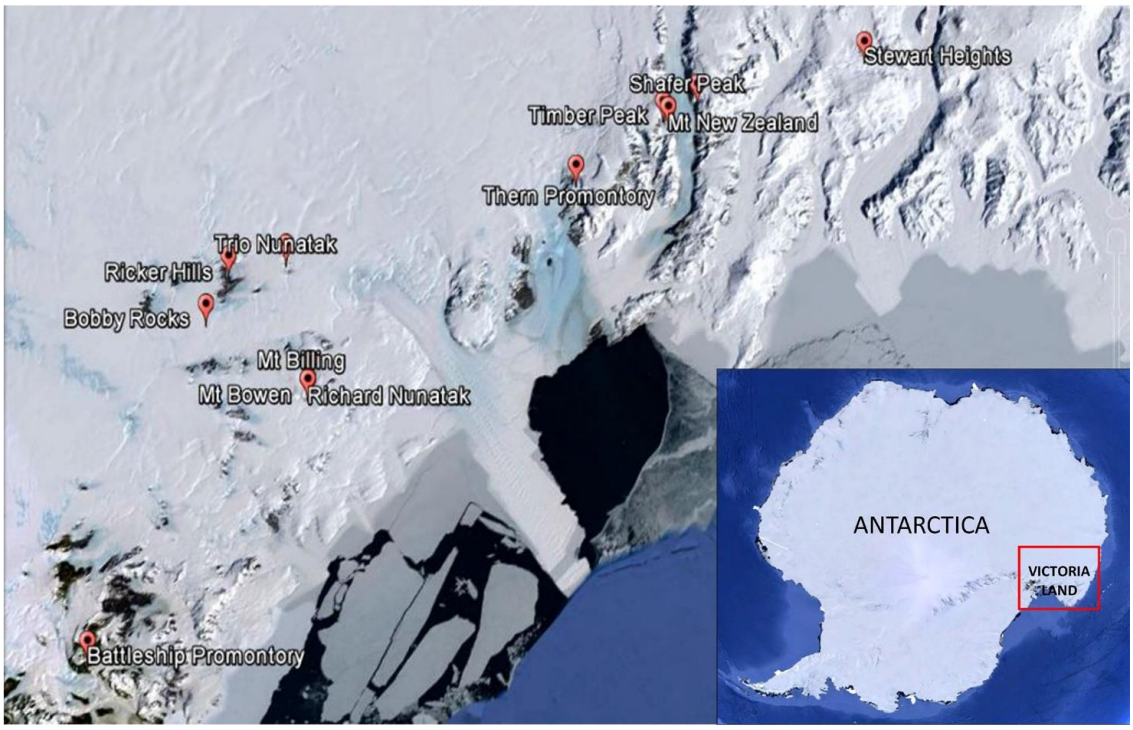
Supplementary figure 4.2 The frequency of observed OTUs for each site was used to calculate bacterial rarefaction curves using the 'rarecurve' function in the R library vegan.



Supplementary figure 4.3 Relative abundances of the dominant bacterial OTUs in cryptoendolithic communitiesin Victoria Land, Antarctica. All abundances are based upon sequences identified at order level. Other included taxa recorded at ≤1%: Acidimicrobiales, Bacteroidales, Clostridiales, Cytophagales, Flavobacteriales, Fusobacteriales, Gemmatimonadales, Gp4, Gp6, Gp16, Sneathiellales, Sphingomonadales and Verrucomicrobiales.



Supplementary figure 4.4 Spearman's correlation ranks of biodiversity indices (S, H′ and 1-D) correlated to the distance from sea gradient.



Supplementary figure 4.5 Map of the study area (Victoria Land) showing the location of the sampling sites, reported in Coleine *et al.* (2018a).

Chapter 5

Exchangeable cations and pH drive diversity and functionality of fungal communities in biological soil crusts from coastal sites of Victoria Land, Antarctica.

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Abstract

Ice-free regions in coastal areas of Victoria Land, Antarctica, are patchily distributed, limited in extent and characterized by a simple vegetation of lichens and mosses, growing only for a short period during the austral summer. These organisms are associated with soil particles and microorganisms (e.g., algae, microfungi and bacteria) to make up biological soil crusts (BSCs), found worldwide in cold and/or arid and semi-arid regions, where plant growth is impaired. Despite BSCs being among the most widespread ecosystems throughout coastal ice-free areas of continental Antarctica, fungal components of these communities have received little focus. Through ITS1 DNA metabarcoding of samples from 17 sites of six different localities from 73 to 77 °S, in a distance scale from 29 to 411 km among different sites, we provide insights into the diversity, community composition, and functionality of fungal communities of these peculiar ecosystems, deepening our knowledge on how they are related to different edaphic variables (i.e. chemical properties and texture). Although fungal richness was low (59 ± 27 OTUs per sample), we found numerous previously unsequenced, putatively unknown fungal species representing a great part of the sampled communities. Community composition was spatially auto-correlated and appeared to be driven by site-specific differences in environmental conditions, particularly edaphic factors, such as exchangeable cations and pH. These results are of particular interest, as they give a wide characterization of the parameters determining soil colonization in a such limiting environment, especially in the light of global changes that are expected to deeply modify the conditions of this environment.

Keywords: DNA metabarcoding; soil fungi; ITS1; environmental filtering; functional groups; soil properties.

5.1 Introduction

Antarctica is the coldest, windiest and driest continent, with the highest mean elevation on the planet and nearly all its surface is covered by ice, with an average thickness of about 2,400 m on the central polar plateau of East Antarctica. Victoria Land, on the western side of the Ross Sea and the Ross Ice Shelf, along a latitudinal gradient spanning 8° from Cape Adare (71°S) to Darwin Glacier (79°S), comprises multiple ecosystems with a great diversity of soil types and minimal human perturbations. McMurdo Dry Valleys, west of McMurdo Sound, in Southern Victoria Land, form the largest ice-free area in Antarctica, while in Northern Victoria Land ice-free regions mainly occur during the warmer summer months, are limited in extent and patchily distributed, and are mostly confined in coastal regions on the fringe of the continent or in isolated nunataks and mountain peaks (Barrett *et al.*, 2006). Microorganisms are the prevalent life forms in the Antarctic soil ecosystems, more than in other continents, with species well adapted to survive and thrive in one of the most hostile environments on Earth (Nienow and Friedmann, 1993) and sometimes endemic, due to the long-time geographic isolation of Antarctica (Vyverman *et al.*, 2010). The simple structure of these communities makes it easier to study the biotic and abiotic interactions with the surrounding environment, than in lower latitudes.

Along the coastal sites of Northern Victoria Land, the vegetation, composed of lichens and mosses (Ochyra *et al.*, 2008), has only a short growth period during the austral summer largely because of the ephemeral water availability (Zucconi *et al.*, 2016). These ice-free areas have been defined "Antarctic oases" (Pickard, 1986) for the presence of mosses, lichens and algal associations growing isolated from each other as islands in the glaciated landscape and hosting distinct groups of organisms. The intimate associations of lichens and bryophytes with soil particles and other microorganisms, such as free-living bacteria, cyanobacteria, algae and microfungi, composes the biological soil crusts (BSCs), that are found worldwide in cold and/or arid and semi-arid regions, where plant growth is impaired and they become the only stable vegetal coverage (Belnap *et al.*, 2001; 2003; Pointing *et al.*, 2015). BSCs, after their establishment, undergo long successional processes that lead to the creation of complex topological and functional relationships among their mineral and biological components. This type of succession has been described in continental Antarctica since 1970, when Cameron and Devaney reported a soil colonization starting with cyanobacteria and algae, followed by lichens

and ending with mosses. The photoautotrophic components, namely bryophytes, and free-living and lichenized cyanobacteria and algae, support the trophic hierarchy of the community by providing a food resource for heterotrophs and by leaching some fixed carbon compounds into the soil (Belnap and Lange 2001; Belnap *et al.*, 2001; Yoshitake *et al.*, 2010). Mosses, for example, are known to contribute considerably to the total carbon (C) pools in high arctic desert soils (Arndal *et al.*, 2009). In addition, some cyanobacterial components of BSCs can fix atmospheric nitrogen (Stewart *et al.*, 2011) in an amount that is known to be adequate to support the needs of mosses and the associated community (Dickson, 2000; Breen and Levesque, 2008). Moreover, the interplay of species growing in filaments or sheaths with others excreting extracellular polymeric substances, leads to the stabilization of the soil and prevents erosion and cryoturbation (Evans and Johansen, 1999; Belnap *et al.*, 2001). Moreover, it is well-known how BSCs are able to increase soil temperature in dry and cold conditions (Xiao *et al.*, 2016), mainly due to their dark colour and increased surface roughness, that decrease surface albedo and increase absorption of solar radiation (Kidron and Tal, 2012). For these reasons, BSCs are often regarded as ecosystem engineers, because their establishment can lead to the development of protosoils, increasing the amount of organic matter (OM) and moisture retention (Bowker *et al.*, 2006; Pointing and Belnap, 2012). Recently, the potential applications of BSCs in controlling soil and water content, e.g., in reversing desertification processes and in restoration of polluted soils (Xiao *et al.*, 2015; Guan *et al.*, 2018), are attracting considerable attention.

Despite being among the most widespread biological communities throughout ice-free areas of continental Antarctica, very few studies have been carried out on BSCs composition in Antarctica. Usually, studies focused on the identification of organisms present in Antarctic soils, without taking into consideration whether they might form specific associations or even biocoenoses (reviewed in Büdel and Colesie, 2014). Green and Broady (2001) provided a first review of BSCs in Antarctica, highlighting how these communities have lower biodiversity, biomass and growth rates compared to analogous ecosystems at lower latitudes. This review and more recent works (Colesie *et al.*, 2014a, b and 2016; Pushkareva *et al.*, 2018) focused mainly on the description of the photosynthetic components and their activity.

Despite the well-known role of fungi in soil ecosystems in recycling C sources, especially in oligotrophic environments and in mutualistic symbioses, and their high resistance to desiccation and UV radiations, their role in Antarctic BSCs has never been investigated, with some exception to lichenized fungi (Smykla *et al.*, 2011; Pérez-Ortega *et al.*, 2012). Free-living

fungi associated with continental Antarctic mosses have been assessed only recently through an isolation approach (Hirose *et al.*, 2016). Even in other environments, the description of free-living fungi associated to BSCs is limited (for example, Bates *et al.*, 2010 and 2012; Steven *et al.*, 2015; Zhang *et al.*, 2016; Maier *et al.*, 2016) and all the works carried out are mostly descriptive of taxonomic composition, without taking into account the environmental factors determining the diversity and distribution of these microorganisms.

Moreover, in spite of the well-known resilience of BSC ecosystems to stress, climate change is expected to alter their composition and abundance (Evans and Lange, 2001; Johnson *et al.*, 2012; Zelikova *et al.*, 2012). Furthermore, especially in polar regions, increased temperatures and altered precipitation patterns are expected to induce an invasion of foreign species (Chown *et al.*, 2012; Pushkareva *et al.*, 2016; Lee *et al.*, 2017). It is still unclear how Antarctic communities will respond to potential expansion of suitable habitats (deglaciation) and changes in biotic interactions linked to ongoing climate change (Convey, 2013; Chown *et al.*, 2015). Hence, the investigation and base-line assessment of these peculiar ecosystems and the description of environmental factors determining their establishment and development is essential to better understand climate-driven changes in BSC communities.

To this extent, we analysed the total fungal community (both symbiotic and free-living fungi) associated with biological soil crusts from 17 sites of six localities in Victoria Land, where different stages of crust development have been identified. All sampled localities are located along the coast, ranging from 73 to 77°S, in a distance scale from 29 to 411 km among different sites. The main aims were to provide a first extensive taxonomic and functional characterization of the fungal communities of these peculiar ecosystems and to understand how diversity, composition and functionality of soil fungal communities are related to nutrient availability and physicochemical variables, in order to give some insights into the landscape-level dynamics of BSC communities in relation to the extreme environment to which they are adapted.

5.2 Material and methods

5.2.1 Sampling

Samples were collected in 17 sites of six coastal localities of Victoria Land, Antarctica, along a latitudinal gradient ranging from 73°31'S (Apostrophe Island) to 77°00'S (Botany Bay) (figure 5.1). All localities are in Northern Victoria Land, except for Botany Bay, located in

Southern Victoria Land. Being north facing and well protected from winds, Botany Bay harbours BSC communities (Seppelt *et al.*, 2010), physiognomically similar to the ones of northern coastal localities. This site and Edmonson Point provide exceptional locations for research on BSC communities, and for this unique ecological status they have been designated as ASPA (Antarctic Specially Protected Areas, N. 154 and N. 165, respectively). Soil samples at Edmonson Point were collected at about 500 m far from the penguin rockery, to limit its influence. In each locality, two to four sampling sites (for a total number of 17 sampling sites) were selected, depending on the presence of different stages of BSC colonization and development, in order to give the widest possible description of these ecosystems (figure 5.1).

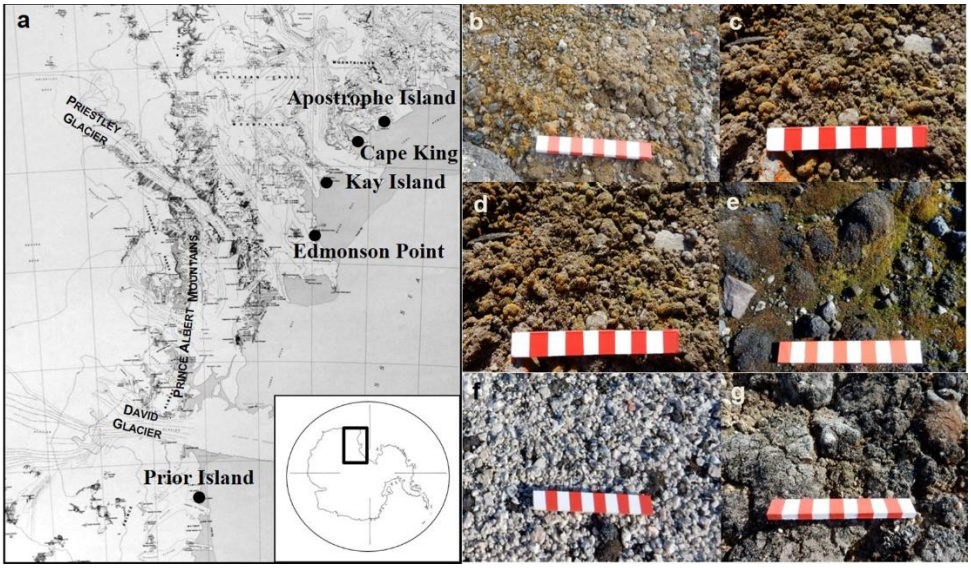


Figure 5.1: Map of the sampling sites (points) in Northern Victoria Land, with the exclusion of Botany Bay located in Southern Victoria Land (a); details of some samples collected: Apostrophe Island site 2 (b); Cape King site 1 (c); Kay Island, site 2 (d); Edmonson Point, site 4(e); Prior Island, site 1 (f); Botany Bay, site 2 (g). Scale bar 1 cm.

Three soil samples representative of each selected area were collected. The top soil (0-5 cm depth) after BCS removal was sampled, resulting in a total of 50 samples. Samples for molecular analyses were stored at -20 °C in sterile bags. The list of the samples, their geographic coordinates and a description of each sampling site are listed in supplementary table 5.1.

5.2.2 Soil physicochemical parameters

Soil samples were air dried and <2 mm sieved. Total soil organic C and N were measured by combustion with an elemental analyzer NA 1500 CHNS (Carlo Erba, Milan, Italy). Particle size distribution, cation exchange capacity (CEC) and pH in a 1:2.5 soil:water suspension were determined according to the SISS (Società Italiana della Scienza del Suolo) methods (Colombo and Miano, 2015). Soil particle size distribution was characterized by sand (0.5-2 mm), coarse silt (0.02-0.05 mm), fine silt (0.002-0.02mm) and clay (<0.002mm). Soil exchangeable bases (Na^+, Ca^{2+}, K^+ and Mg^{2+}) were determined by flame atomic absorption spectrometry (ASS Perkin Elmer 1100B).

5.2.3 DNA extraction, amplification and sequencing

For each sample, metagenomic DNA was extracted from 0.5 g of soil using DNEasy Powersoil kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The ITS1 region was amplified using ITS1F (Gardes and Bruns, 1993) and ITS2 (White *et al.*, 1990) primers, following the protocol described in Smith and Peay (2014). An equimolar pool of uniquely barcoded amplicons was paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform, at the Vincent J. Coates Genomics Sequencing Laboratory at University of California, Berkeley.

5.2.4 Bioinformatic analyses

Bcl files were converted to Fastq files, were demultiplexed and primers were removed using bcl2fastq (v 2.18). Dual-matched 8-bp indexes were used to eliminate the occurrence of "barcode bleed" (or tag-switching) between samples.

Demultiplexed sequences were then processed with the Amplicon ToolKit (AMPtk) for NGS data (formally UFITS) v.1.2.1 (Palmer *et al.*, 2018). 7,172,088 starting reads were subjected to quality trimming and PhiX screening using USEARCH with default parameters (v. 9.2.64; Edgar, 2010). Reads with less than 100 bp were removed, ones longer than 300 bp were trimmed and paired-end reads were merged in one step, obtaining 3,583,937 contigs. Individual sample sequence files were merged into a single file and clustered with a 97% identity threshold into Operational Taxonomic Units (OTUs) using VSEARCH v 2.7.0 (Rognes *et al.*, 2016), simultaneously removing putative chimeras. A total of 3,351,306 (93.5%) reads were mapped in 1,476 OTUs. Taxonomy was assigned to OTUs based on the curated UNITE+INSD reference

database dynamic Species Hypotheses (UTAX release of October 10, 2017) using USEARCH (v. 9.2.64; Edgar, 2010). Singletons (namely, OTUs with only one read in the dataset) and OTUs with less than 70% identity to a fungal SH (Species Hypothesis) were excluded for the following analyses. Representative sequences of each OTU were submitted to GenBank (MK536607 - MK537296). The OTU table was normalized for subsequent statistical analyses by rarefying the number of sequences per sample to the smallest library size (15 921 reads) using the *rrarefy* function implemented in the vegan R package v. 2.5-2 (Oksanen *et al.*, 2015), resulting in 690 OTUs retained. The initial functional assignments were made by FunGuild (Nguyen *et al.*, 2016) and then manually checked based on ecological metadata of the corresponding SHs in UNITE, for genera that are known to comprise species with diverse functions. OTUs with more than 90% similarity to a fungal SH with known ecological function were assigned to one of two functional groups: lichenized fungi and saprotrophs. The assignment lead to the identification of some elements, known to act as animal pathogens, ectomycorrhizal (ECM) fungi, mycoparasites and plant pathogens, that were not considered in subsequent analyses for the low number of OTUs.

5.2.5 Statistical analyses

Unless otherwise specified, all the analyses were carried out with the *vegan* package (Oksanen *et al.*, 2015) in R (R Core Team, 2018). Linear regression analyses were used to examine relationships between edaphic physicochemical parameters listed before and the richness and relative abundance of the two functional groups and of different taxonomic groups of fungi, with 45 degrees of freedom. Bonferroni adjustment has been applied to obtained p-values.

We ran non-metric multidimensional scaling (NMDS) on the Hellinger-transformed OTU table. Ordinations were run separately for the total community, the functional groups, and the two most abundant phyla, following specifications: distance measure = Bray-Curtis, dimensions = 2, initial configurations = 100, model = global, maximum number of iterations = 200, convergence ratio for stress = 0.999999. We used the *envfit* R function to fit edaphic physicochemical parameters listed above and the latitude values onto the NMDS ordinations. In addition, we tested whether fungal communities were statistically different among different localities using the Multi-Response Permutation Procedure (MRPP).

Partial Mantel tests (Smouse *et al.*, 1986) were carried out in PC-ORD v. 6.0 (McCune *et al.*, 2002), to differentiate the effects of spatial distance (in terms of geographical coordinates) and soil parameters on community structure.

Permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001) was carried out on Bray-Curtis distance matrices obtained of Hellinger-transformed OTU tables with 9999 permutations, with the *adonis* function, in order to determine the effect of each soil parameter on the observed variance of the total community, the two functional groups, and the three dominant phyla. Finally, to account for correlations among environmental variables, we performed a forward selection of parameters, based on the previous results, including only significant environmental variables in the final models.

5.3 Results

The metabarcoding analysis led to the construction of a dataset consisting of 690 quality-filtered and rarefied OTUs, with at least 70% identity with known fungal SH. 304 OTUs out of 690 were present in only one sample, and only 13 in at least 50% of the samples, indicating that the composition of each sample is highly localized. Fungal richness was generally low (59 ± 27 OTUs per sample; supplementary table 5.2) and varied greatly among samples, often even within a plot, ranging from 15 in a poorly developed BSC from Prior Island, to 196 for site 2 in Edmonson Point (supplementary table 5.2).

With respect to fungal phyla, 363 OTUs (52.6% of the total) were assigned to Ascomycota, 114 (16.5%) to Basidiomycota, and 36 (5.3%) to Chytridiomycota; other less representative phyla were Glomeromycota (3 OTUs), Kickxellomycota (1 OTU), Mortierellomycota (20 OTUs), Mucoromycota (7 OTUs) and Rozellomycota (9 OTUs). 137 OTUs (19.8%) matched fungal reference sequences that were not referred to a phylum. Regarding the relative abundance of the different phyla, Ascomycota (mean abundance per sample 62.0±24.8% of the total reads) were the dominant group in all samples, followed by Basidiomycota (mean abundance per sample 14.4±13.3% of the total reads) and Chytridiomycota (mean abundance per sample: 3.9±8.0% of the total reads). In six samples of different localities, more than 50% of the reads belonged to OTUs not identified at phylum level.

At class level, 192 OTUs (28% of the total) remained unidentified, while seven classes represented 75% of identified OTUs. These classes were Lecanoromycetes (79 OTUs),

Eurotiomycetes (63 OTUs), Dothideomycetes (62 OTUs), Leotiomycetes (51 OTUs), Agaricomycetes (50 OTUs), Sordariomycetes (36 OTUs) and Tremellomycetes (34 OTUs). In many of the samples more than 50% of the reads belonged to OTUs not identified at class level.

383 OTUs (55.5% of the total), with identity to known SH higher than 90%, were assigned to fungal functional groups: 261 OTUs as saprotrophs and 53 as lichenized fungi. Additionally, 15 OTUs were assigned to fungal pathogens, 23 to mycorrhizal fungi, 11 to mycoparasites and 20 to plant pathogens or other undefined plant-associated fungi; these groups were not analysed statistically because of their low abundance and sporadic presence in different samples.

5.3.1 Correlation between fungal richness/abundance and edaphic parameters

The C/N rate in soil samples was highly variable, ranging from 3.3 in Edmonson Point site 2, showing an apparent very primitive colonization, to values close to 30 in some samples (Kay Islands sites 1 and 2 and Apostrophe Island site 1) collected in areas with abundant moss coverage (supplementary table 5.3). The pH ranged from slightly acidic to neutral values (minimum 5.1 for Apostrophe Island and maximum 7.4 for Edmonson Point; supplementary table 5.3). Regarding soil texture, almost all the sites were dominated by sand (from 50% to 98%), with a relatively small amount of clay (supplementary table 5.3). However, fine and coarse silt showed a varying trend.

We found no significant correlations between total fungal richness and the measured soil parameters (supplementary figures 5.1 and 5.2). On the other hand, the richness of lichenized fungi was positively correlated with CEC (slope=0.9794 and r^2=0.2658; figure 5.2) and negatively correlated with both Na^+ (slope=-5.0553 and r^2=0.1188, marginally significant; fig. S5.1) and K^+ content (slope=-14.510 and r^2=0.2376; figure 5.2 and supplementary figure 5.1). The relative abundance of this group was positively correlated with CEC (slope=1.3056 and r^2=0.1109, marginally significant), but not correlated with Na^+ and K^+ content (figure 5.3 and supplementary figure 5.3). Finally, an increase of pH from acidic to neutral values lead to a significant decrease in richness (slope=-3.9355 and r^2=0.3522; figure 5.2), and a not significant decrease in abundance (figure 5.3).

As for the total community, neither richness nor abundance of saprotrophic fungi, the dominant functional category, showed significant correlations with measured edaphic parameters (figures 5.2 and 5.3; supplementary figures 5.1 and 5.3).

Neither N, nor C content correlated with ascomycete richness and abundance (supplementary figures 5.1 and 5.3), and with the richness and abundance of the classes belonging to this phylum (Table 5.1). Both richness (slope=0.3992 and r2=0.1299) and abundance (slope=0.9287 and r2=0.01337) of Ascomycota were positively correlated with C/N ratio (supplementary figures 5.1 and 5.3), and a similar trend was observed for the richness of Eurotiomycetes (Table 5.1).

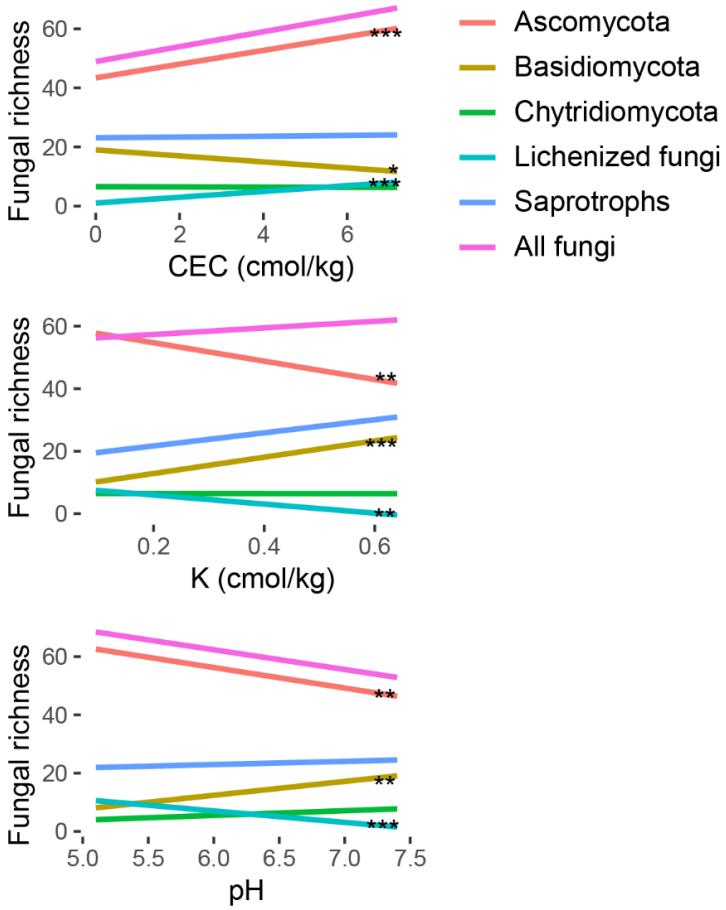


Figure 5.2: Regression lines for the variation of richness (y-axis) of the total fungal community, the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla (for the phyla the relative richness has been plotted), in response to soil chemical parameters: CEC, exchangeable K^+, and pH (x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, m. s. (marginally significant) $p<0.1$. Single graphs of the regressions with the points corresponding to all the samples are in supplementary figure 5.1.

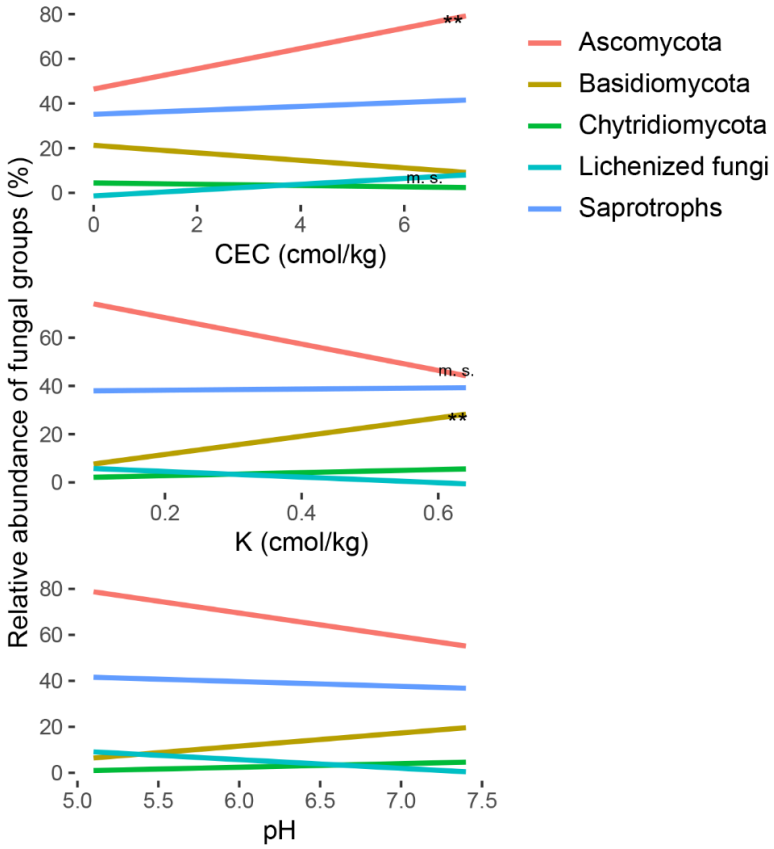


Figure 5.3: Regression lines for the variation of relative abundance (y-axis) of the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla, in response to soil chemical parameters: CEC, exchangeable K^+, and pH (x-axis). The significance of the regressions is indicated as ** $p<0.01$ and m. s. (marginally significant) $p<0.1$. Single graphs of the regressions with the points corresponding to all the samples are in supplementary figure 5.3.

Regarding cation availability, the CEC had a strong positive relationship with Ascomycota richness (slope=2.3280 and r2=0.3466; figure 5.2) and abundance (slope=4.554 and r2=0.2028; figure 5.3), as did Mg^{2+} for richness (slope=4.661 and r^2=0.1679) but not significantly for abundance (Supplementary figures 5.1 and 5.3, respectively). On the contrary, Na^+ and K^+ showed strong negative correlations with both richness (Na^+: slope=-15.558 and r2=0.2805, fig. S1; K^+: slope=-29.415 and r2=0.2198, figure 5.2) and abundance (Na^+: slope=-44.228 and r2=0.3681, fig S3; K^+: slope=-54.712 and r2=0.1113, marginally significant; figure 5.3). These trends were observed in several classes (Table 5.1). Finally, pH was negatively correlated with Ascomycota richness (slope=-7.038 and r2=0.2492, figure 5.2), but not significantly with abundance (figure 5.3). A negative correlation was also reported for Dothideomycetes abundance and Lecanoromycetes richness (Table 5.1).

Table 5.1: Regression slopes for the variation of relative richness (S) and abundance of seven dominant classes in response to edaphic parameters (significant for p<0,05; §marginally significant, p<0.1; n. s. non-significant).

		Dothideomycetes		Eurotiomycetes		Lecanoromycetes		Leotiomycetes		Sordariomycetes		Agaricomycetes		Tremellomycetes	
		S	Abun-dance	S	Abun-dance	S	Abun-dance	S	Abun-dance	S	Abun-dance	S	Abun-dance	S	Abun-dance
Chemical parameters	C	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	N	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	C/N ratio	n. s.	n. s.	0.163	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-0.121§	n. s.	n. s.	n. s.
	CEC	n. s.	2.912	n. s.	n. s.	1.500	1.587	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-1.7579
	Na^+	n. s.	n. s.	-4.953	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	K^+	n. s.	n. s.	n. s.	n. s.	-26.944	n. s.	13.246	n. s.	n. s.	n. s.	9.034	n. s.	n. s.	33.532
	Ca^{2+}	n. s.	n. s.	1.256	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-0.981§	-0.888§	-0.912§	n. s.
	Mg^{2+}	3.231	n. s.	1.437§	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-1.354	-1.441	-1.357	n. s.
	pH	n. s.	-9.272	n. s.	n. s.	-6.989	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
Physical parameters	Sand	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	0.159	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	Coarse silt	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-0.334	n. s.	0.1598§	0.5231	n. s.	n. s.	n. s.	n. s.
	Fine silt	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	-0.265	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	Clay	n. s.	n. s.	0.590	2.293	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

Basidiomycota, compared to Ascomycota, displayed opposite trends with respect to the impact of many of the parameters considered, with negative correlations between richness and CEC (slope=-1.0148 and r^2=0.129), and positive correlations between richness and both K^+ (slope=26.088 and r^2=0.390) and pH (slope=4.773 and r^2=0.248) (figure 5.2). Relative abundance of Basidiomycota positively correlated with K^+ (slope=37.901 and r^2=0.195) (figure 5.3). The two Basidiomycota classes considered (Agaricomycetes and Tremellomycetes) showed similar trends and were also negatively correlated with Ca^{2+} and Mg^{2+} content (Table 5.1). Finally, Chytridiomycota richness was only positively correlated with Ca^{2+} content (slope=1.4972 and r^2=0.166; supplementary figure 5.1).

The soil texture did not show any significant trend with many of the groups considered (supplementary figure 5.2), except for the negative correlation between coarse silt and relative richness of Basidiomycota (slope=-0.3057 and r^2=0.158) and Chytridiomycota (slope=-0.26648 and r^2=0.1290). Basidiomycota richness positively correlated with sand content (slope=0.18235 and r^2=0.156) and negatively with fine silt (slope=-0.4353 and r^2=0.130). Finally, the richness of Chytridiomycota had a positive relationship with clay content (slope=0.9277 and r^2=0.323).

5.3.2 Fungal communities composition

Total fungal community composition differed significantly among the six localities, as indicated by the MRPP analysis (p=0.001, A=0.1409) and illustrated by the two-dimensional NMDS ordination (figure 5.4a). Among the soil chemical parameters measured, pH showed the strongest correlation with fungal community structure, followed by Na^+ and CEC (supplementary table 5.4). All four soil texture classes correlated significantly with fungal community composition, in order of decreasing importance: coarse silt, sand, clay, and fine silt content (supplementary able S5.4).

The NMDS ordinations revealed different structuring of fungal communities at the level of functional groups (figure 5.4 b and c) and phyla (figure 5.4 d and e). The ordinations resulted in a 3-dimensional solution (stress: 0.156) for saprotrophic fungi and in 2-dimensional solution (stress: 0.155) for lichenized fungi, Ascomycota (0.180), and Basidiomycota (0.183). As for the total fungal community, MRPP revealed significant structuring among the sampling sites for saprotrophs (A=0.1692), lichenized fungi (A=0.1678), Ascomycota (A=0.1485) and Basidiomycota (A=0.167), all with p=0.001 significance. The CEC and the pH were the only edaphic parameters that showed significant correlation with the community structure in all

groups (supplementary table 5.4). Also latitude was significant for all the groups (supplementary table 5.4).

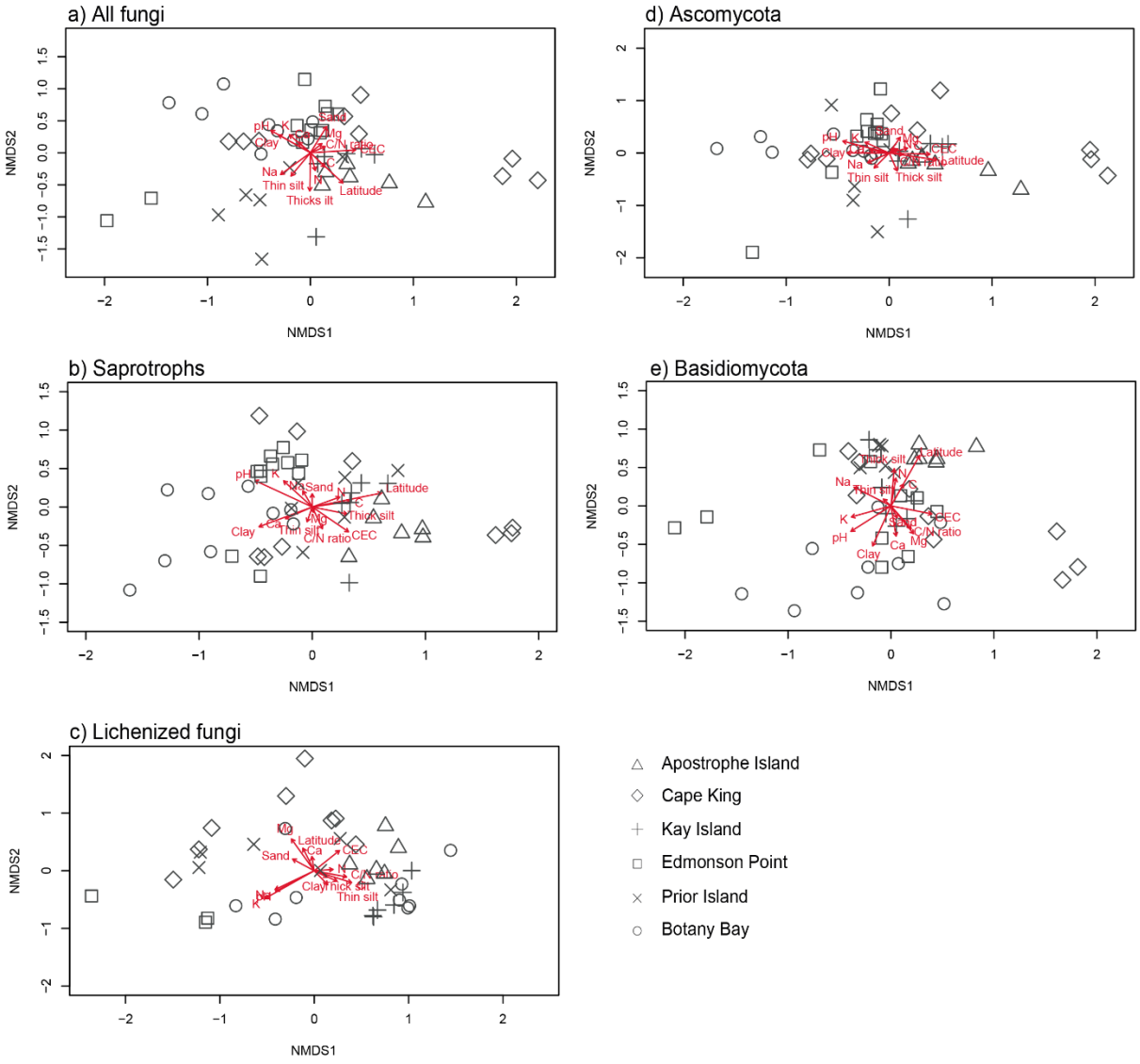


Figure 5.4: Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in fungal communities composition (Hellinger transformed OTUs abundances) in the different localities for: the total fungal community (a), the two functional groups of saprotrophic and lichenised fungi (b, c) and the two dominant phyla (d, e). The significance and the strength of the correlation of all the variables in the figure are reported in supplementary table 5.4.

5.3.3 Effects of environmental parameters on fungal community composition

Partial mantel tests indicated that soil physicochemical parameters had a strongly significant effect on community structure ($r = 0.245$, $p < 0.001$) when the geographic distance was accounted for (control matrix), while the effect of the geographic distance was substantially weaker, though still significant ($r = 0.155$, $p = 0.013$), when soil parameters matrix was controlled.

PerMANOVA analysis was used to quantify the degree to which edaphic physicochemical parameters could explain the distances in community composition among samples. First, variables were considered individually for the total community, the two functional groups, and the three phyla. For the total community, the K^+ content explained the greatest amount of variance, followed by the Mg^{2+} content and the pH (Table 5.2). Three out of the four soil texture classes (sand, coarse silt, and fine silt) explained a significant portion of variance, while the effect of clay content was not significant. When considering the functional groups and the three phyla separately, the percentages of variance explained for each soil parameter were lower, but in many cases they remained significant for shaping the structure of the communities.

When the environmental variables were combined to account for correlation among them, most of the edaphic parameters still contributed significantly to explain proportions of variance in the combined model in all fungal groups (Table 5.3). For the total community, K^+ content explained more than 53% of the observed variance in composition, Mg^{2+} content explained an additional 14%, followed by sand content (7.43%) and CEC (6.48%), while the remaining variables explained less than 5% each in the combined model. For saprotrophic fungi, pH, CEC and different exchangeable cations explained most of the variation in community composition, followed by clay and coarse silt content. At phylum level, pH and soil texture (in particular, silt and clay) explained the most variance, although the contribution of individual variables to the combined model differed among the phyla (Table 5.3).

Table 5.2. Proportion of variation in fungal community composition, at level of the total community (all fungi), the two functional groups (saprotrophs and lichenized fungi) and the three major phyla (Ascomycota, Basidiomycota, and Chytridiomycota), explained by soil physicochemical parameters calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed fungal community matrix. Significant variables (in bold) were included in the final model for each fungal group (Table 5.3).

	All fungi		**Saprotrophs**		**Lichenized fungi**		**Ascomycota**		**Basidiomycota**		**Chytridiomycota**	
Variable	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
C	**17.94**	**0.001**	**6.40**	**0.001**	**8.81**	**0.001**	**5.79**	**0.002**	**4.36**	**0.043**	**11.96**	**0.001**
N	**9.94**	**0.017**	**4.02**	**0.028**	3.87	0.101	3.31	0.079	**4.88**	**0.026**	**10.29**	**0.001**
C/N ratio	**19.70**	**0.001**	**4.78**	**0.006**	2.67	0.425	**4.12**	**0.031**	4.12	0.051	**4.49**	**0.045**
CEC	**25.22**	**0.001**	**9.50**	**0.001**	**5.52**	**0.018**	**7.34**	**0.001**	**4.41**	**0.032**	3.79	0.085
Na^+	**16.31**	**0.003**	**7.37**	**0.001**	**5.12**	**0.029**	**5.93**	**0.004**	**5.39**	**0.014**	3.60	0.112
K^+	**53.11**	**0.001**	**7.76**	**0.001**	**8.33**	**0.001**	**7.73**	**0.001**	**6.10**	**0.004**	**9.10**	**0.001**
Ca^{2+}	**19.92**	**0.002**	**6.49**	**0.001**	**5.44**	**0.030**	**5.39**	**0.002**	**4.61**	**0.026**	3.94	0.082
Mg^{2+}	**30.57**	**0.001**	**6.38**	**0.001**	**9.36**	**0.002**	**5.66**	**0.004**	**4.85**	**0.019**	1.95	0.564
pH	**29.20**	**0.001**	**11.52**	**0.001**	**8.61**	**0.001**	**9.70**	**0.001**	**6.55**	**0.007**	**10.49**	**0.001**
Sand	**19.14**	**0.001**	**4.18**	**0.016**	4.31	0.076	**5.51**	**0.002**	**4.22**	**0.048**	**8.35**	**0.001**
Coarse silt	**15.50**	**0.004**	**6.85**	**0.001**	4.37	0.061	**6.27**	**0.001**	**8.23**	**0.001**	**16.04**	**0.001**
Fine silt	**19.19**	**0.002**	**4.07**	**0.018**	4.29	0.096	**5.41**	**0.006**	3.82	0.073	**6.61**	**0.004**
Clay	5.82	0.078	**6.89**	**0.001**	**5.06**	**0.032**	**5.13**	**0.010**	**7.07**	**0.003**	**4.59**	**0.034**

Table 3: Proportion of variation in fungal community composition, at level of the total community (all fungi), the two functional groups (saprotrophs and lichenized fungi) and the three major phyla (Ascomycota, Basidiomycota, and Chytridiomycota) explained by soil physicochemical parameters added sequentially (first to the last) in a model, depending on their independent influence in the variance, as reported in table 5.2. Significant values in bold.

All fungi			**Saprotrophs**			**Lichenized fungi**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
K^+	**53.12**	**0.0001**	pH	**11.52**	**0.0001**	Mg^{2+}	**9.36**	**0.0001**
Mg^{2+}	**14.28**	**0.0001**	CEC	**7.80**	**0.0001**	C	**6.50**	**0.0003**
pH	**3.21**	**0.0001**	K^+	**4.38**	**0.0001**	pH	**6.49**	**0.0001**
CEC	**6.48**	**0.0001**	Na^+	**3.63**	**0.0011**	K^+	**6.09**	**0.0003**
Ca^{2+}	**1.77**	**0.0002**	Clay	**4.67**	**0.0001**	CEC	**7.40**	**0.0001**
C/N ratio	**1.60**	**0.0001**	Coarse silt	**4.54**	**0.0001**	Ca^{2+}	**3.64**	**0.0222**
Fine silt	**4.41**	**0.0001**	Ca^{2+}	**5.85**	**0.0001**	Na^+	**4.98**	**0.0008**
Sand	**7.43**	**0.0001**	C	**3.29**	**0.0016**			
C	0.24	0.1115	Mg^{2+}	**3.84**	**0.0005**			
Na^+	**3.58**	**0.0001**	C/N ratio	2.01	0.0909			
N	0.16	0.2069	N	2.07	0.0740			
Ascomycota			**Basidiomycota**			**Chytridiomycota**		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
pH	**9.70**	**0.0001**	Coarse silt	**8.23**	**0.0001**	Coarse silt	**16.03**	**0.0001**
K^+	**4.40**	**0.0003**	Clay	**6.67**	**0.0001**	C	2.60	0.0652
CEC	**4.95**	**0.0004**	pH	**5.19**	**0.0007**	N	1.64	0.3361
Coarse silt	2.26	0.0674	K^+	**4.39**	**0.0037**	pH	**5.80**	**0.0003**
Na^+	**4.59**	**0.0001**	Na^+	**6.65**	**0.0001**	K^+	**5.28**	**0.0010**
C	**5.97**	**0.0001**	N	0.38	0.9751	Sand	**3.88**	**0.0082**
Mg^{2+}	**4.56**	**0.0002**	Mg^{2+}	**3.93**	**0.0065**	Fine silt	**4.71**	**0.0013**
Sand	**4.77**	**0.0001**	Ca^{2+}	**8.25**	**0.0001**	Clay	**6.30**	**0.0003**
Fine silt	**4.97**	**0.0001**	CEC	**4.61**	**0.0020**	C/N ratio	2.71	0.0620
C/N ratio	**2.96**	**0.0141**	C	0.92	0.7356			

5.4 Discussions

Our study presents a first deep molecular investigation of both symbiotic and free-living fungi associated with BSCs in continental Antarctica. The relatively low fungal richness values recorded can be compared to the ones of cyanobacterial components of Antarctic BSCs (Pushkareva *et al.*, 2018) and are similar to values recorded in other Antarctic fungal community surveys, as those associated with cryptoendolithic lichen-dominated communities (Coleine *et al.*, 2018a). The low biodiversity of Antarctic ecosystems is mainly due to the low temperature and water availability (Green and Broady, 2001), and complies with the trends reported globally for fungi, i.e., decreasing their richness from mid-latitudes to the poles (Tedersoo *et al.*, 2014; Bahram *et al.*, 2018), even though these studies did not include Antarctic samples.

This study also reveals the occurrence of several sequences that could not be identified even to phylum level, many of which could play important roles in these communities. Additionally, more than 40% of the OTUs found were present in only one out of 50 samples processed, indicating the high degree of patchiness of fungi, often seen in other biomes as well, and the need to carry out more BSC studies in these environments.

Most organisms in the analysed mycobiomes, both in terms of abundance and biodiversity, belong to Ascomycota, followed by Basidiomycota, as previously reported from both molecular and culture-dependent studies for soils of the Antarctic peninsula (Cox *et al.*, 2016; Ji *et al.*, 2016) and the McMurdo Dry Valleys (Wei *et al.*, 2016), for rock communities in Victoria Land (Coleine *et al.*, 2018a) and soils under mosses in ice-free coastal areas of the same region (Hirose *et al.*, 2016).

Lecanoromycetes, Eurotiomycetes and Dothideomycetes are the prevalent classes in the BSCs analysed. Lecanoromycetes and Dothideomycetes were already counted as the dominant classes within the Antarctic endolithic microbial communities, known to include some of the most resistant and extremotolerant organisms (Coleine *et al.*, 2018a).

Ascomycota comprise lichenized, saprotrophic and parasitic fungi (Dahlberg and Bültmann, 2013), which are the main functional groups in our dataset. Lichenized fungi are fundamental components of BSCs, while saprotrophic fungi are important for OM decomposition and nutrient recycling and can utilize the organic compounds released from the photosynthetic component of BSCs. These two functional groups have also been reported as the major colonizers of Antarctic lichen-dominated cryptoendolithic communities (Coleine *et*

al., 2018b). Other groups with lower incidence and abundance in the samples, such as plant pathogens on mosses, and fungal and animal parasites, could be metabolically active in these ecosystems, while fungi known to be associated with vascular plants (mycorrhizal fungi) likely occur in these soils as dormant spores dispersed from other continents. It is well known that DNA metabarcoding also detects fungi that are present only as spores in the given sample. This has frequently been addressed in culture-based studies on Antarctic fungal communities, because of the ability of many fungi to remain viable in a dormant state for extended periods. Therefore, the detection of a particular fungal species in soil or other substrates is not considered a definitive evidence that the species could have an ecological role in these ecosystems (Arenz *et al.*, 2014). The presence of cosmopolitan species of genera such as *Alternaria*, *Penicillium*, *Aspergillus*, also quite common in our dataset, is often attributed to their wide dispersal potential and even to the ubiquitous association with human structures and materials (Ruisi *et al.*, 2007). Metabarcoding of DNA does not distinguish active organisms from relic DNA, which may represent a greater proportion of total DNA in low biomass soils (Carini et al., 2016). This effect could be exacerbated by the preservation of relic DNA in cold Antarctic soils (Willerslev et al., 2004). A recent study on maritime Antarctic soils showed that DNA-based studies may be biased towards cosmopolitan fungi, perhaps due to a higher proportion of inactive molecules for these fungi (Cox et al., 2019).

The simple Antarctic ecosystems, characterized by limiting harsh environmental conditions and presumably lower level of competition than at lower latitudes, offer an ideal setting to study the role of edaphic parameters in shaping the diversity and functionality of fungal communities. Richness did not statistically differ among the sampling sites and localities and it was not correlated with the apparent complexity of BSCs at the sampling sites either. Rather, fungal community composition differed significantly among localities and even among sites within the same locality. As shown via partial Mantel tests, the geographic distance had a significant effect on community composition, even if weaker than the edaphic parameters tested. These patterns are in agreement with certain levels of regional endemism previously reported for non-fungal Antarctic microorganisms, such as diatoms, green algae, cyanobacteria and other bacteria (Vyverman *et al.*, 2010), and are likely driven by the environmental filtering due to differences in mesoclimatic and edaphic parameters both among and within localities.

Among the main edaphic parameters driving the composition of fungal communities, soil pH was among the most influential ones, affecting the composition of all functional and

taxonomic groups studied. However, although soil pH was among the main driver of fungal and bacterial diversity in both polar regions (Siciliano *et al.*, 2014) and of fungal diversity and distribution at global scale (Tedersoo *et al.*, 2014; Bahram *et al.*, 2018), it did not show a significant effect on the fungal richness in this study. Similarly, while soil C and N are known to affect fungal richness and C/N is a major predictor of fungal abundance and gene function on global and regional scales (Bahram *et al.*, 2018), in this study their correlations with richness, abundance and community composition were weaker than that of several other edaphic parameters. PerMANOVA analysis highlighted that C and N content, and their ratio, even being significant for the communities structure, were not independent from the other physicochemical parameters considered. For these reasons, the distribution of fungi appears to be more linked to the mineral characteristics of the environment than to the presence of OM derived from BSCs.

Soil parameters found to be determinant for fungal community composition included the exchangeable cations, as K^+, explaining more than 53% of the observed variance, followed by Mg^{2+}. Previous reports on the effects of exchangeable cations on fungal diversity are that of Tedersoo *et al.* (2014) on the global diversity of fungi, and the one of Bahram *et al.* (2018) on global microbial diversity and function, although neither of them included samples from Antarctica. Tedersoo *et al.* (2014) reported Ca^{2+} as one of the most important predictors of fungal diversity at global scale, even though it was not determinant for community composition. Ca^{2+} is important for many physiological processes in plants and microorganisms and can influence the turnover rate of soil OM (Reich *et al.*, 2005). It had a significant influence on community composition in our dataset as well, but K^+ and Mg^{2+} had stronger effects, not reported at all at global scale. The other cations affecting the composition or functionality of fungal communities in our study were not determinant neither in Tedersoo *et al.* (2014), nor in Bahram *et al.* (2018). Similar to the results of this study, a significant effect of K^+ on the diversity of fungi in maritime Antarctic soils has also been documented (Newsham et al 2016), although other cations appeared more important in driving fungal composition in this Antarctic region.

In an earlier study carried out on different Antarctic soil environments with a culture-dependent approach, soil C and N were reported to have a direct effect on fungal community composition, while pH and salinity were thought to influence fungi indirectly (Arenz and Blanchette, 2011). Interestingly, in the same work, fungal diversity and abundance were negatively correlated with the total salinity, estimated through the electrical conductivity. Our

study confirmed this trend for Na and K on Ascomycota, while abundance and diversity of Basidiomycota were positively influenced by both these cations. In Antarctic soils, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} contents have been shown to be less influenced by biological activity than by a suite of physical factors, including the proximity of marine sources, soil age and the degree of mineral weathering, which is extremely slow due to the scarcity of available liquid water (Campbell and Claridge, 1987). The high potential of fungi in promoting mineral weathering (reviewed in Hoffland *et al.*, 2004) can support the nutrient need of the total BSCs community and their symbiotic primary producers, explaining the strong connection of the fungal community with the presence of such cations.

The CEC and the content of minerals had a profound influence on the composition of the total fungal community and of some taxonomic and functional groups studied, as well as on their richness and relative abundance. The CEC of soils varies according to the clay percentage, pH, OM amount, and nature of soil substrate (Brady and Weil, 2002). The greater the amount of clay in soils, the greater its ability to retain water and nutrients and provide more favourable conditions for microbial activity. Instead, sandy soils, such as the ones in our samples, are characterized by very low OM and CEC. Therefore, it could be hypothesized that in these extreme conditions the low capacity of the soil to make cations available for living organisms is likely to be a limiting factor for the colonization and survival of fungi and other microbes. In such limiting environments, even small changes in pH values, due to environmental changes, can dramatically affect the CEC and the consequent availability of exchangeable cations, strongly influencing fungal communities. On the other hand, pH, soil texture and associated soil CEC have all been shown to be influenced by the development of BSCs in arid environments (Anderson *et al.*, 1982; Chamizo *et al.*, 2012), which could also explain their link with fungal community composition, given the effect that they could exert on primary production and the entire BSCs community.

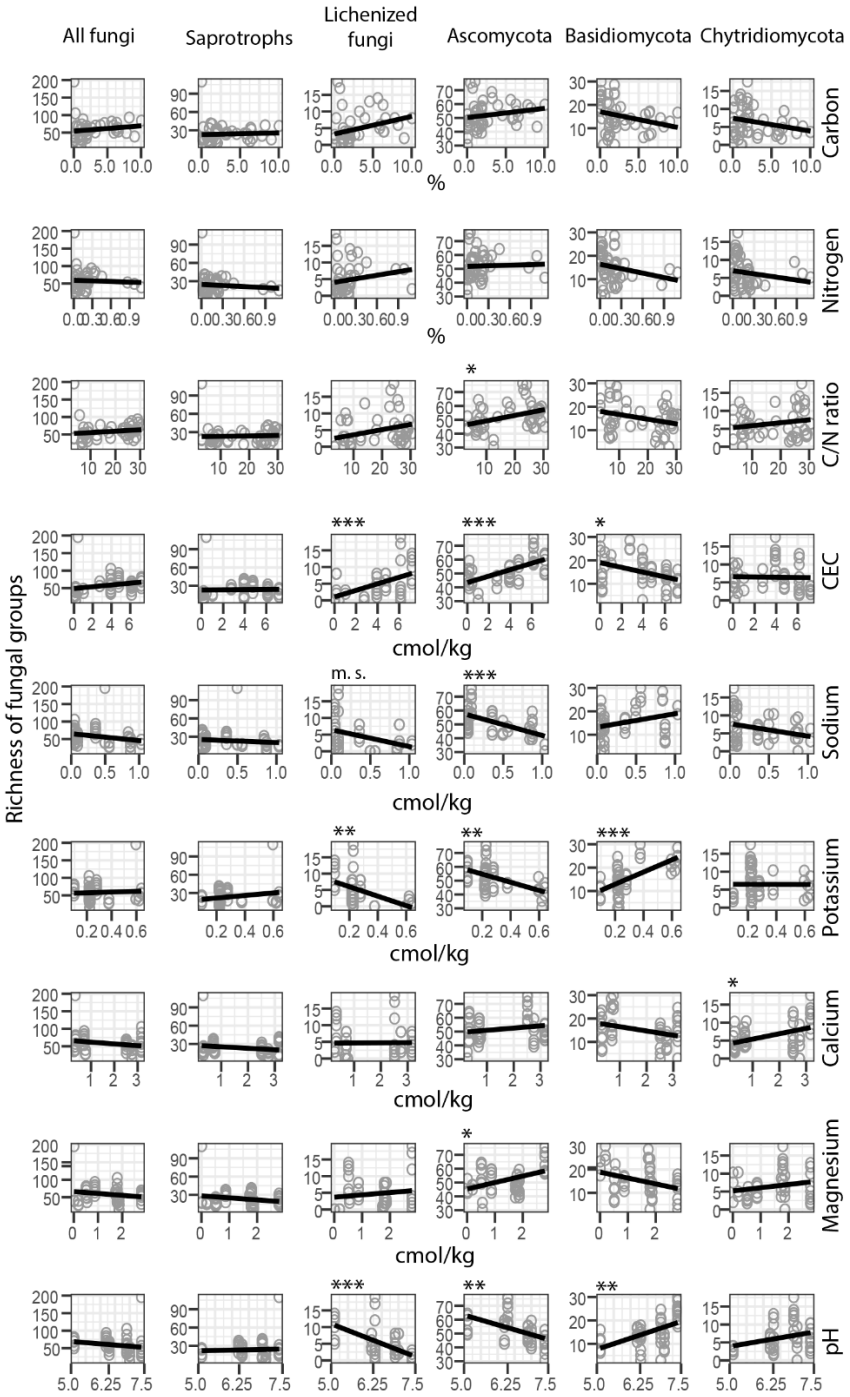
Regarding the functionality of the communities, even if direct reports on saprotrophic fungi are not available for Antarctic soils, there have been a few studies on the influence of environmental factors on decomposition rates. One of these studies reported that pH and C/N ratio, mainly dependent on the quality of the substratum, affected the decomposition rates in two moss communities in maritime Antarctica (Davis, 1986). In our study, the saprotrophic community was mainly influenced by abiotic parameters, particularly soil pH, followed by CEC, exchangeable cations and soil texture, while C/N ratio, although significant, was not

independent from other parameters in determining community composition. Globally, saprotrophic fungal community composition is strongly influenced by pH, and richness by Ca^{2+} content (Tedersoo *et al.*, 2014), while in our dataset Ca content only correlated with community composition and not with richness.

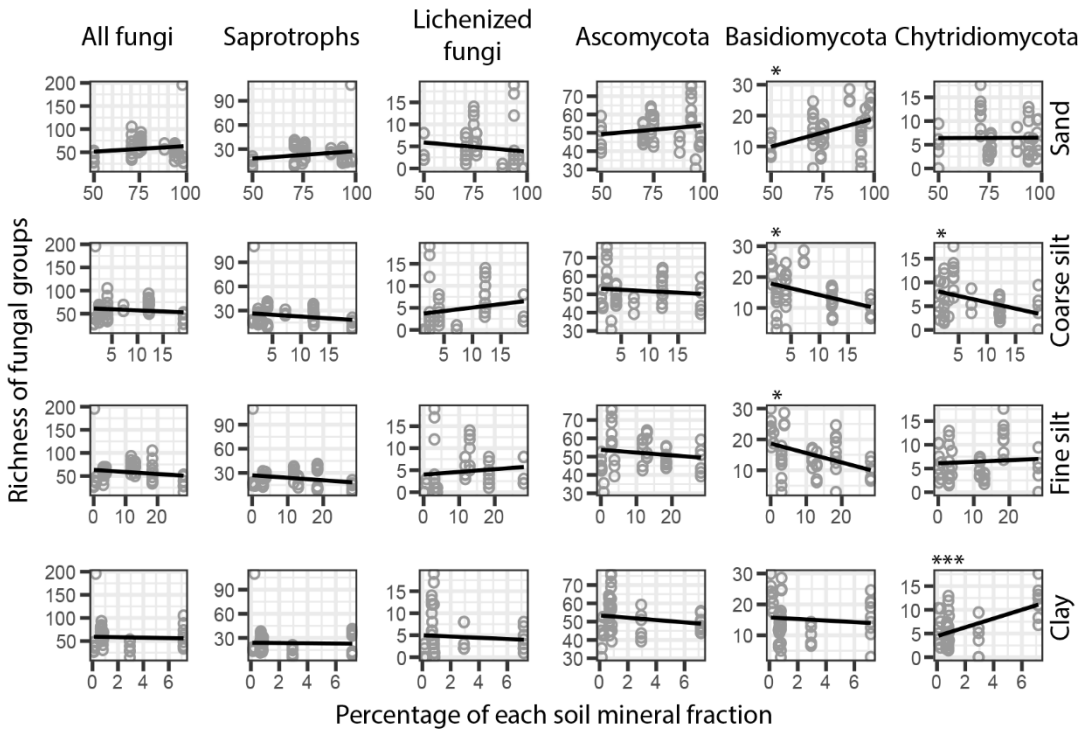
For lichenized fungi, Mg^{2+} and K^{+} were the main cations driving the community composition, in agreement with previous studies (Kranner *et al.*, 2008). In fact, taking into account the well-known drought tolerance of lichens, it has been reported that the recovery of activity after rewetting depends on the quality of the habitat, and is mainly due to the loss of K^{+} and Mg^{2+} during desiccation (Kranner *et al.*, 2008). We hypothesize that in Antarctica, where lichens have to cope with a combination of fluctuating conditions of water availability and low CEC, K^{+} and Mg^{2+} cations availability may be further limiting factors for their development. Additionally, one further factor connected with the community composition of lichenized fungi is soil C content, that also has a positive correlation with richness. This may be connected with primary production capacity of lichens, that also affects the quality of the soil OM, resulting in a positive correlation between C/N ratio and lichenized fungal richness and, to a lesser extent, their abundance.

This study is the first comprehensive taxonomic and functional characterization of fungal communities in the continental Antarctica biological soil crust system. Although the richness values reported here are substantially lower than those reported from soils in various biomes, including the High Arctic, using the same DNA metabarcoding methodology (e.g., Geml *et al.*, 2012; Tedersoo *et al.*, 2014; Timling *et al.*, 2014; Grau *et al.*, 2017; Bahram *et al.*, 2018; Canini *et al.*, 2019; Geml, 2019), they are nonetheless impressive when considering the very thin soil layer, the harshness of the environmental conditions and the paucity of vegetation and other eukaryotes (Zucconi *et al.*, 2016). Our study provides further insights into the effects of abiotic factors on fungal diversity and community composition, highlighting a stronger effect of pH and the presence of exchangeable cations, namely K^{+} and Mg^{2+}, as driving factors. This baseline characterization is of particular relevance also in the context of global warming, as temperature increase is expected to affect fungal community assemblages, mainly due to increases in the amount of available water that will affect soil conditions and to the possible invasion of more competitive alien species (Turner et al., 2003).

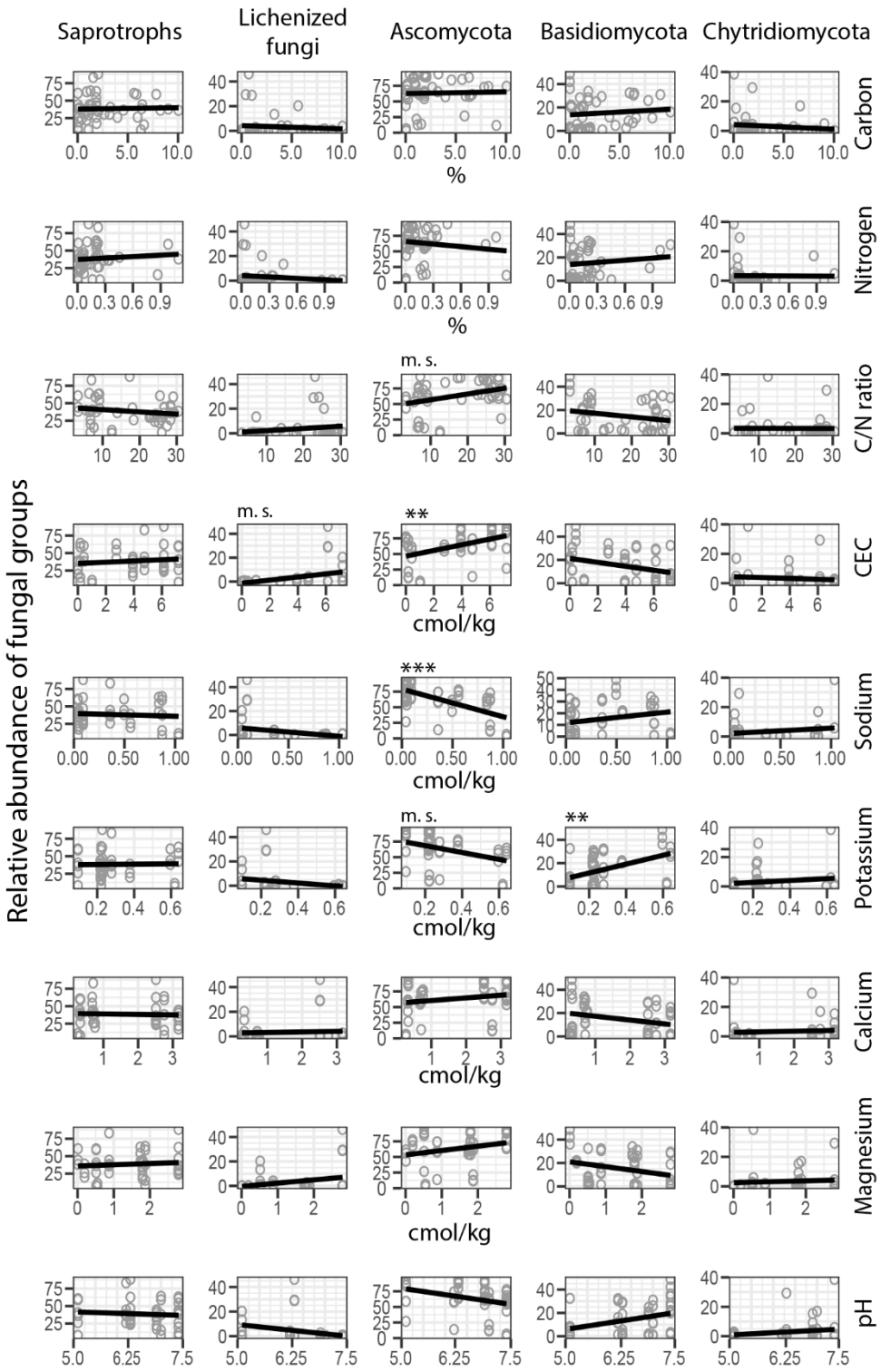
Supplementary Material



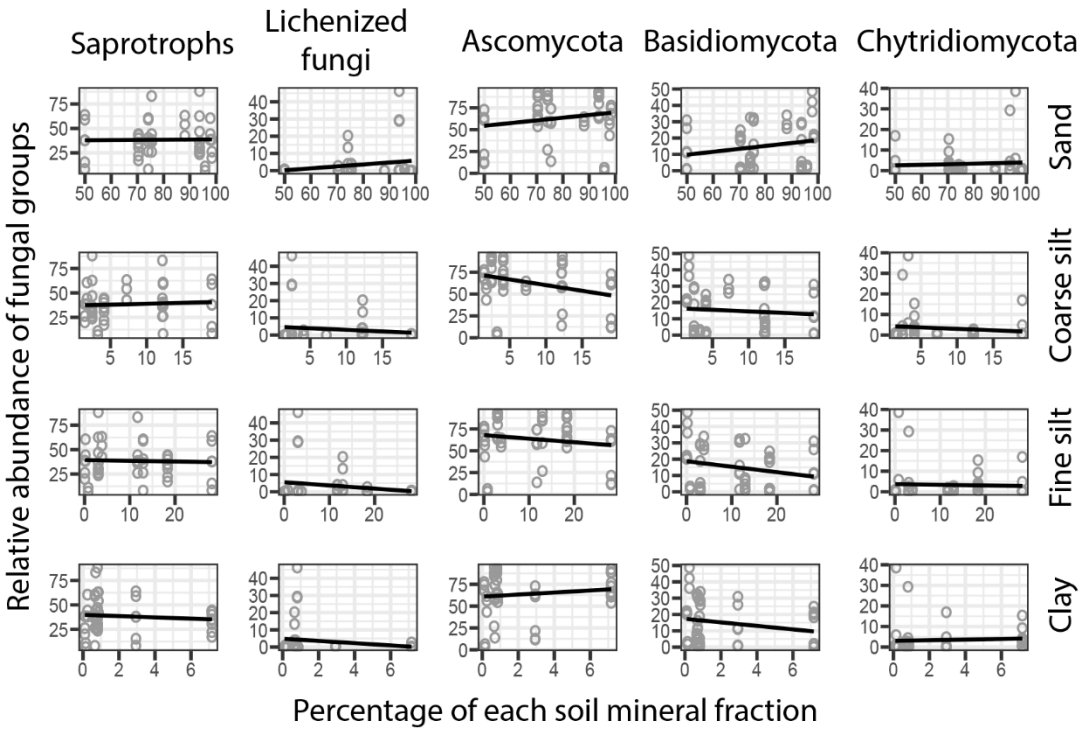
Supplementary figure 5.1: Linear regression plots for the variation of richness (y-axis) of the total fungal community, the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla (for the phyla the relative richness has been plotted) in response to soil chemical parameters: C, N, C/N ratio, pH, CEC, exchangeable Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} (x-axis). The significance of the regressions is indicated as *** p<0.001, ** p<0.01, * p<0.05, m. s. (marginally significant) p<0.1.



Supplementary figure 5.2: Linear regression plots for the variation of richness (y-axis) of the total fungal community, the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla (for the phyla the relative richness has been plotted) in response to soil particles size distribution, reported as relative content of sand, coarse silt, fine silt, and clay (x-axis). The significance of the regressions is indicated as *** $p<0.001$, * $p<0.05$.



Supplementary figure 5.3: Linear regression plots for the variation of the relative abundance (y-axis) of the two functional groups of saprotrophic and lichenized fungi and the three dominant phyla, in response to soil chemical parameters: C, N, C/N ratio, pH, CEC, exchangeable Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} (x-axis). The significance of the regressions is indicated as *** $p<0.001$, ** $p<0.01$, m. s. (marginally significant) $p<0.1$.



Supplementary figure 5.4: Linear regression plots for the variation of the relative abundance (y-axis) of the two functional groups of saprotrophic and lichenized fungi and the three dominant phyla, in response to soil particles size distribution, reported as relative content of sand, coarse silt, fine silt, and clay (x-axis).

Supplementary table 5.1: Coordinates, altitude and description of 17 sampling sites in 6 different localities

Sampling site	Latitude	Longitude	Altitude (m a.s.l.)	Description
Apostrophe Island site 1	73°31'9.5"S	167°25'55.3"E	41	Grey crust with few mosses propagules
Apostrophe Island site 2	73°31'10.5"S	167°25'55.3"E	41	Brown thin crust with mosses and lichens
Apostrophe Island site 3	73°31'14.2"S	167°25'56.0"E	37	Low developed brown crust
Cape King site 1	73°35'08.2"S	166°37'19.2"E	144	Black highly developed crust with mosses
Cape King site 2	73°35'08.9"S	166°37'3.5"E	124	Dry thin and black crust with very few mosses
Cape King site 3	73°35'08.5"S	166°37'9.0"E	100	Highly developed crust dominated by mosses, with lichens
Kay Island site 1	74°04'12.6"S	165°18'59.5"E	190	Low developed crust, with some mosses
Kay Island site 2	74°04'11.8"S	165°18'58.7"E	61	More developed crust, with more mosses
Edmonson Point site 1	74°19'45.0"S	165°07'35.0"E	31	At Edmonson Point 4 sites have been selected, with four increasing degrees of crust development, starting from a gravelled soil with any apparent colonization in the site 1, to an highly developed crust completely covered by mosses
Edmonson Point site 2	74°19'45.0"S	165°07'39.0"E	30	
Edmonson Point site 3	74°19'45.0"S	165°07'40.0"E	30	
Edmonson Point site 4	74°19'44.8"S	165°07'42.0"E	29	
Prior Island site 1	73°40'52.9"S	162°53'38.3"E	102	Grey, thin and low developed crust
Prior Island site 2	73°40'54.5"S	162°53'45.8"E	98	Wet highly developed crust dominated by mosses
Botany Bay site 1	77°00'26.0"S	162°32'39.4"E	115	Black thin crust disconnected from the below soil
Botany Bay site 2	77°00'26.7"S	162°32'41.5"E	94	Black, highly developed crust
Botany Bay site 3	77°00'22.9"S	162°32'30.4"E	69	Brown thin crust with white efflorescence

Supplementary table 5.2: Richness of fungal groups considered in the analyses. Values obtained after the rarefaction of the dataset. For the phyla relative richness (%) is reported.

Samples	All fungi	Saprotrophs	Lichenized fungi	Ascomycota	Basidiomycota	Chytridiomycota
Edmonson Point site 1 sample 2	36	14	3	30.55	22.22	2. 78
Edmonson Point site 1 sample 3	48	16	2	35.42	18.75	6.25
Edmonson Point site 2 sample 1	40	17	0	42.50	17.50	7.50
Edmonson Point site 2 sample 2	39	18	0	43.59	20.51	10.26
Edmonson Point site 2 sample 3	196	110	0	52.55	23.47	2.04
Edmonson Point site 3 sample 1	29	15	0	48.28	24.14	10.34
Edmonson Point site 3 sample 2	40	18	0	45.00	30.00	5.00
Edmonson Point site 3 sample 3	27	15	0	48.15	25.93	3.70
Edmonson Point site 4 sample 1	69	31	1	44.93	24.64	8.70
Edmonson Point site 4 sample 2	56	24	1	39.29	28.57	5.36
Edmonson Point site 4 sample 3	56	26	0	48.21	25.00	3.57
Apostrophe Island site 1 sample 1	70	26	10	64.29	11.43	2.86
Apostrophe Island site 1 sample 2	69	24	5	49.27	15.94	2.90
Apostrophe Island site 1 sample 3	77	24	13	51.95	11.69	3.90
Apostrophe Island site 2 sample 1	57	12	12	59.65	7.02	1.75
Apostrophe Island site 2 sample 2	81	25	14	62.96	6.17	2.47
Apostrophe Island site 2 sample 3	50	16	6	50.00	12.00	4.00
Apostrophe Island site 3 sample 1	65	19	8	44.61	12.31	6.15
Apostrophe Island site 3 sample 2	83	33	8	45.78	20.48	4.82
Apostrophe Island site 3 sample 3	57	21	10	54.39	12.28	3.51
Kay Island site 1 sample 1	54	18	3	42.59	12.96	7.41
Kay Island site 1 sample 2	78	34	4	44.87	16.67	6.41
Kay Island site 1 sample 3	71	28	8	59.15	14.08	7.04
Kay Island site 2 sample 1	84	37	8	59.52	16.67	3.57
Kay Island site 2 sample 2	64	31	4	54.69	17.19	4.69

Kay Island site 2 sample 3	93	38	6	56.99	10.75	3.23
Prior Island site 1 sample 1	15	6	2	53.33	6.67	0.00
Prior Island site 1 sample 2	28	8	3	39.29	7.14	3.57
Prior Island site 1 sample 3	29	10	2	41.38	10.34	0.00
Prior Island site 2 sample 1	39	15	2	43.59	12.82	5.13
Prior Island site 2 sample 2	53	17	8	50.94	7.55	9.43
Prior Island site 2 sample 3	49	21	8	59.18	14.29	6.12
Cape King site 1 sample 1	31	14	0	58.06	16.13	6.45
Cape King site 1 sample 2	51	23	3	68.63	13.73	1.96
Cape King site 1 sample 3	56	32	2	62.50	17.86	3.57
Cape King site 2 sample 1	42	16	1	57.14	11.90	11.90
Cape King site 2 sample 2	62	26	3	56.45	12.90	12.90
Cape King site 2 sample 3	60	28	4	58.33	15.00	10.00
Cape King site 3 sample 1	59	13	12	76.27	5.08	5.08
Cape King site 3 sample 2	69	15	19	75.36	2.90	2.90
Cape King site 3 sample 3	67	19	17	71.64	7.46	1.49
Botany Bay site 1 sample 1	105	35	8	46.67	14.29	12.38
Botany Bay site 1 sample 2	48	9	7	45.83	10.42	8.33
Botany Bay site 1 sample 3	40	10	5	55.00	12.50	12.50
Botany Bay site 2 sample 1	38	13	2	50.00	21.05	13.16
Botany Bay site 2 sample 2	34	11	2	44.12	2.94	17.65
Botany Bay site 2 sample 3	88	40	4	55.68	15.91	6.82
Botany Bay site 3 sample 1	57	33	2	50.88	24.56	14.04
Botany Bay site 3 sample 2	73	41	1	43.84	20.55	10.96
Botany Bay site 3 sample 3	63	37	3	47.62	19.05	11.11

Supplementary table 5.3: Soil physicochemical parameters. Bold values have been obtained as mean of the other two values available for the same plot.

Samples	N (%)	C (%)	C/N ratio	pH	CEC (cmol/kg)	Na^+ (cmol/kg)	K^+ (cmol/kg)	Mg^{2+} (cmol/kg)	Ca^{2+} (cmol/kg)	Sand (%)	Coarse silt (%)	Fine silt (%)	Clay (%)
Edmonson Point site 1 sample 2	0.004	0.05	12.5	7.4	1.01	1.03	0.62	0.55	0.27	95.80	3.25	0.80	0.15
Edmonson Point site 1 sample 3													
Edmonson Point site 2 sample 1	0.009	0.03	3.33		0.42	0.50	0.60	0.01	0.32	97.79	1.76	0.20	0.25
Edmonson Point site 2 sample 2													
Edmonson Point site 2 sample 3													
Edmonson Point site 3 sample 1	0.021	0.15	6.74		0	0.56	0.38	0.19	0.76	98.39	1.46	0.05	0.10
Edmonson Point site 3 sample 2													
Edmonson Point site 3 sample 3													
Edmonson Point site 4 sample 1	0.204	1.86	9.12		2.75	0.84	0.64	1.74	0.68	88.08	7.24	3.82	0.86
Edmonson Point site 4 sample 2													
Edmonson Point site 4 sample 3													
Apostrophe Island site 1 sample 1	0.45	3.26	7.20	5.1	7.19	0.04	0.09	0.51	0.33	74.14	12.26	12.95	0.65
Apostrophe Island site 1 sample 2	0.20	5.87	29.06										
Apostrophe Island site 1 sample 3	**0.33**	**4.56**	13.94										
Apostrophe Island site 2 sample 1	0.24	6.31	25.97										
Apostrophe Island site 2 sample 2	0.22	5.63	25.47										
Apostrophe Island site 2 sample 3	**0.23**	**5.97**	25.73										
Apostrophe Island site 3 sample 1													
Apostrophe Island site 3 sample 2													
Apostrophe Island site 3 sample 3													
Kay Island site 1 sample 1	0.22	1.55	6.98	6.2	4.75	0.36	0.28	0.87	0.71	75.41	12.17	11.72	0.7
Kay Island site 1 sample 2	0.21	6.37	30.40										
Kay Island site 1 sample 3	**0.215**	**3.96**	18.37										
Kay Island site 2 sample 1	0.34	10.05	29.30										
Kay Island site 2 sample 2	0.23	6.44	28.37										

Kay Island site 2 sample 3	**0.285**	**8.24**	28.93										
Prior Island site 1 sample 1	0.16	1.08	6.71	7.0	0.17	0.87	0.22	1.87	2.75	49.92	19	28.15	2.93
Prior Island site 1 sample 2	0.20	1.29	6.35										
Prior Island site 1 sample 3	**0.18**	**1.18**	6.51										
Prior Island site 2 sample 1	1.09	9.04	8.27										
Prior Island site 2 sample 2	0.87	6.62	7.63										
Prior Island site 2 sample 3	**0.98**	**7.83**	7.98										
Cape King site 1 sample 1	0.10	0.91	8.83	6.3	6.14	0.09	0.23	2.79	2.51	93.69	2.47	3.03	0.81
Cape King site 1 sample 2	0.13	3.19	23.81										
Cape King site 1 sample 3	**0.12**	**2.05**	17.30										
Cape King site 2 sample 1	0.066	1.87	28.33										
Cape King site 2 sample 2													
Cape King site 2 sample 3													
Cape King site 3 sample 1	0.045	1.02	22.67										
Cape King site 3 sample 2	0.017	0.41	24.12										
Cape King site 3 sample 3	**0.031**	**0.715**	23.06										
Botany Bay site 1 sample 1	0.043	0.24	5.58	6.9	3.92	0.05	0.22	1.79	3.16	70.38	4.1	18.36	7.16
Botany Bay site 1 sample 2	0.085	2.07	24.35										
Botany Bay site 1 sample 3	**0.064**	**1.155**	18.05										
Botany Bay site 2 sample 1	0.035	0.88	25.14										
Botany Bay site 2 sample 2	0.069	1.89	27.39										
Botany Bay site 2 sample 3	**0.052**	**1.385**	26.63										
Botany Bay site 3 sample 1	0.038	1.01	26.58										
Botany Bay site 3 sample 2	**0.070**	**1.835**	26.03										
Botany Bay site 3 sample 3	0.103	2.66	25.82										

Supplementary table 5.4. Regression r^2 and significance of each variable fitted in the NMDS analyses (figure 5.4). Significant values in bold.

	All fungi		**Saprotrophs**		**Lichenized fungi**		**Ascomycota**		**Basidiomycota**	
Variable	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*
C	0.0681	0.218	0.1188	0.073	**0.1990**	**0.026**	0.0411	0.399	0.0955	0.128
N	0.0796	0.164	0.0758	0.184	0.0404	0.480	0.0135	0.716	0.1328	0.054
C/N ratio	0.0298	0.508	0.0890	0.129	0.1296	0.086	0.0189	0.661	**0.1262**	**0.049**
CEC	**0.1950**	**0.011**	**0.2090**	**0.009**	**0.1957**	**0.012**	**0.1583**	**0.021**	**0.1681**	**0.014**
Na^+	**0.1987**	**0.010**	0.0523	0.315	**0.2774**	**0.003**	0.0790	0.149	**0.1883**	**0.009**
K^+	0.1208	0.073	**0.1800**	**0.012**	**0.5074**	**0.001**	0.0968	0.092	**0.1682**	**0.020**
Ca^{2+}	0.0409	0.412	0.0786	0.182	0.0611	0.328	0.0386	0.433	**0.1559**	**0.023**
Mg^{2+}	0.0326	0.488	0.0052	0.881	**0.3628**	**0.001**	0.0113	0.802	**0.1784**	**0.012**
pH	**0.2688**	**0.001**	**0.3370**	**0.001**	**0.2904**	**0.003**	**0.2539**	**0.003**	**0.2610**	**0.002**
Sand	**0.1944**	**0.008**	0.0290	0.531	0.0934	0.175	0.1023	0.102	0.0275	0.567
Coarse silt	**0.3558**	**0.002**	0.1022	0.104	0.0318	0.554	**0.1255**	**0.050**	**0.2343**	**0.003**
Fine silt	**0.1668**	**0.015**	0.0384	0.428	0.0919	0.186	0.1017	0.102	0.0135	0.760
Clay	**0.1741**	**0.014**	**0.2863**	**0.002**	0.0788	0.232	**0.1672**	**0.015**	**0.3013**	**0.002**
Latitude	**0.3234**	**0.001**	**0.4081**	**0.001**	**0.1669**	**0.041**	**0.2330**	**0.004**	**0.5053**	**0.001**

Chapter 6

Conclusions and future perspectives

Polar environments represent unique ecosystems, and microbes adapted to these extreme conditions are optimal models for studying the environmental factors driving community's survival, resistance and resilience. With this work I aimed to describe the soil microbial diversity associated to different polar environments, still poorly studied, and to describe the effect of some deterministic parameters affecting the composition and the potential functionality of these communities, first to give a baseline description of their diversity and then to try to give some clues on the potential effect of the changes of environmental conditions, even in view of the ongoing climate changes particularly affecting these areas.

Regarding the Arctic, given the increasing interest for this region for the pressing effects of global warming (Bevis *et al.*, 2019), among which the well documented expansion of shrub vegetation is reducing other terrestrial habitats, as those dominated by mosses and lichens (Vowles and Björk, 2019), I analysed both the fungal and bacterial (chapters 2 and 3, respectively) communities associated to three different soil habitats, representative of a plant coverage gradient, in Western Greenland area, where these phenomena have been widely described (Hollesen *et al.*, 2015), but information regarding the contribute of associated microbial communities are completely lacking. While for fungi I could highlight an increase of diversity connected with the increasing complexity of the coverage, the same was not true for bacteria; for both the compartments I found that the main factor determining communities composition was the type of coverage and I could highlight an higher number of unique indicator species associated to the less complex environment, letting hypothesize a possible local loss of diversity connected to the expansion of shrubs at the expense of other habitats, as already speculated in similar studies (Grau *et al.*, 2017). Also, regarding the fungal functional guilds, the type of habitat was the main determinant. For bacteria I found a higher proportion of oligotrophic taxa associated to bare grounds, while vascular vegetation dominated soils had a higher abundance of copiotrophic species. In parallel, for fungi I found an higher abundance of saprotrophic fungi in fully vegetated plots. These data are particularly alarming, given the Arctic vast carbon stocks

that will be exposed to microbial degradation, following to deglaciation (Crowther *et al.*, 2016). In fact, shrub encroachment, especially of deciduous species, such as *Betula nana*, may produce labile compounds from leaf litter poorly resistant to decomposition, that may promote the spreading of microorganisms with enhanced decomposition abilities for old stocks of organic matter (Walker *et al.*, 2015). This is in line with the evidence that the quality of substratum, in terms of C/N ratio, was one of the main edaphic determinants for both the compartments. Among other edaphic parameters, pH was one of the main predictors of the composition of both bacterial and fungal communities. This parameter has been diffusely reported as determinant for both fungi and bacteria in the Arctic (see for example Chu *et al.*, 2010; 2011; Männistö *et al.*, 2007; Timling *et al.*, 2014), but soil pH is also influenced by water content and the type of vegetation and on the other hand it can influence the availability of many nutrient and cations fundamental for life (Lauber *et al.*, 2009). Finally, not only the type of coverage, but also the plant composition has been highlighted to be determinant for the composition of the belowground communities, suggesting that not only the expansion of shrubs, but also the type of vegetation coverage, as for example the different balance of deciduous and evergreen species, has to be taken into account in the frame of a global warming.

In Antarctica I took into account two different microbial ecosystems from the area of Victoria Land, both still scarcely studied and for which the relative importance of environmental gradients in shaping communities remains unexplored. For Antarctica as well, it is extremely important to give a baseline description of existing microbiomes, in order to monitor any possible fluctuation due to global changes. Then, the evaluation of the parameters allowing the survival of microbial species, resulting particularly facilitated due to the extremely simplified composition of the communities examined, may shed light on their astonishing adaptations to this extreme environment.

First, I examined how the bacterial component of lichen-dominated cryptoendolithic communities exposed to different degrees of environmental pressures in gradients from 1.000 to 3.300 m a.s.l. and from 29 to 96 km distance from sea are affected by these two parameters, as well as by the different composition of fungal communities in the same samples (Coleine *et al.*, 2018a) (chapter 4). I found that the establishment and development of bacteria in these communities are not influenced by sea distance, but altitude explained a high proportion of variation, suggesting that the combination of UV irradiance and temperature regimes may impact these ecosystems and should be taken into account in future studies. Additionally, I found a strong correlation between fungal and bacterial compartments, as expected in such highly adapted and self-standing ecosystems.

Finally, I studied the composition of free-living and symbiotic fungi associated to biological soil crusts spreading during the austral summer along the coasts of Victoria Land, never studied

before, in a latitudinal gradient from 73 to 77 °S. I found, as expected, a low biodiversity for these communities and lot of sequences impossible to be identified. I found that the geographical distance, even being significant for the community composition, had a limited effect when compared to the soil physicochemical characteristics. Among the parameters tested, the abiotic variables resulted to be the main determinants of community composition, in particular potassium and magnesium soils content explained the highest proportion of variation, maybe due to the fundamental role of these cations in the resistance to desiccation of many organisms, as lichens, dominant in these landscapes (Kranner *et al.*, 2008). Also soil pH and cation exchange capacity had a significant impact on these communities. Given that cation exchange capacity varies due to soil texture, pH, organic matter content and nature of substrate (Brady and Weil, 2002), in such limiting environments, even small changes in these parameters can dramatically affect nutrient retention and availability, influencing the colonization. Therefore, even minimal changes in the actual conditions can dramatically affect these ecosystems. Further studies are still needed to deeply characterize the soil food webs of these peculiar environments and to identify all the organisms involved. In this optic, the characterization of the bacterial counterpart of the same samples is ongoing.

All my studies highlighted the needing of future monitoring activities to characterize all the possible changes of these communities and the connected parameters in the next years. Additionally, a deeper characterization of the metabolic adaptations in both the environments is still lacking, making evident the needing of further studies based on approaches targeting the functionality of the communities, as metagenomics and metatranscriptomic studies, in order to determine specific functions that could be altered in response to variations in environmental parameters. In this perspective, in Antarctica, some sites have been selected for the installation of remote sensing stations, provided with air, soil and rock sensors, to provide information on the climatic variation influencing diversity and functionality of both rocks and soils communities that will be analysed with molecular techniques.

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