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

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Abstract:

Patterned ground (PG) is one of the most evident expressions of cryogenic processes affecting periglacial soils, where macroscopic, repeated variations in soil morphology seem to be associated with small-scale edaphic and vegetation gradients, potentially influencing also microbial communities. While for high latitude environments only few studies on PG microbiology are available, the alpine context, where PG features are rarer, is almost unexplored under this point of view. We followed a double approach, based on Denaturing Gradient Gel Electrophoresis (DGGE) and quantitative PCR (qPCR), in order to investigate microbial community composition and ~~to evaluate the~~ abundance of phylogenetic markers and functional genes (bacterial and archaeal *amo*A) within single PG features and among different sites from four areas in the Western Italian Alps, characterized by different lithotypes. Bacterial, archaeal and fungal community composition was quite homogeneous within single features, with more differences among samples collected from different lithologies. The abundance of phylogenetic and functional markers was uniform at different sites, except for the highest altitude one showing the lowest bacterial, archaeal and ammonia-oxidizing archaea abundance. Nevertheless, at a small-scale level, a concentric distribution of microbial markers was described within single features, paralleling soil chemical properties trends. These first results support the hypothesis that microbial ecology in alpine, periglacial ecosystems is driven by a complex series of environmental factors, such as lithology, altitude and cryogenic activity, acting simultaneously on community shaping both in terms of diversity and abundance.

Keywords:

Periglacial alpine soils; Cryoturbation; Lithology; Community structure; Microbial abundance

Introduction

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

Until now, several works recognized the presence of a strong interaction between soil processes and vegetation in patterned ground formation and functioning (D'Amico et al. 2015; Michaelson et al. 2012; Walker et al. 2008), but the effects on soil microbial properties are poorly known. Microorganisms are able to survive, grow and be metabolically active in very harsh conditions, such as subzero temperatures, presence of ice and freeze-thaw cycles (Margesin and Miteva 2011; Steven et al. 2006). Moreover, they play key roles in weathering processes, pedogenesis, biogeochemical cycling and plant colonization of permafrost soils and recently deglaciated areas, like glacier forefields (Bajerski and Wagner 2013; Jansson and Taş 2014; Nemergut et al. 2006). Considering the impact of microbial communities on soil

ecosystem properties, it is necessary to increase our comprehension of the role of microbial communities in a complex and dynamic pedo-environment like patterned ground.

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

While patterned ground landscapes are extensively distributed in Arctic and subarctic regions, in mid-latitude mountain ranges their diffusion is limited to few areas characterized by flat surfaces, rapid snow removal by wind and high water availability (Bockheim and Munroe 2014). For this reason, the identification of sites suitable for the study of the combined action of climatic conditions and cryoturbation disturbances on soil microbial communities is rather complex. On the other hand, the presence of the same phenomenon replicated in sites geographically close to each other gives the opportunity to investigate the influence of other environmental drivers potentially involved in communities and ecosystem modelling, like altitude, parent material lithology and soil properties. The parent material

lithology, in particular, has a strong impact not only on the morphology of patterned ground features, due to the different resistance to ice-driven weathering, but also on soil chemical properties and plant colonization (Michaelson et al. 2008). This suggests that also the composition and distribution of microbial communities might be influenced by these properties, as already reported for other cold ecosystems (Boyd et al. 2007; Larouche et al. 2012; Nyyssönen et al. 2014; Reith et al. 2015).

With this study, we performed a preliminary investigation -the first, on our best knowledge- on microbial communities inhabiting patterned ground features in an alpine context, in terms of composition, overall diversity and abundance. We chose four active patterned ground sites in the North-Western Italian Alps, developed on different lithotypes creating large gradients in chemical soil properties such as available nutrients and heavy metal contents. Our hypothesis was that, as for chemical soil properties and plant distribution, cryoturbation should have an impact also on microbial population, both in terms of biomass distribution and community composition. The intensity of this influence should be modulated by site-specific edaphic properties linked to parent material lithology. This work had thus three main objectives: 1) to give a first insight in the microbial ecology of a fascinating and previously almost unexplored ecosystem; 2) to describe and compare microbial diversity and distribution both at a small-scale, within single PG features, and among different sites; 3) to define soil properties potentially involved in shaping microbial communities composition.

Materials and methods

Sample collection

For this study, four active patterned ground areas, located in the Western Italian Alps and dominated by stripes, sorted and nonsorted circles, were chosen. All areas were located in protected areas (Mont Avic Natural Park and Gran Paradiso National Park).

The different sites were characterized by different parent material (Table 1): calcschists (CS site), serpentinite and metamorphic gabbros respectively in SP and GB sites, and frost shattered gneiss at GN site. In CS and GB, the parent materials were enriched in small quantities of serpentinite derived from upslope areas.

For each site, one typical PG feature was examined in order to minimize the sampling impact on these ecosystems. Five surface samples (0-10 cm) were collected equally spaced along a north-south transect drawn across the circle/stripe. Hence, we obtained two external

samples, taken from the stony/vegetated rims (N and S), one central sample (C) and two intermediate samples (NC and SC), as shown in Fig. 1. A total of 20 samples was obtained.

Sampling took place in late September 2012. In that period, nighttime air temperatures were expected to drop below freezing point, enhancing freeze-thaw cycling; below the sampled depth, in fact, the soils were completely frozen. All the samples were collected in the early afternoon, stored at 4 °C in the field and during the transport and at – 20 °C in the laboratory prior to further analysis.

Climatic conditions of the study areas and morphological, mineralogical and textural characteristics of PG soils, as well as vegetation type and distribution, are described in detail in D'Amico et al. 2015. Soil chemical properties and percentage of vegetation cover, measured at each sampling point and reported in the same study, are summarized in Table 2.

Soil DNA extraction

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

PCR-DGGE

Polymerase chain reaction (PCR) products for denaturing gradient gel electrophoresis (DGGE) were obtained by amplifying total bacterial and archaeal 16S rRNA genes and fungal 26S rRNA genes. While bacterial and fungal genes were amplified directly from the extracted DNA, a nested approach was followed for Archaea. Primer pairs were 357F-GC and 518R ~~(Muyzer et al. 1993)~~ for bacteria (Muyzer et al. 1993), NL1 and LS2 ~~(O'Donnell 1993; Cocolin et al. 2000)~~ for fungi (O'Donnell 1993; Cocolin et al. 2000), A2F and 1492R ~~(Reysenbach et al. 1995; Lane 1991)~~ and SaF-GC and PARCH519R ~~(Nicol et al. 2003; Øvreås et al. 1997)~~ for the first and second step of archaeal PCR respectively (Reysenbach et al. 1995; Lane 1991; O'Donnell 1993; Cocolin et al. 2000). Primer sequences and reaction conditions are reported in Online Resource 2.

All PCR reactions were carried out in a DNAEngine® Peltier Thermal Cycler (Bio-Rad Laboratories) in a 25 µl reaction volume containing 1 x reaction buffer (Bioline), 3 mM

$MgCl_2$, 0.02 bovine serum albumin (BSA), 0.2 mM of each dNTP, 0.4 µM of each primer, 1.25 U of BIOTAQ™ DNA polymerase (Bioline) and 2 µl of soil DNA diluted 1:10 in sterile DNase-treated water (Sigma). Second steps of nested PCR were performed without BSA, using 1 µl of the first step product as template.

DGGE was carried out as previously described by Webster et al. (2006) using a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories), with a gradient from 30 to 60%. Electrophoresis was run at 200 V for 5 h (with an initial 10 min at 80 V) at 60°C in 1 x TAE buffer. Gels were stained for 30 min with SYBR® Gold nucleic acid gel stain (Invitrogen) and visualized under UV with an UVIpro Platinum Gel Documentation System (UVItec).

Reproducibility of DGGE profiles was tested by comparing PCR products obtained by using DNA extracted in triplicate from the same sample as template. Considering that good reproducibility was achieved, DGGE gels were organized in order to compare single samples within PG features and among different sites (Online Resource 1). DGGE bands recurrent at site level, or shared among different sites were excised, incubated one night at -20°C, washed and crushed in 10-20 µl of molecular-grade water. Supernatant (1 µl) was used as template and PCR was performed as above except for the elimination of BSA and the employment of modified linker-PCR archaeal and bacterial primers described in O'Sullivan (2008). PCR products were sequenced and searched for sequence similarities in the National Center for Biotechnology Information database using nucleotide Basic Local Alignment Search Tool (BLASTn) analysis (Altschul 1990).

Obtained 16S bacterial rRNA gene sequences were submitted to the European Nucleotide Archive ~~EMBL database~~ (http://www.ebi.ac.uk/ena) under accession numbers. ~~Submission code was Hx2000054952.~~ LT613607-LT613635.

Quantitative PCR

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

For standard curves construction, the reference genes were amplified from genomic DNA extracted from pure cultures of standard organisms: *Lactococcus lactis* subsp. *cremoris* for bacterial 16S, *Methanococcoides methylutens* for archaeal 16S, *Saccharomyces cerevisiae* for eukaryotic 26S and *Nitrosomonas europaea* for bacterial *amoA*. PCR products were than

purified with the PCRExtract Mini Kit (5 Prime), in accordance with the manufacturer's instructions, quantified by NanoDrop and used to prepare serial dilutions in molecular-grade water.

Primer pairs used for standard preparation were 27F and 1492R ~~(Lane 1991)~~ for bacteria (Lane 1991), S-D-Arch-0025-a-S-17 and 1517R ~~(Vetriani et al. 1999)~~ for archaea (Vetriani et al. 1999), NL1 and LS2 ~~(O'Donnell 1993; Cocolin et al. 2000)~~ for fungi (O'Donnell 1993; Cocolin et al. 2000) and amoA-1F and amoA-2R ~~(Mc Tavish et al. 1993)~~ for bacterial *amo*A genes (Mc Tavish et al. 1993). Primer sequences and references for PCR conditions are reported in Online Resource 3, while master mix composition was as described above (excluding BSA).

Only for archaeal amoA gene, PCR products obtained by amplifying total DNA extracted from PG sample 2N with primer pair Arch-amoAF and Arch-amoAR (Francis et al. 2005) were pooled, purified, quantified by NanoDrop, serially diluted and used for standard curve construction.

qPCR reactions were performed using a Chromo4™ Real Time PCR Detection System (Bio-Rad Laboratories), and data were analysed with the MJ Opticon Monitor software (version 3.1). Primer pairs were the same as for standard preparation, except for Bacteria and Archaea. The first were substituted by the pair 519F and 907R (Lane 1991; Muyzer et al. 1995), the second by the pair S-D-Arch-0025-a-S-17 and S-D-Arch-0344-a-S-20 (Vetriani et al. 1999). The PCR mixture contained 0.3 µM of each primer, 10 µl of SsoAdvanced™ SYBR® Green Supermix and 2 µl of soil DNA diluted 1:10 - 1:100, in a total volume of 20 µl. Optimal DNA dilution was chosen in order to minimize inhibition problems linked to low A260/A230 ratio of extracted DNA. All the samples and the standards were analysed in triplicate on PCR strip tubes (Bio-Rad Laboratories) with the following thermal cycling conditions: 95° for 5 min, followed by 40 cycles of 95°C for 30 sec, annealing for 30 sec and 72°C for 1 min. Different annealing temperatures are reported in Online Resource 3. PCR specificity was verified by melting curves analysis. Standard curves R^2 value was always higher than 0.996, and all the reactions showed efficiencies higher than 70%.

Statistical analysis

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

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

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

Gradients in microbial community composition within the different patterned ground sites were observed using unconstrained ordination methods (NMDS, Kruskal, 1964, distance Bray-Curtis). The analysis was carried out with metaMDS within R vegan, using a Wisconsin double standardization and a maximum number of 100 runs to reach the best solution (two axis). To visualize relationships between microbial community composition and environmental parameters, the resulting NMDS biplot was interpreted using a post-hoc correlation with significant soil and environmental parameters (function envfit).

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

Results

Community structure (PCR-DGGE)

Bacterial DGGE profiles showed a quite homogeneous community composition within single PG features, with more differences among the four sites (Online Resource 1). A pool of ubiquitous, brightly stained bands was clearly recognizable beside several site-specific, often weaker bands. Sequence analysis of excised bands highlighted the presence of at least 7 different phyla (*Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Chloroflexi*), with all the ubiquitous phylotypes belonging to *Acidobacteria* or *Alphaproteobacteria* (Table 3). Only two of the detected bacterial phylotypes detected were closely related to described bacterial species (>97% sequence similarity), while the majority was related to uncultured bacteria previously found in soils or periglacial ecosystems. Cluster analysis confirmed the presence of distinct bacterial community composition at all the sites (Fig. 2).

Archaeal DGGE profiles appeared more heterogeneous compared to Bacteria at a small-scale level, both in terms of bands number and intensity, and few dominant bands recurred in samples collected from different sites (Online Resource 1). Archaeal community ~~structure~~composition in PG sites separated in different groups, as indicated by cluster analysis. Only at SP site a separation between centre and rims was detected (Fig. 2). All the sequences obtained from excised bands belonged to *Thaumarchaeota*, and showed 93-96% of sequence similarity with Candidatus *Nitrososphaera gargensis* or Candidatus *Nitrososphaera evergladensis*. Nearly all the phylotypes were closely related to uncultured Archaea previously detected in high-altitude soils (as reported in Table 4) but also with DNA sequences retrieved from temperate agricultural and forest soils.

Our PCR-DGGE approach was able to detect only low fungal diversity. As for Bacteria, the main differences among profiles seemed to be linked to the site, rather than the position within PG features. Cluster analysis supported this interpretation, even if for GB and GN samples a clear separation has not been found. Sample 3N was excluded from the analysis due to the impossibility to obtain a clear DGGE profile (Fig. 2). Only few sequences were obtained from fungal excised bands, including bands recurrent at site level, or shared among different sites, all corresponding to *Ascomycota* or *Basidiomycota* (Table 5); interestingly, four of them were strictly related (97-98% similarity) to uncultured *Ascomycota* detected in PGs from North American Arctic by Timling et al. (2014).

Microbial abundance

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

Comparing samples from different lithologies, significant differences ($P<0.05$) were only found for DNA, bacterial and archaeal markers and for archaeal *amo*A genes (Fig. 3). In general, lower abundances were reported for GN samples (average of 9.10, 6.31 and 6.67 Log copies per g of dry soil for Bacteria, Archaea and AOA respectively), while CS, SP and GB showed similar values (average of 9.92-10.12, 6.69-7.57 and 7.21-7.87 Log copies per g of dry soil for Bacteria, Archaea and AOA respectively). Fungal marker abundance ranged from an average of 8.57 to 9.27 Log copies per g of dry soil, without significant differences among

sites. Neither for bacterial *amo*A genes abundance, ranging from an average of 4.34 to 5.59 Log copies per g of dry soil, significant differences were detected. Comparing only functional genes, a predominance of archaeal over bacterial *amo*A genes, with AOA/AOB Log copies ratio ranging from 1.4 to 1.9, was detected in all the four sites.

At a small-scale level, a slightly concentric variation was reported for all the phylogenetic markers: the abundance of bacterial and archaeal genes decreasing from the rims toward the centre of single features in CS, GB and GN, and showing an opposite trend in SP; fungal markers reaching the highest values in intermediate positions (Fig. 4). Also DNA concentration followed a similar trend, decreasing from the rims to the centre in CS, GB and GN and from the centre to the rims in SP. No clear repeated patterns were described observing the distribution of AOA and AOB markers within single PG features. The only recognizable trends were a concentric decreasing of AOB in GB and AOA in GN or the north-to-south decreasing of AOB in GN and AOA in SP.

Discussion

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

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

microbial complexity, the more represented groups and evidenced the ecosystem properties possibly involved in shaping and influencing the community.

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

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

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

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

In general, analysis of bacterial, archaeal and fungal phylogenetic markers revealed a quite homogeneous community composition within single PGFs, without a clear separation between samples collected from the vegetated rims and the central, nearly bare soil portion. This is also coherent with the results that Timling et al. (2014) obtained for fungal phylogenetic markers investigating patterned grounds in the northernmost bioclimatic subzone of the North American Arctic Transect.

Differences in community composition were related more consistently to the sampling site than to the position across the PGFs. Also the NMDS analysis (Fig. 5), performed on the average band distribution of all the phylogenetic markers, supported this separation among sites, pointing out the main parameters (data from D'Amico et al. 2015) involved in site differentiation and, potentially, in shaping the composition of microbial community (Table 6). For instance, the GN site was located at the highest altitude, and was characterized by highest P content and exchangeable Ca/Mg ratio. Conversely, high levels of exchangeable Mg and Ni fitted with the SP community; CS sites were mainly characterized by high levels of Ca, while GB communities were correlated with intermediate levels of most soil parameters. This seems to suggest that in this mid-latitude, alpine context parent material lithology can be a strong driver for microbial community differentiation in terms of community composition, overcoming the effect of strong, small-scale gradients in edaphic properties produced by cryoturbation and patterned ground development. The importance of parent material lithology on composition of microbial communities has been described in a variety of different ecosystems, like soils (Reith et al. 2015), continental crystalline crust (Nyyssönen et al. 2014), pristine aquifers (Boyd et al. 2007) and arctic streams (Larouche et al. 2012). D'Amico et al. (2015), analysing the same study sites, reported a similar vegetation diversity pattern, with a lack of differentiation between rims and centres, and a strong separation of plant communities developed on different matrixes. Therefore, parent material lithology and the associated soil chemical properties, plant colonization and microbial community composition seem to be strictly linked.

Quantitative analysis, performed by qPCR, presents a different picture. In fact, microbial abundance resulted quite homogeneous among different sites. The only one showing

significantly lower abundances (in terms of Bacteria, Archaea, AOA markers and DNA) was GN site. This is probably linked to the lower temperatures, associated to highest altitude, which affects also total vegetation cover (D'Amico et al. 2015).

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

Finally, exploring more in detail the relationships existing between microbial abundances and soil chemical parameters (Table 7), Bacteria showed the highest number of significant correlations with different chemical properties (vegetation cover and contents of Ca, Mg, K, TN and TOC and C/N). On the other hand, Archaea abundances only correlated with the soil C/N, but correlated to all the microbial markers except for bacterial *amo*A. Similar situations have been previously reported for alpine forest soils (Siles and Margesin 2016) and for tundra soils (Blaud et al. 2015), with bacterial abundance following chemical soil properties trend, and archaeal abundance independent from them. However, in those cases, all the microbial markers resulted positively correlated to each other, suggesting an indirect action of environmental parameters on the whole microbial population. In our case we can hypothesize that,

despite the presence of a bacterial population apparently more sensitive to variations in substrate composition, Archaea seems to represent the link among the different microbial domains, and so the real keystone of the ecosystem. Moreover, considering that Thaumarchaeota seem to represent an important portion of archaeal community in this ecosystem, the low level of correlation with any environmental parameter but C/N, reported for both archaeal and AOA markers, could be linked to the wide ecophysiological potential of this group, including not only autotrophy but also mixotrophy and heterotrophy lifestyles (Pester et al. 2011, Prosser and Nicol 2012). Concerning fungal abundances, the only correlation with soil properties was found with C/N. Nevertheless, the presence of a unique intra-feature distribution in gene-abundance repeated in all the sites suggests the presence of other factors, not considered here but suitable for further investigations, driving fungal distribution, such as organic matter composition and quality.

Conclusions

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

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

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

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

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Figure captions

Fig. 1 Sampling scheme on PG features. A north-south transect was drawn across the circles (or the stripe, at site SP) and five surface samples (0-10 cm), equally spaced, were collected: one central (C), two external (N and S), taken from the stony/vegetated rims and two intermediate (NC and SC)

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

Fig. 3 DNA concentration and abundance of bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, archaeal and bacterial *amo*A genes in the four sites. Different letters indicate significant differences ($P < 0.05$) among sites according to Games Howell *post hoc* test

Fig. 4 Distribution of different biological markers within single PG features in the four sites: DNA concentration, bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, archaeal and bacterial *amo*A genes

Fig. 5 NMDS ordination of the four sites (1=CS; 2=SP; 3=GB; 4=GN), based on DGGE profiles (for each sampling point information obtained from archaeal, bacterial and fungal profiles were combined). Vectors show the direction and strength of environmental variables

Figure 1

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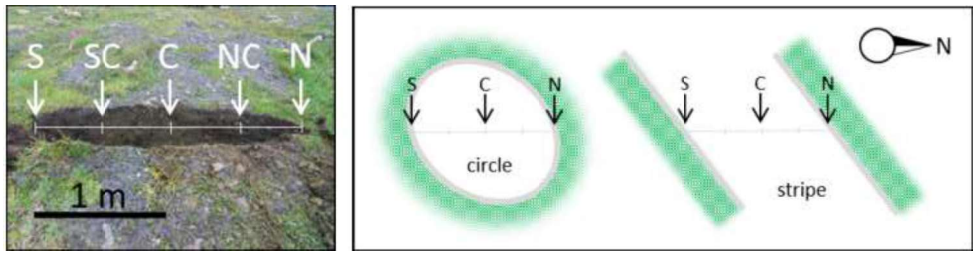

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Figure 2

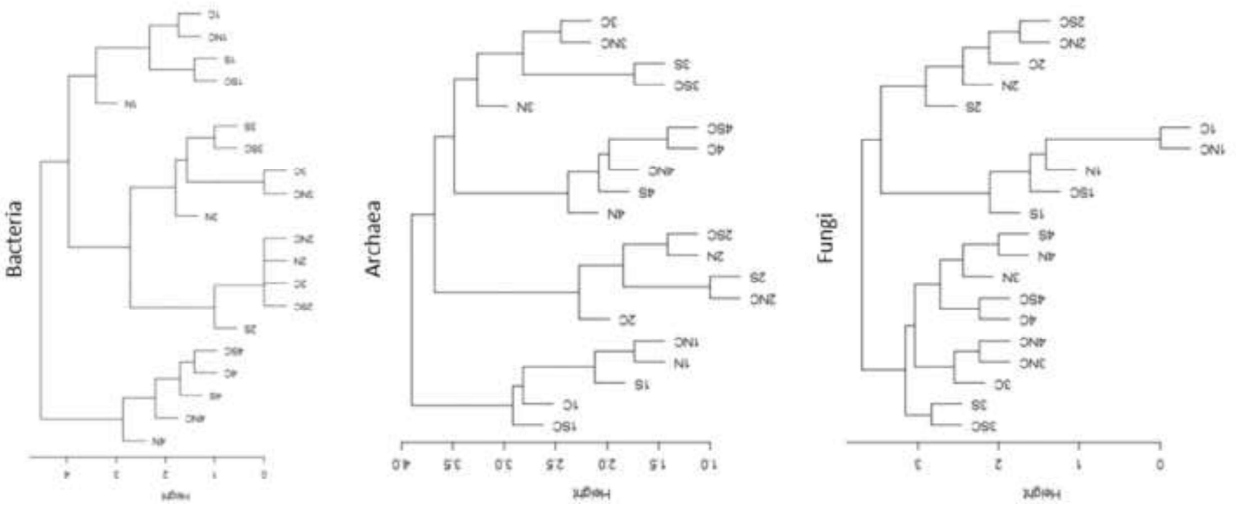

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Figure 3

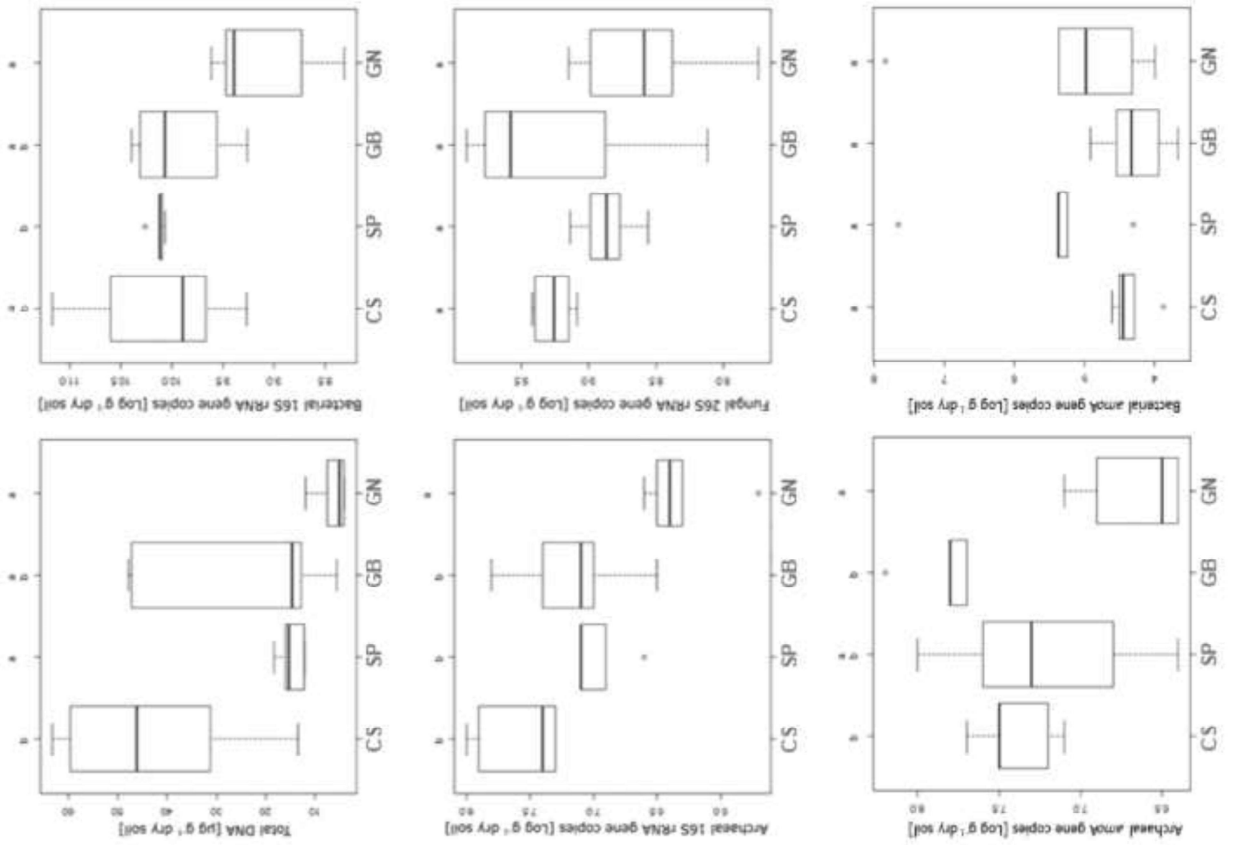

Figure 4

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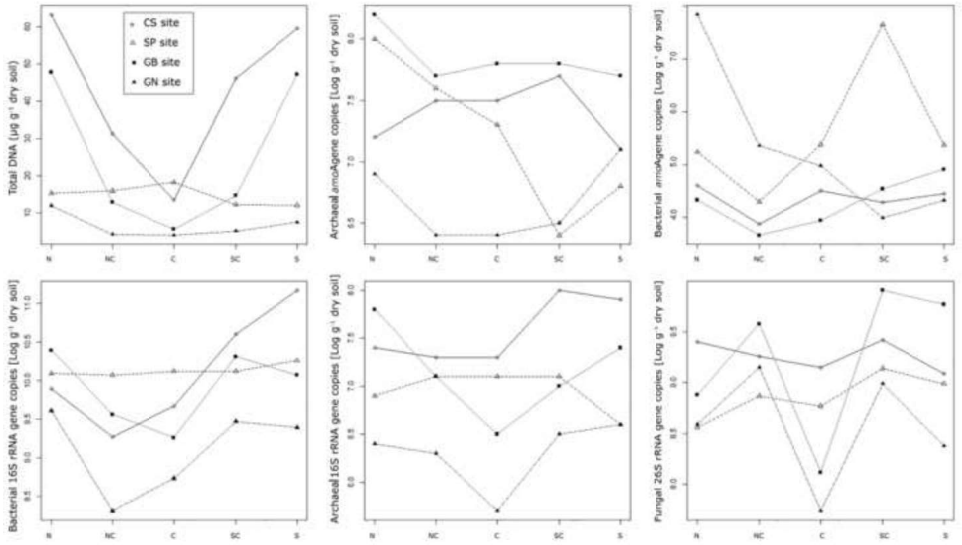

Figure 5

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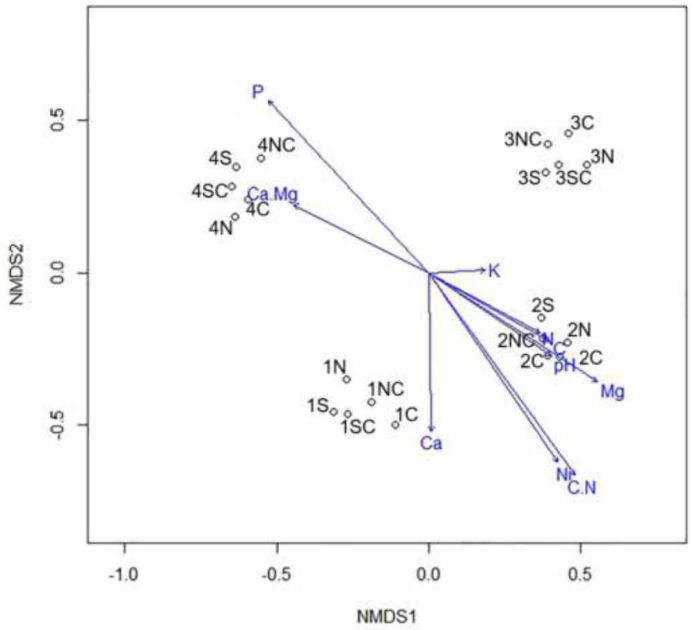


Table 1

Table 1 Localization and environmental properties of the study sites

	Site localization	Coordinates	Elevation (m a.s.l.)	Parent material	PG type	Dimensions (m)
1 - (CS)	Fenetre de Champorcher (Champorcher, AO)	45°35'57.41" 07°30'18.90"	2705	Calcschists (serpentinite in traces)	Nonsorted circles, hummocks	0.8/1.5
2 - (SP)	Colle di Raye Chevrere (Champdepraz, AO)	45°40'04.70" 07°32'31.32"	2710	Serpentinite	Sorted stripes	0.8/1.5-3/8
3 - (GB)	Lac des Heures (Champdepraz, AO)	45°29'36.14" 07°32'53.77"	2780	Gabbro (serpentinite in traces)	Sorted elongated circles	1.2/2.5
4 - (GN)	Piata Lazin (Ronco Canavese, TO)	45°29'21.74" 07°26'21.30"	3054	Gneiss	Sorted circles	0.8/2

Table 2

Table 2 ~~C~~Main chemical soil properties and plant cover distribution in the sampling sites (data from D'Amico et al. 2015)

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

Table 3

Table 3 Closest 16S rRNA gene sequence matches to excised bacterial DGGE bands using the NCBI BLASTN search tool

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest sequence match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs			
							CS	SP	GB	GN
B3	*Edaphobacter aggregans* (NR_043898)	forest soil	95	*Acidobacteria*	*Edaphobacter aggregans* (NR_043898)	95	x	x	x	x
B1	*Nitrobacter winogradskyi* (NR_074324)	-	100	*Alphaproteobacteria*	*Nitrobacter winogradskyi* (NR_074324)	100	x	x	x	x
B17	Uncultured bacterium clone KA13 (JQ973360)	forest soil	96	*Alphaproteobacteria*	*Microvirga guangxiensis* (NR_044563)	95	x	x	x	x
B5	Uncultured bacterium isolate DGGE gel band 02_F2 clone 01 (JX986102)	soil	99	*Alphaproteobacteria*	Phaeospirillum fulvum (NR_025836)	94	x	x	x	x
B2	Uncultured bacterium clone B8-80 (KF494605)	permafrost	99	*Alphaproteobacteria*	*Sinorhizobium fredii* (NR_102919)	97	x	x		x
B7	Uncultured SOIL BACTERIUM clone GC0AA4ZA03PP1 (JQ919779)	soil	96	*Bacteroidetes*	*Pedobacter borealis* (NR_044381)	88		x	x	
B16	Chloroflexi bacterium Ellin7237 (AY673403)	soil	86	*Chloroflexi*	Chloroflexi bacterium Ellin7237 (AY673403)	82				x
B4	*Arthrobacter ramosus* (KF387693)	Himalaya	90	*Actinobacteria*	*Arthrobacter ramosus* (KF387693)	90		x		
B15a	Bacterium PE03-55G21 (AB127830)	eutrophic lake	97	*Alphaproteobacteria*	*Filomicrobium insigne* (NR_044095)	95	x		x	
B24	Uncultured bacterium clone HF127 (KF037272)	soil	97	*Betaproteobacteria*	*Nitrosospira multiformis* (NR_074736)	93		x		
B15	Chroococcales cyanobacterium PE5G6 (HE805950)	lake water	86	*Cyanobacteria*	*Cyanobium gracile* (NR_102447)	85				x
B8, B9	*Pseudomonas antarctica* (NR_025586)	McMurdo Valley, Antarctica	100	*Gammaproteobacteria*	*Pseudomonas antarctica* (NR_025586)	100				x
B12, B13	Uncultured bacterium clone P1s-43 (GQ287601)	periglacial soil Himalaya	100	*Verrucomicrobia*	*Prosthecobacterfluviatilis* (NR_041608)	86	x			
B14	Uncultured bacterium isolate 111286933975 (HQ11871)	forest soil	95	*Verrucomicrobia*	Bacterium Ellin507 (AY960770)	90				x

Table 4

Table 4 Closest 16S rRNA gene sequence matches to excised archaeal DGGE bands using the NCBI BLASTN search tool

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

Table 5

Table 5 Closest 26S rRNA gene sequence matches to excised fungal DGGE bands using the NCBI BLASTN search tool

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

Table 6

Table 6 Correlation values and significance between the soil chemical properties and the NMDS factors shown in Fig.5

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

Table 7 Correlation analysis among chemical soil properties and DNA content or microbial abundances estimated by qPCR (reported Pearson's coefficient and significance level of the correlation)

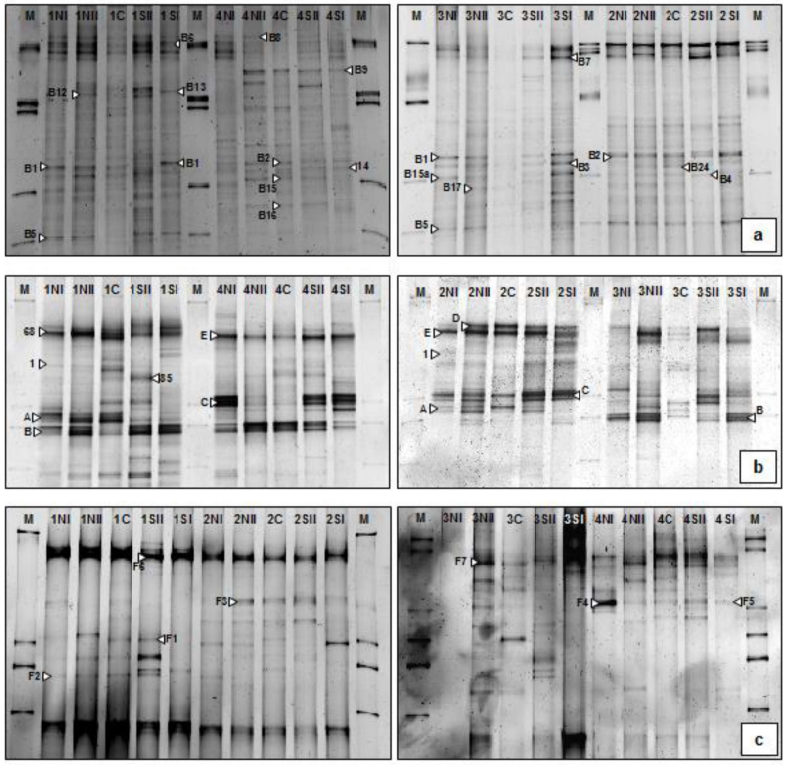
Variables	Bact	Arch	Fun	AOB	AOA	DNA
Plant coverage %	0.698**	0.666**	0.321	0.094	0.281	0.802**
pH	-0.167	-0.129	-0.396	-0.089	0.047	-0.187
Ca	0.588**	0.717**	0.368	-0.164	0.257	0.710**
Mg	0.478*	0.534*	0.362	0.060	0.275	0.594**
K	0.606**	0.349	0.343	0.224	-0.108	0.414
Ni	0.412	0.352	0.009	0.163	0.123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

*P < 0.05, **P < 0.01,

Online Resource 1

Comparison among sites and sampling points

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



(Biology and Fertility of Soils)
Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)
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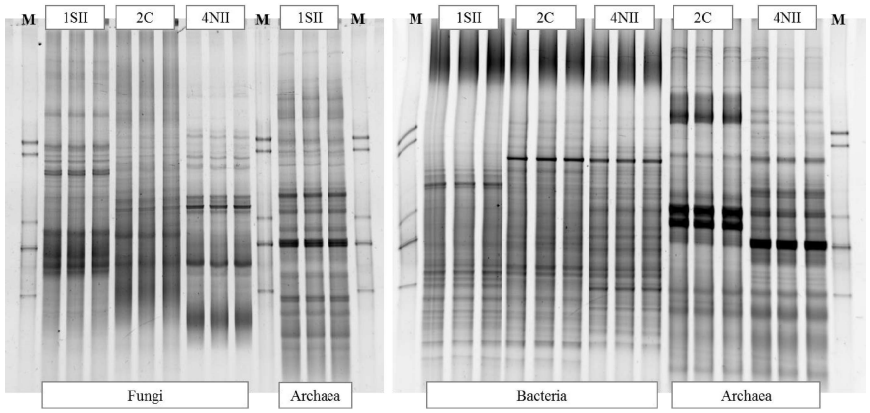
DGGE reproducibility test

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



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Online Resource 2

Primer sets and amplification details used for PCR-DGGE analysis

Primer pair	Sequence (5'-3')[a]	Target gene	Fragment lenght	Reference	Amplification details	Protocol reference
357F-GC[b]	CCT ACG GGA GGC AGC AG	*Bacteria* 16S rRNA	193	Muyzer et al. 1993	95 °C 5 min; 10 cycles: 94 °C 30 s, 55 °C 30 s, 72 °C 1 min; 25 cycles: 92 °C 30 s, 52 °C 30 s, 72 °C 1 min; 72 °C 10 min	Webster et al. 2006
518R	ATT ACC GCG GCT GCT GG					
A2F	TTC CGG TTG ATC CYG CCG GA	*Archaea* 16S rRNA (PCR step I)	c. 1500	Reysenbach et al. 1995	94 °C 5 min; 36 cycles: 95 °C 1 min, 50 °C 1 min, 72 °C 1 min; 72 °C 5 min	-
1492R	GGT TAC CTT GTT ACG ACT T			Lane 1991		
SaF-GC[b,c]	Sa1f-CCT AYG GGG CGC AGC AGG Sa2f-CCT ACG GGG CGC AGA GGG	*Archaea* 16S rRNA (PCR step II)	192	Nicol et al. 2003	95 °C 5 min; 5 cycles: 94 °C 30 s, 53.5 °C 30 s, 72 °C 1 min; 30 cycles: 92 °C 30 s, 53.5 °C 30 s, 72 °C 1 min; 72 °C 10 min	Nicol et al. 2003
PARCH519R	TTA CCG CGG CKG CTG			Øvreås et al. 1997		
NL1-GC[b]	GCC ATA TCA ATA AGC GGA GGA AAA G	Fungal 26S rRNA	c. 250	O'Donnell 1993	95 °C 5 min; 36 cycles: 95 °C 1 min, 52 °C 45 s, 72°C 1 min; 72 °C 7 min	Cocolin et al. 2000
LS2	ATT CCC AAA CAA CTC GAC TC			Cocolin et al. 2000		

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

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

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

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Electronic Supplementary Material 3

Online Resource 3

Primer sets and amplification details used for qPCR analysis

Primer pair	Sequence (5'-3')[a]	Target gene	Fragment length	Reference	Amplification details	Application
27F	AGA GTT TGA TCM TGG CTC AG	*Bacteria* 16S rRNA	c. 1500	Lane 1991		qPCR standard
1492R	GGT TAC CTT GTT ACG ACT T					
S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	*Archaea* 16S rRNA	c. 1500	Vetriani et al. 1999		qPCR standard
1517R						
NL1	GCC ATA TCA ATA AGC GGA GGA AAA G	Fungal 26S rRNA	c. 250	O'Donnell 1993	95 °C 5 min; 44 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
LS2	ATT CCC AAA CAA CTC GAC TC			Cocolin et al. 2000		
amoA-1F	GGG GTT TCT ACT GGT GGT	Bacterial *amo*A gene	491	Mc Tavish et al. 1993	94 °C 3 min; 40 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 45 s	qPCR standard, qPCR
amoA-2R	CCC CTC KGS AAA GCC TTC TTC					
Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Archaeal *amo*A gene	635	Francis et al. 2005	95 °C 30s; 40 cycles: 95 °C 30 s, 53 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
Arch-amoAR	GCG GCC ATC CAT CTG TAT GT					
519F	CCA GCA GCC GCG GTA AT AC	*Bacteria* 16S rRNA	c. 390	Lane 1991	95 °C 30 s; 40 cycles: 95 °C 30 s, 50 °C 30 s, 72 °C 1 min	qPCR
907R	CCG TCA ATT CMT TTR AGT TT			Muyzer et al. 1995		
S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	*Archaea* 16S rRNA	c. 320	Vetriani et al. 1999	95 °C 30 s; 40 cycles: 95 °C 30 s, 48 °C 30 s, 72 °C 1 min	qPCR
S-D-Arch-0344-a-S-20	TCG CGC CTG CTG CGC CCC GT					

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

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