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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 *amoA*) 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; Nemerut 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; Nyssönen 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):

caeschists (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/strip. Hence, we obtained two external

1 samples, taken from the stony/vegetated rims (N and S), one central sample (C) and two
2 intermediate samples (NC and SC), as shown in Fig. 1. A total of 20 samples was obtained.
3 Sampling took place in late September 2012. In that period, nighttime air temperatures
4 were expected to drop below freezing point, enhancing freeze-thaw cycling; below the
5 sampled depth, in fact, the soils were completely frozen. All the samples were collected in the
6 early afternoon, stored at 4 °C in the field and during the transport and at – 20 °C in the
7 laboratory prior to further analysis.

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

12

13 **Soil DNA extraction**

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

19

20 **PCR-DGGE**

21 Polymerase chain reaction (PCR) products for denaturing gradient gel electrophoresis
22 (DGGE) were obtained by amplifying total bacterial and archaeal 16S rRNA genes and fungal
23 26S rRNA genes. While bacterial and fungal genes were amplified directly from the extracted
24 DNA, a nested approach was followed for Archaea. Primer pairs were 357F-GC and 518R-
25 ~~(Muyzer et al. 1993)~~ for bacteria ~~(Muyzer et al. 1993)~~, NL1 and LS2 ~~(O'Donnell 1993)~~;
26 ~~Coccolin et al. 2000)~~ for fungi ~~(O'Donnell 1993; Coccolin et al. 2000)~~, A2F and 1492R-
27 ~~(Reysenbaech et al. 1995; Lane 1994)~~ and SaF-GC and PARCH519R ~~(Nieol et al. 2003)~~;
28 ~~Øvreås et al. 1997)~~ for the first and second step of archaeal PCR respectively ~~(Reysenbaech et~~
29 ~~al. 1995; Lane 1991; O'Donnell 1993; Coccolin et al. 2000)~~. Primer sequences and reaction
30 conditions are reported in Online Resource 2.

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

1 MgCl₂, 0.02 bovine serum albumin (BSA), 0.2 mM of each dNTP, 0.4 µM of each primer,
2 1.25 U of BIOTAQ™ DNA polymerase (BioLine) and 2 µl of soil DNA diluted 1:10 in sterile
3 DNase-treated water (Sigma). Second steps of nested PCR were performed without BSA,
4 using 1 µl of the first step product as template.

5 DGGE was carried out as previously described by Webster et al. (2006) using a
6 DCode™ Universal Mutation Detection System (Bio-Rad Laboratories), with a gradient from
7 30 to 60%. Electrophoresis was run at 200 V for 5 h (with an initial 10 min at 80 V) at 60°C
8 in 1 x TAE buffer. Gels were stained for 30 min with SYBR® Gold nucleic acid gel stain
9 (Invitrogen) and visualized under UV with an UVpro Platinum Gel Documentation System
10 (UVItec).

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

22 Obtained 16S bacterial rRNA gene sequences were submitted to the [European](#)
23 [Nucleotide Archive/EMBL database](#) (<http://www.ebi.ac.uk/ena>) under [accession numbers](#):-
24 [Submission code was Hx20000054952](#). LT613607-LT613635.

25

26 **Quantitative PCR**

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

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

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

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

11 Only for archaeal amoA gene, PCR products obtained by amplifying total DNA
 12 extracted from PG sample 2N with primer pair Arch-amoAF and Arch-amoAR (Francis et al.
 13 2005) were pooled, purified, quantified by NanoDrop, serially diluted and used for standard
 14 curve construction.

15 qPCR reactions were performed using a Chromo4™ Real Time PCR Detection
 16 System (Bio-Rad Laboratories), and data were analysed with the MJ Opticon Monitor
 17 software (version 3.1). Primer pairs were the same as for standard preparation, except for
 18 Bacteria and Archaea. The first were substituted by the pair 519F and 907R (Lane 1991;
 19 Muyzer et al. 1995), the second by the pair S-D-Arch-0025-a-S-17 and S-D-Arch-0344-a-S-
 20 20 (Vetriani et al. 1999). The PCR mixture contained 0.3 µM of each primer, 10 µl of
 21 SsoAdvanced™ SYBR® Green Supremix and 2 µl of soil DNA diluted 1:10 - 1:100, in a total
 22 volume of 20 µl. Optimal DNA dilution was chosen in order to minimize inhibition problems
 23 linked to low A260/A230 ratio of extracted DNA. All the samples and the standards were
 24 analysed in triplicate on PCR strip tubes (Bio-Rad Laboratories) with the following thermal
 25 cycling conditions: 95° for 5 min, followed by 40 cycles of 95°C for 30 sec, annealing for 30
 26 sec and 72°C for 1 min. Different annealing temperatures are reported in Online Resource 3.
 27 PCR specificity was verified by melting curves analysis. Standard curves R² value was always
 28 higher than 0.996, and all the reactions showed efficiencies higher than 70%.

29 **Statistical analysis**

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

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

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

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

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

19 **Results**

20 **Community structure (PCR-DGGE)**

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

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

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

21 Microbial abundance

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

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

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

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

15 Discussion

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

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

1 microbial complexity, the more represented groups and evidenced the ecosystem properties
2 possibly involved in shaping and influencing the community.
3 Overall, the composition of bacterial communities found on these patterned ground
4 features results quite similar to those described [more in](#) detail on alpine soils (Nemergut
5 et al. 2005), or in other periglacial landscapes like polygonal soils (Frank-Fahle et al. 2014),
6 ice wedges (Wilhelm et al. 2012), or glacier forefields (Bajerski and Wagner 2013), in
7 accordance with Delmont et al. (2014) that showed how similar habitats may lead to the
8 development of communities with similar composition.
9 The presence of phylotypes belonging to at least seven different phyla indicates quite
10 complex bacterial communities. Among primary producers, phototrophic Cyanobacteria and
11 Alphaproteobacteria families including chemolithotrophic and chemoorganotrophic organisms
12 (Bradyrhizobiaceae, Rhodospirillaceae, Hyphomicrobiaceae) were detected. Moreover, in all
13 the sites several Acidobacteria-related phylotypes were found. The ability to grow at low
14 nutrient conditions and tolerate variations in soil humidity often characterize Acidobacteria
15 (Ward et al. 2009), giving a potential explanation for their ubiquity in the examined patterned
16 ground ecosystems. Finally, the presence of at least one representative of Bacteroidetes in all
17 the sites suggests that these communities can host also a group of degraders of complex
18 substrates (Nemergut et al. 2005).
19 Archaea showed lower differentiation, with all the investigated phylotypes belonging
20 to Thaumarchaeota division. Considering that Thaumarchaeota includes all known archaeal
21 ammonia oxidizers, this result is also consistent with the high abundance of *amoA* gene
22 copies, which exceed their bacterial analogues in all the samples. Similar situations are quite
23 common in different ecosystems, particularly in acidic soils (Prosser and Nicol 2012; Qin et
24 al. 2013; Tian et al 2014; Xu et al. 2012), and has been reported by Frank-Fahle et al. (2014)
25 for polygonal tundra. Nevertheless, the same study pointed out a clear predominance of
26 methanogens, not detected in this study. Previous studies highlighted the importance of
27 waterlogging, common phenomenon affecting polygonal soils, in driving permafrost
28 microbial community [composition](#) [structure](#) (Ollivier et al. 2014). However, the study sites
29 present quite different characteristics, in terms of water content, 12-28% (unpublished data),
30 from those reported for high latitude patterned ground ecosystems. Therefore, the
31 predominance of aerobic phylotypes over anaerobic is not surprising and could indicate a
32 relevant role of Thaumarchaeota in influencing [Nitrogen](#) availability in mid-latitude PGs.

1 In terms of archaeal sequences, the presence of identical sequences (100% similarity)
2 in our samples and in both cold or rock-associated ecosystems and temperate agricultural and
3 forest soils might suggest the cosmopolitan nature of at least a part of the community.
4 In general, analysis of bacterial, archaeal and fungal phylogenetic markers revealed a
5 quite homogeneous community composition within single PGFs, without a clear separation
6 between samples collected from the vegetated rims and the central, nearly bare soil portion.
7 This is also coherent with the results that Timling et al. (2014) obtained for fungal
8 phylogenetic markers investigating patterned grounds in the northernmost bioclimatic
9 subzone of the North American Arctic Transect.
10 Differences in community composition were related more consistently to the sampling
11 site than to the position across the PGFs. Also the NMDS analysis (Fig. 5), performed on the
12 average band distribution of all the phylogenetic markers, supported this separation among
13 sites, pointing out the main parameters (data from D'Amico et al. 2015) involved in site
14 differentiation and, potentially, in shaping the composition of microbial community (Table 6).
15 For instance, the GN site was located at the highest altitude, and was characterized by highest
16 P content and exchangeable Ca/Mg ratio. Conversely, high levels of exchangeable Mg and Ni
17 fitted with the SP community; CS sites were mainly characterized by high levels of Ca, while
18 GB communities were correlated with intermediate levels of most soil parameters. This seems
19 to suggest that in this mid-latitude, alpine context parent material lithology can be a strong
20 driver for microbial community differentiation in terms of community composition,
21 overcoming the effect of strong, small-scale gradients in edaphic properties produced by
22 cryoturbation and patterned ground development. The importance of parent material lithology
23 on composition of microbial communities has been described in a variety of different
24 ecosystems, like soils (Reith et al. 2015), continental crystalline crust (Nyyssönen et al. 2014),
25 pristine aquifers (Boyd et al. 2007) and arctic streams (Larouche et al. 2012). D'Amico et al.
26 (2015), analysing the same study sites, reported a similar vegetation diversity pattern, with a
27 lack of differentiation between rims and centres, and a strong separation of plant communities
28 developed on different matrixes. Therefore, parent material lithology and the associated soil
29 chemical properties, plant colonization and microbial community composition seem to be
30 strictly linked.
31 Quantitative analysis, performed by qPCR, presents a different picture. In fact, mi-
32 crobial abundance resulted quite homogeneous among different sites. The only one showing

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

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

22 Finally, exploring more in detail the relationships existing between microbial abun-
 23 dances and soil chemical parameters (Table 7), Bacteria showed the highest number of signifi-
 24 cant correlations with different chemical properties (vegetation cover and contents of Ca, Mg,
 25 K, TN and TOC and C/N). On the other hand, Archaea abundances only correlated with the
 26 soil C/N, but correlated to all the microbial markers except for bacterial *amoA*. Similar situa-
 27 tions have been previously reported for alpine forest soils (Siles and Margesin 2016) and for
 28 tundra soils (Blaud et al. 2015), with bacterial abundance following chemical soil properties
 29 trend, and archaeal abundance independent from them. However, in those cases, all the micro-
 30 bial markers resulted positively correlated to each other, suggesting an indirect action of envi-
 31 ronmental parameters on the whole microbial population. In our case we can hypothesize that,

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

14 Conclusions

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

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

29 In conclusion, our results offer a picture quite in accordance with previous studies focused on
 30 Arctic PGFs, adding lithology to the complex hierarchy of controls modulating the effect of
 31 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 exposure to freeze-thaw activity.

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1 **Figure captions**
2
3 **Fig. 1** Sampling scheme on PG features. A north-south transect was drawn across the circles
4
5 (or the stripe, at site SP) and five surface samples (0-10 cm), equally spaced, were collected:
6
7 one central (C), two external (N and S), taken from the stony/vegetated rims and two
8
9 intermediate (NC and SC)
10
11 **Fig. 2** Cluster analysis of DGGE profiles obtained for bacterial, archaeal and fungal PG
12
13 communities (site 1=CS; 2=SP; 3=GB; 4=GN), based on Bray-Curtis dissimilarity algorithm
14
15 **Fig. 3** DNA concentration and abundance of bacterial and archaeal 16S rRNA genes, fungal
16
17 26S rRNA genes, archaeal and bacterial *amoA* genes in the four sites. Different letters indi-
18
19 cate significant differences ($P < 0.05$) among sites according to Games Howell *post hoc* test
20
21 **Fig. 4** Distribution of different biological markers within single PG features in the four sites:
22
23 DNA concentration, bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, ar-
24
25 chaeal and bacterial *amoA* genes
26
27 **Fig. 5** NMDS ordination of the four sites (1=CS; 2=SP; 3=GB; 4=GN), based on DGGE pro-
28
29 files (for each sampling point information obtained from archaeal, bacterial and fungal pro-
30
31 files were combined). Vectors show the direction and strength of environmental variables

Figure 1

Figure 2

Figure 3

Figure 5

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Figure 4

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Table 1

1	2	Table 1 Localization and environmental properties of the study sites				
		Site localization	Coordinates	Elevation (m a.s.l.)	Parent material	PG type
3	4	1 - (CS)	Fenetre de Champorecher (Champorecher, AO)	2705	Calcschists (serpentinite in traces)	Nonsorted circles, hummocks
5	6					
7	8	2 - (SP)	Colle di Raye Chevrere (Champdepraz, AO)	2710	Serpentinite	Sorted stripes
9	10					
11	12	3 - (GB)	Lac des Heures (Champdepraz, AO)	2780	Gabbro (serpentinite in traces)	Sorted elongated circles
13	14					
15	16	4 - (GN)	Piata Lazin (Ronco Canavese, TO)	3054	Gneiss	Sorted circles
17	18					

2

Table 2

1	2	Table 2 @Main chemical soil properties and plant cover distribution in the sampling sites (data from D'Amico et al. 2015)										
3	4	5	6	7	8	9	10	11	12	13	14	15
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	IN	5.2	2.65	14.7	6.32	2.05	11.77	8.09	98			
	INC	5.7	2.05	14.6	3.27	1.25	5.86	2.26	30			
	IC	6	1.12	18.7	1.15	0.31	6.94	1.35	5			
	ISC	5.3	3.01	14.3	3.76	1.23	3.50	2.66	20			
SP	IS	5.5	3.12	14.2	6.57	1.18	1.94	7.18	100			
	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			
GB	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			
	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			
GN	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			
	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

1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6

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	<i>Alphabacter aggregans</i> (NR_074324)	forest soil	95	<i>Acidobacteria</i>	<i>Alphabacter aggregans</i> (NR_074324)	95	x x x x
B8	<i>Nitrospira sibirica</i> (NR_074324)	-	100	<i>Alphaproteobacteria</i>	<i>Nitrospira sibirica</i> (NR_074324)	100	x x x x
B17	Uncultured bacterium clone KA13 (JQ973360)	forest soil	96	<i>Alphaproteobacteria</i>	<i>Micromonospora guangxiensis</i> (NR_044563)	95	x x x x
B5	Uncultured bacterium isolate DGGE gel band (L12 clone H1 (JX591052))	soil	99	<i>Alphaproteobacteria</i>	<i>Phaeosiphonidium thuyum</i> (NR_023836)	94	x x x x
B2	Uncultured bacterium clone B18-80 (KF394065)	permafrost	99	<i>Alphaproteobacteria</i>	<i>Phaeosiphonidium thuyum</i> (NR_023836)	97	x x x x
B7	Uncultured SOIL BACTERIUM clone G03AAZ03DP1 (JQ919759)	soil	96	<i>Bacteroidetes</i>	<i>Psychrobacter borealis</i> (NR_064381)	88	x x
B16	Chloroflexi bacterium Ellin727 (AY673463)	soil	86	<i>Chloroflexi</i>	<i>Chloroflexi bacterium Ellin727</i> (AY673463)	82	x
B4	<i>Arthrobacter ramosus</i> (KF338765)	Himalaya	90	<i>Actinobacteria</i>	<i>Arthrobacter ramosus</i> (KF338765)	90	x
B15a	Uncultured bacterium clone (AB127883)	eutrophic lake	97	<i>Alphaproteobacteria</i>	<i>Arthrobacter ramosus</i> (KF338765)	95	x x
B24	Uncultured bacterium clone HPI27 (K837272)	soil	97	<i>Betaproteobacteria</i>	<i>Nitrospira multiformis</i> (NR_074736)	93	x
B15	<i>Crocococcus cyanobacterium</i> PFS66 (HE809540)	lake water	86	<i>Cyanobacteria</i>	<i>Cyanidium gracile</i> (NR_102447)	85	x
B8, B9	<i>Pseudomonas anarctica</i> (NR_025586)	McMurdo Valley, Antarctica	100	<i>Gammaproteobacteria</i>	<i>Pseudomonas anarctica</i> (NR_025586)	100	x
B12, B13	Uncultured bacterium clone periphytic soil Himalaya	periphytic soil	100	<i>Feromonibacteria</i>	<i>Psychrobacter cephalotus</i> (NR_023836)	86	x
B14	Uncultured bacterium isolate 111260339975 (BQ1871)	forest soil	95	<i>Feromonibacteria</i>	<i>Psychrobacter cephalotus</i> (NR_023836)	90	x

Table 4

1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6

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-11-10 (KJ534140)	permafrost sediments (Olan mudflat)	98	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	94	x x x x
A	Uncultured archaeon clone (KF444583)	Western Himalaya	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera evergladenensis</i> SRI (CF080774)	94	x x x x
B	Uncultured archaeon clone AS.A17 (GJ288213)	glacial cryonite (Sury Island, Antarctica)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	96	x x x x
C	Uncultured clone DT-14Q-2T-17 (KJ066470)	permafrost soil (Oughliss, Tibetian Plateau)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	95	x x x x
D	Uncultured archaeon clone DZ2-14Q-2T-17 (KJ066484)	permafrost soil (Oughliss, Tibetian Plateau)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	95	x x x x
E	Uncultured archaeon clone ABCde-50 (GQ26886)	soil (Mount Mita, Tibetian Plateau)	97	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	93	x x x x
68	Uncultured archaeon clone ABCde-227 (GQ272580)	soil (Mount Mita, Tibetian Plateau)	96	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	94	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 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 PCs			
							CS	SP	GB	GN
F1	Uncultured fungus clone 126, NA10 PF3, C19 (KC966197)	Petwood Ground (North American Ascle)	98	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	
F2	<i>Phytophthora</i> sp. (JX244063)	<i>Populus deltoides</i> roots	95	<i>Ascomycota</i>	<i>Phytophthora</i> sp. (JX244063)	95	x	x	x	
F3, F4, F5	Uncultured fungus clone 112, NA3 PF1, PT1 (KC966078)	Petwood Ground (North American Ascle)	97	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	x
F6	<i>Ceratomyces</i> sp. (F8687648)	alpine soil (root apex of <i>Salix herbacea</i>)	97	<i>Basidiomycota</i>	<i>Ceratomyces</i> sp. (F8687648)	97	x	x		
F7	<i>Glioma</i> sp. (JX244063)	Guillem Massif	89	<i>Basidiomycota</i>	<i>Glioma</i> sp. (JX244063)	89			x	x
F8	Uncultured fungus clone 126, NA10 PF3, C19 (KC966197)	Petwood Ground (North American Ascle)	98	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	
F2	<i>Phytophthora</i> sp. (JX244063)	<i>Populus deltoides</i> roots	95	<i>Ascomycota</i>	<i>Phytophthora</i> sp. (JX244063)	95	x	x	x	

Variables	Dances				DNA			
	Plant coverage %	Bact	Arch	Fun	AOB	AOA	DNA	
pH	0.698**	0.321	0.666**	0.094	0.281	0.802**		
Ca	-0.167	-0.396	-0.129	-0.089	0.047	-0.187		
Mg	0.588**	0.368	0.717**	-0.164	0.257	0.710**		
K	0.478*	0.362	0.534*	0.060	0.275	0.594**		
Ni	0.606**	0.343	0.349	0.224	-0.108	0.414		
Cu/Mg	0.412	0.009	0.352	0.163	0.123	0.430		
P	-0.089	0.071	0.075	-0.144	-0.230	0.024		
N	-0.162	-0.620	-0.347	0.326	-0.439	-0.264		
C	0.789**	0.526*	0.643**	0.186	0.216	0.695**		
C/N	0.785**	0.492*	0.654**	0.205	0.229	0.712**		
Bact	0.510*	0.526*	0.815**	-0.033	0.287	0.747**		
Arch		0.284	0.626**	0.185	0.329	0.672**		
Fun		0.556*		-0.323	0.480*	0.916**		
AOB				-0.195	0.197	0.454*		
AOA					-0.450*	-0.125		
						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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Online Resource 2

Primer sets and amplification details used for PCR-DGGE analysis

Primer pair	Sequence (5'-3')	Target gene	Fragment length	Reference	Amplification details	Protocol reference
37F-QC ^b	CCTACGAGGAGGCAGCAG	<i>Bacteria</i> 16S rRNA	193	Majzer 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, 55 °C 30 s, 72 °C 1 min; 72 °C 10 min	Webster et al. 2006
518R	ATT ACC GCG GCT GCT GG					
31						
32						
A2F	TTC CGG TTG ATC CYG CCG GA	<i>Archaea</i> 16S rRNA (PCR step I)	c. 1500	Reysenbach et al. 1995	94 °C 5 min; 36 cycles: 95 °C 1 min, 56 °C 1 min, 72 °C 1 min; 72 °C 5 min	-
1492R	GGT TAC CTT GTT ACG ACT T					
S1F-GC ^{b,c}	Sal I/ECI AY GGG CGC AGC AGG	<i>Archaea</i> 16S rRNA (PCR step II)	192	Nicod et al. 2003	95 °C 5 min; 5 cycles: 94 °C 30 s, 30 cycles: 92 °C 30 s, 53.5 °C 30 s, 72 °C 1 min; 72 °C 10 min	Nicod et al. 2003
41	Sa2/ECI ACG GGG CGC AGA GGG					
42	TTA/CCT CGG CKG CTG					
PARCH519R						
N1-LQC ^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	Coscllin et al. 2000
L52	ATT CCC AAA CAA CTC GAC TC					

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61 (Biology and Fertility of Soils)
62 **Driving factors of soil microbial ecology in alpine, mid-latitude**
63 **patterned grounds (NW Italian Alps)**
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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
27	27F	AGA GTT TGA TCM TGG CTC AG	<i>Bacteria</i> 16S rRNA	c. 1500	Lane 1991		qPCR standard
28	1492R	GGT TAC CTT GTT ACG ACT T					
29							
30	S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	<i>Archaea</i> 16S rRNA	c. 1500	Verriani et al. 1999		qPCR standard
31							
32	1517R						
33							
34		GCC ATA TCA ATA AGC GGA GGA AAA G	Fungal 26S rRNA	c. 250	OTDonnell 1993	95 °C 5 min; 44 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
35	NLI				Coccolin et al. 2000		
36	LS2	ATT CCC AAA CAA CTC GAC TC					
37							
38							
39	amoA-1F	GGG GTT TCT ACT GGT GGT	Bacterial <i>amoA</i> 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
40							
41	amoA-2R	CCC CTC KGS AAA GCC TTC TTC					
42							
43							
44	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Archaeal <i>amoA</i> gene	635	Francis et al. 2005	95 °C 30s; 40 cycles: 95 °C 30 s, 50 °C 30 s, 30 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
45		GCG GCC ATC CAT CTG TAT GT					
46	Arch-amoAR						
47							
48							
49	519F	CCA GCA GCC GCG GTA AT AC	<i>Bacteria</i> 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
50							
51							
52	907R	CCG TCA ATT CMT TTR AGT TT			Muyzer et al. 1995		
53							
54	S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	<i>Archaea</i> 16S rRNA	c. 320	Verriani et al. 1999	95 °C 30 s; 40 cycles: 95 °C 30 s, 48 °C 30 s, 72 °C 1 min	qPCR
55							
56	S-D-Arch-0344-a-S-20	TCG CGC CTG CCG CCC GT					
57							

^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

Biology and Fertility of soils

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