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Effects of slope exposure on soil physico-chemical and microbiological properties along an altitudinal climosequence in the Italian Alps

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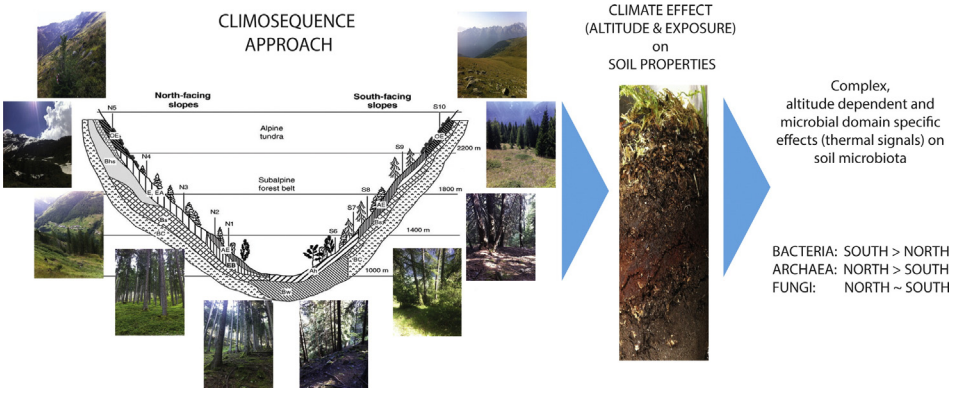
HIGHLIGHTS

- Slope aspect on microbial diversity and activity is often neglected.
- Slope exposure effect was enzyme-specific.
- Bacteria were more abundant on the south-facing slope irrespective of the altitude.
- Exposure did not significantly affect fungal abundance along the climosequence.
- Exposure effects on archaeal abundance were altitude-dependent.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to their sensitivity to changing environmental conditions sub- and alpine soils are often monitored in the context of climate change, usually, however, neglecting slope exposure. Therefore, we set up a climosequence-approach to study the effect of exposure and, in general, climate, on the microbial biomass and microbial diversity and activity, comprising five pairs of north (N)- and south (S)-facing sites along an altitudinal gradient ranging from 1200 to 2400 m a.s.l. in the Italian Alps (Trentino Alto Adige, Italy). Soil physico-chemical properties were related to microbiological properties (microbial biomass: double strand DNA yield *vs.* substrate-induced respiration; diversity of bacterial, fungal and archaeal communities: genetic fingerprinting DGGE *vs.* real-time PCR; microbial activity: basal respiration *vs.* multiple hydrolytic enzyme assays) to monitor shifts in the diversity and activity of microbial communities as a function of slope exposure and to evaluate the most determinant chemical parameters shaping the soil microbiota. The exposure-effect on several hydrolytic key-enzymes was enzyme-specific: e.g. acid phosphomonoesterase potential activity was more pronounced at the N-facing slope

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Bacteria-fungi-archaea
Multiple hydrolytic enzyme activities
Soil depth

while the activities of alkaline phosphomonoesterase, pyrophosphate-phosphodiesterase and arylsulfatase were higher at the S-facing slope. Furthermore, this exposure-effect was domain-specific: bacteria (S > N, altitude-independent); fungi (N ~ S); and archaea (N > S; altitude-dependent). Additionally, the abiotic parameters shaping the community composition were in general depending on soil depth. Our multidisciplinary approach allowed us to survey the exposure and altitudinal effects on soil physico-chemical and microbiological properties and thus unravel the complex multiple edaphic factor-effects on soil microbiota in mountain ecosystems.

1. Introduction

The microbial response to changing climatic conditions requires further research because up to now, most studies have had a limited ability to differentiate between the complex climate effects on the soil ecosystem (Sun et al., 2013). This is partly due to its heterogeneity and discontinuity (micro-, meso-, macroscale), which makes soil a cryptic (micro)habitat even when considering the new knowledge gained from molecular screening approaches, including recent high-throughput next generation DNA sequencing (Insam, 2001; Nannipieri et al., 2003; Orgiazzi et al., 2015).

It has been shown that thermal conditions at high altitudes and unfavourable soil conditions (e.g. very low pH in strongly leached soils) affect the structure and function of soil microbial communities (Margesin et al., 2009; Ascher et al., 2012; Zhang et al., 2013; Siles and Margesin, 2016; Siles et al., 2016). To date, however, contradictory findings have been reported on soil microbial biomass and microbial diversity along altitudinal gradients in mountain ecosystems. A decrease in bacterial and fungal biomass (assessed by phospholipid fatty acid biomarkers) with increasing altitude was observed in the Austrian Central Alps (1500–2530 m above sea level, a.s.l.; Margesin et al., 2009). On the contrary, Siles and Margesin (2016) found a greater bacterial and fungal biomass, using quantitative real-time PCR, as well as an increased microbial activity, assessed by basal respiration and enzymatic activities, at higher altitudes along an altitudinal gradient from 545 to 2000 m a.s.l. in the Italian Alps. Accordingly, these latter authors observed that the higher microbial abundances were significantly and positively related to an increase in the levels of soil organic matter and nutrients with altitude. This phenomenon could be explained by the greater recalcitrance of coniferous litter, resulting in a higher C sequestration and lower nutrient immobilization rates at higher altitudes (Berger et al., 2015). Nevertheless, any clear pattern in bacterial and fungal diversity/richness was recorded along the above-mentioned altitudinal gradient. Interestingly, Siles and Margesin (2016) observed that environmental and soil chemical properties, primarily soil pH and C/N ratio, explained the variations in microbial communities' properties better than altitude itself.

Slope aspect is considered an important topographic factor affecting local microclimate (Egli et al., 2006, 2009; Carletti et al., 2009; Barbosa et al., 2015). The amount of solar irradiation influences soil temperature, soil water retention and availability, nutrient dynamics (Carletti et al., 2009; Egli et al., 2009), composition and activity of soil microbial communities (Carletti et al., 2009; Margesin et al., 2009; Ascher et al., 2012) and soil mesofauna (Ascher et al., 2012). Therefore, we set up a climosequence approach comprising five pairs of north- and south-facing sites along an altitudinal gradient from 1200 to 2400 m a.s.l., so as to evaluate potential effects of the state factor *climate* (thermal conditions due to differences in exposure and altitude) on (i) microbial biomass (dsDNA content; substrate-induced respiration); (ii) microbial abundance (bacteria, fungi and archaea; real-time PCR); (iii) microbial activity (basal respiration); (iv) microbial community in terms of diversity and phylotype richness of the three microbial domains (denaturing gradient gel electrophoresis-DGGE genetic fingerprinting); and (v) multiple hydrolytic enzyme activities involved in the C, N, P and S cycles. We also assessed how these microbial properties vary within the topsoil (0–5, 5–10 and 10–15 cm) and, in addition, which physico-chemical factors are the most important drivers for soil microbial diversity along the climosequence scenario.

We hypothesised that: (1) soils at lower elevations and south exposure provide favourable climatic conditions for the autochthonous soil microbiota (higher biomass and activity); (2) the abundance, richness and diversity of the three microbial domains – bacteria, fungi and archaea – will be affected by the slope exposure, and this exposure-effect will be dependent on the altitude; (3) the climate effects will be more pronounced in the uppermost topsoil-layer due to its direct exposure.

2. Material and methods

2.1. Study area and soil sampling

The study area is located in Val di Rabbi (Trentino) in the south Alpine belt in northern Italy. The area was chosen due to (i) its situation in-between a rather warm Insubrien and a cold Alpine climate; (ii) the general suitability of the sites (accessibility of north- and south-facing sites at different altitudes); and (iii) the comprehensive database about soils and GIS data. A detailed map of the study area is given in Egli et al. (2006). The ten selected sites were located between 1200 and 2400 m a.s.l., five sites at north- ($N_{1–5}$) and five sites at south-facing ($S_{6–10}$) slopes (Fig. 1, Table 1), and were sampled in August 2012. To allow the comparison among soils collected on N- and S-facing slopes, the altitudes of the sites on both slopes were selected to be as similar as possible. We selected old-growth forest sites and natural grasslands to minimise the influence of human activities and the grazing by livestock. Overall, all the sites were located in catchments with acidic paragneiss or morainic material consisting of acidic paragneiss (Egli et al., 2006; Table 1), and the soils were classified as Cambisols to Umbrisols or Podzols (WRB, 2014; Table 1). In Val di Rabbi the mean annual temperature ranges from 8.2 (valley floor) to around 0 °C (at 2400 m a.s.l.), whereas the mean annual precipitation ranges from 800 to 1300 mm/year (Sboarina and Cescatti, 2004; Table 1).

Three independent plots (5 × 5 m) placed at ± 50 m a.s.l. from each other were selected in each of the 10 main study sites and five soil subsamples were randomly taken in each plot in 5 cm depth intervals (0–5, 5–10 and 10–15 cm), using a corer device (ø 5 cm; 5 cm correspond to 100 cm^3). A total of 450 soil samples (10 sites × 3 plots × 5 sub-samples × 3 soil depths) were placed in polyethylene bags and transported on ice to the laboratory. They were sieved (<2 mm), carefully separated from root fragments and stones, and kept at 4 °C for physico-chemical analyses and at −20 °C for molecular analyses, respectively.

2.2. Physico-chemical analyses

The volatile solids content was determined from the weight loss following ignition in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Total C and N contents were analysed in dried samples, using a CN analyser (TruSpec CHN; LECO, Michigan, U.S.A.). Particle-size analysis was assessed following the pipette procedure according to Indorante et al. (1990). Soil bulk density was determined according to Grossman and Reinsch (2002). Electrical conductivity (EC) and pH were determined in soil:distilled water extracts (1:10, w/v) by using a conductivity Meter LF 330 WTW (Weilheim, Germany) and a pH Meter Metrohm

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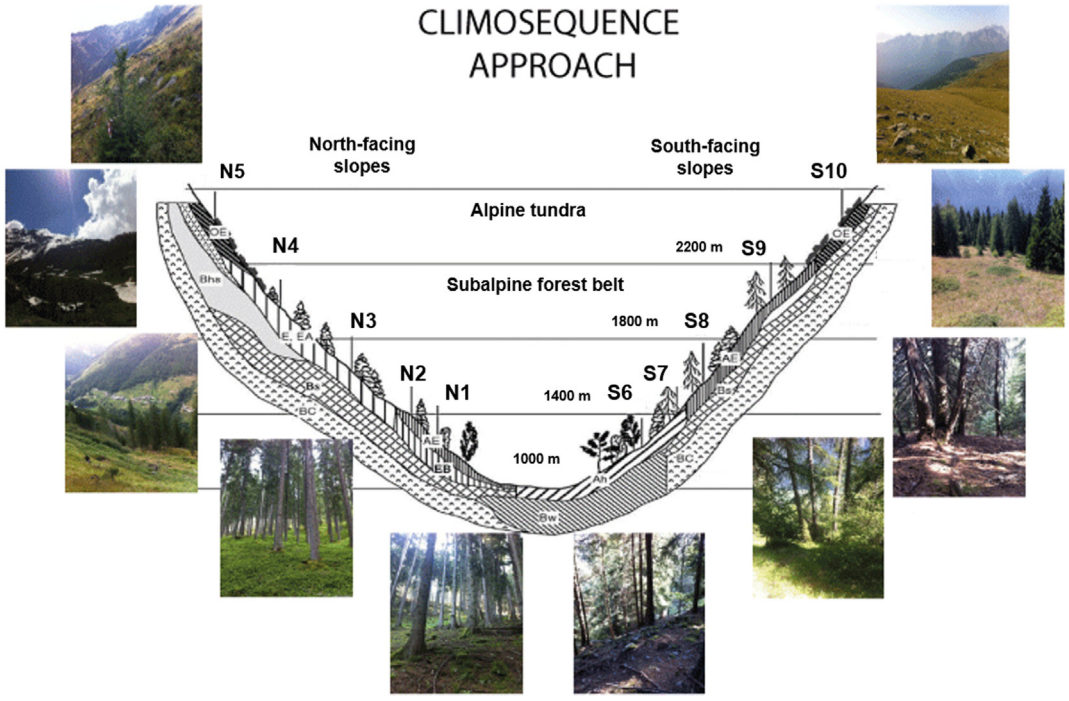

Fig. 1. Overview of the study area comprising five pairs of north (N_1-N_5) and south (S_6-S_7)-facing sites along an altitudinal gradient from 1200 to 2400 m a.s.l. in the Italian Alps (Trentino Alto Adige, Italy). The photos (J. Ascher) were assembled by T. Bardelli on the modified scheme of Egli et al. (2006).

744, respectively. Inorganic nitrogen (NH_4^+ and NO_3^-) was measured in 0.0125 M $CaCl_2$ extracts, as described by Kandeler (1993a, 1993b).

2.3. Soil enzymes activities

Eight hydrolytic enzymes involved in the principal nutrient cycles were determined, namely: (i) *C-cycle*: β-glucosidase (*gluc*), acetate esterase (*ester*); (ii) *N-cycle*: chitinase (*chit*), leucine-aminopeptidase (*leu*), (iii) *P-cycle*: acid (*acP*)- and alkaline phosphomonoesterases (*alkP*), pyrophosphate-phosphodiesterase (*piroP*); (iv) *S-cycle*: arylsulphatase (*aryS*). All of the potential enzymatic activities were measured in duplicate from all the soil samples by a heteromolecular exchange procedure (Fornasier and Margon, 2007), using a 3% solution of lysozyme as desorbant and bead-beating to disrupt soil aggregates and microbial cells. Briefly, 0.4 g of soil (fw) were placed into 2-mL microcentrifuge tubes, together with 1.4 mL of a solution containing 3% lysozyme and glass beads. The tubes were then subjected to bead-beating using a Retsch 400 beating mill at 30 strokes s^{-1} for 3 min, followed by centrifugation at 20,000*g* for 5 min. The supernatant containing desorbed enzymes was dispensed into 384-well white microplates with the appropriate buffer to fluorometrically quantify the enzymatic activities using fluorescent, 4-methyl-umbelliferyl- (MUF) and 4-amido-7-methyl-coumarine (AMC) substrates. All measurements were done in duplicate and the activities were expressed as nanomoles of MUF (or AMC) min^{-1} g^{-1} dry soil.

Table 1
Characteristics of the ten study sites at north-and south-facing slopes (N_{1-5} and S_{6-10}, respectively) in Val di Rabbi (Egli et al., 2006; Petrillo et al., 2015).

Sites	Altitude (m a.s.l.)	Aspect (°N)	Slope (°)	MAP (mm y^{-1})	MAAT (°C)	MAST (°C)	Parent material	Dominating tree species	Land use	Soil classification (WRB)
N_1	1180	340	31	950	5.6	7.3	Paragneiss debris	*Picea abies*	Natural forest (ecological forestry)	Chromi-Episkeletic Cambisol (Dystric)
S_6	1185	160	31	950	7.6	8.1	Paragneiss debris	*Picea abies*	Former-coppice, natural forest (ecological forestry)	Episkeleti-Endoleptic Cambisol (Chromi-Dystric)
N_2	1390	0	28	1000	4.6	6.3	Paragneiss debris	*Picea abies*	Natural forest (ecological forestry)	Chromi-Episkeletic Cambisol (Dystric)
S_7	1400	145	33	1000	6.6	8.7	Paragneiss debris	*Larix decidua*	Natural forest (ecological forestry)	Dystri-Endoskeletic Cambisol
N_3	1620	0	29	1060	3.5	5.8	Paragneiss debris	*Picea abies*	Natural forest (ecological forestry)	Chromi-Endoskeletic Cambisol (Dystric)
S_8	1660	210	33	1060	5.5	6.0	Paragneiss debris	*Picea abies*	Natural forest (ecological forestry)	Skeletic Umbrisol
N_4	1930	20	12	1180	1.4	5.0	Paragneiss debris, Moraine material	*Larix decidua*	Originally used as pasture	Episkeletic Podzol
S_9	1995	160	25	1180	3.4	6.4	Paragneiss debris	*Larix decidua*	Former-pasture, natural forest (ecological forestry)	Skeletic Umbrisol
N_5	2390	30	25	1300	-1.0	2.2	Paragneiss debris	*Rhododendro-vaccinietum*	Natural grassland and shrubs	Enti-Umbric Podzol (Episkeletic)
S_{10}	2420	190	28	1300	1.0	4.5	Paragneiss debris	*Festuca spp.*	Natural grassland	Dystri-Epileptic Cambisol

MAP = mean annual precipitation, MAAT = mean annual air temperature (Sboarina and Cescatti, 2004); MAST = mean annual soil temperature.

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2.4. MicroResp

Basal respiration (BR) and substrate-induced respiration (SIR) were assessed as CO_2 evolution by using the micro-respiration technique (MicroResp™) (Campbell et al., 2003). Briefly, soil samples were placed into a deep-well microplate, the moisture content was adjusted to 60% of their water holding capacity. BR was measured in wells with only deionised water added (25 μL); whilst SIR was determined by adding glucose (1% dry matter basis), applied in a volume of 25 μL. Two technical replicates were run for each field replicate. The absorbance was measured twice at 590 nm (Zenyth 3100, Anthos, Eugendorf, Austria), one immediately prior to sealing the soil microplate, and the other one at the end of 6 h incubation at 25 °C. Absorbance values were converted to CO_2 concentration, using a standard calibration curve as described by Campbell et al. (2003) and Lalor et al. (2007) and CO_2 evolution from individual wells was determined.

2.5. Molecular analyses

2.5.1. Soil microbial biomass index (dsDNA)

Whole community DNA was extracted from soil samples (0.5 g, fw) by mechanical cell disruption (bead-beating) in presence of a sodium phosphate buffer (0.12 M, pH 8 Na_2HPO_4), and *crude* (not purified) double strand DNA (dsDNA) was directly quantified by using PicoGreen fluorescent dye (Life Technologies) as described in Fornasier et al. (2014).

2.5.2. DNA extraction

Whole-community DNA was extracted from soil samples (0.5 g, fw) and purified by using a commercial kit (FastDNA Kit for Soil, MP-Biomedicals) as described in Ascher et al. (2009). DNA was qualitatively characterised by agarose gel electrophoresis (1× Tris Acetate-EDTA buffer; 1:10,000 EtBr; 0.8% w/v; 100 V 60 min) to assess the molecular weight and fragment length distribution in comparison to a DNA Mass Ladder Mix (Fermentas, 80–20,000 bp). The concentration of DNA was assessed using two quantification methods, namely via fluorometric (Quant-iT PicoGreen; specific for dsDNA) and spectrophotometric (Picodrop; DNA absorbance at 260 nm) measurements.

2.5.3. Denaturing gradient gel electrophoresis (PCR-DGGE)

Microbial community level endpoint PCR was performed with 1 μL DNA in 25 μL solution containing a final concentration of 2× MyTaq reaction buffer (Bioline GmbH, Germany); 0.2 μM of each primer to target bacteria (968f-GC/UNI1401r; Nübel et al., 1996), fungi (FF390/FR1-GC; Vainio and Hantula, 2000) and archaea (Parch519f-GC/Arc915r; Coolen et al., 2004); 0.4 mg mL^{-1} bovine serum albumin (BSA; Sigma, Austria); 10 mM TMAC (tetramethylammonium chloride); 1× Enhancer (VWR Internation GmbH, Germany); 0.035 U MyTaq DNA polymerase (Bioline GmbH, Germany), and distilled water (RNase/DNase free, Gibco™, UK). The PCR amplification of SSU rDNA targets of the three domains — bacteria, fungi and archaea — was performed following the cycling conditions described by Ascher et al. (2012), Shemekite et al. (2014), and Coolen et al. (2004), respectively. Proper sizes of amplification products were verified by electrophoresis on 1% agarose gels. The gel electrophoresis was performed by loading 60 ng of PCR products in an 8% (w/v) polyacrylamide gel as described by Shemekite et al. (2014). A denaturing gradient of 40–70% and 30–65% was used for bacteria and for fungi and archaea, respectively. GeneRuler 1 kb DNA ladder (Fermentas, Life Technology) served as marker. Gels were run in an INGENYphorU System (Ingeny International BV, The Netherlands) at a constant temperature (60 °C) and voltage (100 V) for 16 h. Gels were stained with silver nitrate using the Hoefer Automated Gel Stainer (Amersham Pharmacia Biotech, Germany), air dried and scanned for subsequent image analysis. Similarities among microbial communities as a function of exposure and soil depth were assessed by UPGMA (unweighted pair group method with arithmetic mean) cluster analyses based on Dice similarity coefficients (Dice, 1945) using the Quantity One 4.5.1 software (Bio-Rad) according to Ascher et al. (2012). Pairwise comparisons of prominent microbial community members/populations based on absence or presence of unique and shared DGGE bands were assessed and the resulting similarities (%) in microbial community structures among the studied soils were visualized by dendrograms.

The phylotype richness of the bacterial, fungal and archaeal communities was determined as the number of bands within the DGGE gels. The Shannon's diversity index (H′) was calculated as formulated by Eichner et al. (1999), using the following equation:

$$H' = -\sum p_i \ln p_i$$

where p_i expresses the proportional number in a specific group relative to the total number.

2.5.4. Quantitative real-time PCR

Quantitative real-time PCR (qPCR) analysis was performed to determine the 16S rRNA gene copy number of bacteria and archaea, and the 18S rRNA gene copy number of fungi. Real-time PCR was performed with the 1× Sensimix™ SYBR® Hi-rox (Bioline, USA) based on the DNA-intercalating dye SYBR Green I. The Rotorgene 6000 Real Time Thermal Cycler (Corbett Research, Sydney, Australia) was used in combination with the Rotor-Gene Series Software 1.7. Standard curves were constructed with PCR amplified 16S rDNA (bacteria and archaea) and PCR amplified 18S rDNA (fungi) according to the cycling conditions from the section 2.5.3, and by using the following pure cultures as templates: *Nitrosomonas europaea* (DSMZ 21879) as bacteria, *Methanobacterium formicicum* (DSMZ 1535) as archaea and *Fusarium solani* (DSMZ 10696) as fungi. The primer sets used for qPCR were as follows: 1055f/1392r (bacteria, Ferris et al., 1996), FF390/FR1 (fungi, Prévost-Bouré et al., 2011), Parch519f/Arc915r (archaea, Coolen et al., 2004).

Stock concentration [gene copies μL^{-1}] was determined via PicoGreen measurement and freshly prepared for each run. Ten-fold dilutions were used for standard curve construction. The reactions were performed in 20 μL assays with each reaction mix containing 1× Sensimix™ SYBR® Hi-rox (Bioline, USA), forward and reverse primers (200 nM each primer), 0.4 mg mL^{-1} BSA, distilled water (RNase/DNase free, Gibco™, UK) and 2 μL of either 1:10 diluted DNA-extract, and ten-fold diluted standard DNA. All the standards and samples were run in duplicate. After an initial denaturation at 94 °C (bacteria and archaea) and 95 °C (fungi) for 10 min, thermal cycling comprised 40 cycles of 20 s at 95 °C, 15 s at 58 °C and 30 s at 72 °C for bacteria; 15 s at 95 °C, 30 s at 50 °C and 30 s at 72 °C for fungi; and 30 s at 94 °C, 40 s at 57 °C and 30 s at 72 °C for archaea. To check for product specificity and potential primer dimer formation, runs were completed with a melting analysis starting from 60 °C to 95 °C with temperature increments of 0.25 °C and a transition rate of 5 s. The purity of the amplified products was further checked by the presence of a single band of the expected length on a 1% agarose gel stained with the DNA stain Midori Green (Nippon Genetics, Germany) via UV-transillumination (Vilber Lourmat Deutschland GmbH).

2.6. Statistical analyses

Statistical analyses were performed with Statistica 9 (StatSoft, USA). A factorial analysis of variance (ANOVA) was done to evaluate the effects of exposure (north *vs.* south) and soil depth (0–5, 5–10 and 10–15 cm) on the physico-chemical and microbiological parameters along the climosequence. Normality and variance homogeneity of the dataset were tested prior to ANOVA.

by using the Shapiro–Wilk and Levene's tests, respectively. Before analysis, data were log- or square root-transformed to meet the assumptions for ANOVA. Significant differences ($p < 0.05$) in the main effects were further analysed by paired comparisons with the Tukey

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HSD test. This post hoc test also allowed us to determine whether the exposure effects were more pronounced in the uppermost topsoil-layer by comparing the three different soil layers (i.e., 0–5, 5–10, 10–15 cm) from the north-facing slopes with those from the corresponding south-facing slope. Associations between the potential enzymatic activities and the principal chemical and microbiological variables were explored using Pearson's correlation.

Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was carried out using the software PAST to visualize the patterns of bacterial, fungal and archaeal communities along the climosequence. A model of multivariate analysis of variance was constructed using distance-based redundancy analysis (dbRDA) based on the Bray-Curtis distance to determine the environmental variables that were most influential on the bacterial, fungal and archaeal community compositions. Marginal tests were performed to determine the amounts of variation explained by the selected variables. Significance tests were performed through nonparametric permutation, which does not rely on the assumption of multivariate normality (Taylor et al., 2009).

3. Results

To assess the exposure effects (N *vs.* S), the results obtained from the climosequence approach were principally evaluated by pairwise comparison of the sites located at similar altitudes (Fig. 1, Table 1).

3.1. Physico-chemical analyses

An overview of the physico-chemical parameters as a function of the slope exposure and soil depth along the climosequence is given in Table 2. The output of factorial ANOVA with regard to the different experimental factors is shown in Table 4. Slope exposure had a significant impact on the percentage of volatile solids assessed as an estimation of the soil organic matter (SOM) content (Table 4), with higher values on N- than S-facing sites along the altitudinal gradient. This trend is in line with previous studies in the Trentino area (Egli et al., 2006, 2009; Ascher et al., 2012; Nahidan et al., 2015). This parameter decreased significantly with increasing soil depth (Table 4), with the highest OM content in the 0–5 cm soil layer in both slope exposures. Accordingly, we observed that the soil bulk density was significantly higher on the S- than on the N-facing sites between 1200 and 1600 m a.s.l. (Table 4), reflecting the SOM content (assessed as volatile solids; N > S; Table 2); whilst at the highest altitudes no differences were found, resulting in a significant interaction between exposure and altitude (Table 4). The ten study sites were silty-sandy or sandy-silty soils according to the particle-size analysis. As described by Egli et al. (2006, 2010), the cooler and more acidic conditions gave rise to more pronounced weathering processes and increased clay minerals formation at the N-facing sites. Moreover, the clays were found to be negatively correlated to soil pH which indicates that N-facing slopes are often related to more acidic environments (Carletti et al., 2009; Begum et al., 2010). Additionally, due to the accumulation of often weakly-degraded OM at N-slopes, more –COOH and –OH functional groups or phenolic compounds are present (Egli et al., 2009). As such, we found that soil acidity (pH) varied significantly with slope exposure (Table 4), with lower values in N- than in S-facing sites. In accordance with previous studies (Nahidan et al., 2015), the N-facing slopes were characterised by greater EC levels (Table 4), with two times higher values in soils from the N-facing sites between 1600 and 2000 m a.s.l. A plausible explanation could be that the higher OM content results in a higher soil cation exchange capacity and hence increased EC values. Moreover, as shown by Smith et al. (2002), the nitrate content could also contribute to increase the EC levels. In fact, the greatest differences in the NO_3^- content with respect to exposure (12 times higher on the N- than on the S-slope) were recorded for the altitude of 2000 m a.s.l. This was also supported by a positive correlation between EC and NO_3^- content ($R = 0.821$, $p < 0.001$). In both slopes, a significant diminution of EC was found with the soil depth (Table 4), with greater levels in the 0–5 cm soil layer, being three and four times higher than in the 5–10 and 10–15 cm layers, respectively. The total C content was significantly higher (2–3 times) at N- than at S-facing sites between 1200 and 1600 m a.s.l. (Table 4). However, no exposure effects were found for the higher altitudes (2000 and 2400 m a.s.l.), leading to a significant interaction between exposure and altitude (Table 4). Similar trends regarding the slope exposure were found for total N and C:N ratio (Table 4). A significant decrease in this ratio was found with soil depth at both slopes (Table 4). Previous works reported a higher inorganic N pool at the S-facing slopes (Zhang et al., 2012 and Nahidan et al., 2015). Nonetheless, in our study higher NH_4^+ concentrations were recorded in soils at N-facing sites (1–4 times higher) between 1200 and 2000 m a.s.l. (Table 2), while on the highest sites the NH_4^+ levels were greater on south exposure resulting in a significant interaction between both exposure and altitude (Table 4). A similar pattern was observed for NO_3^- content (Table 4), and 12 times higher NO_3^- levels were registered at the N-than at the S-facing soils at 2000 m a.s.l. (Table 2). Overall, both NH_4^+ and NO_3^- levels were significantly higher in the top 5 cm (Table 4).

3.2. Enzymatic activities

An overview of the potential hydrolytic enzyme activities as a function of the slope exposure and soil depth along the climosequence is given in Figs. 2 and 3. The output of factorial ANOVA with regard to the different experimental factors is shown in Table 4. The exposure-effect on β-glucosidase activity was dependent on the altitude and soil depth (Table 4). A two times higher activity was recorded for soils from the N-facing sites at an altitude of 1600 m a.s.l. and for the 5–10 and 10–15-cm soil layers (Fig. 2A); while no significant differences with exposure were found for the remaining altitudes (Fig. 2A). Moreover, a higher potential activity was found in the uppermost soil layer (0–5 cm), followed by the 5–10 cm layer, where the β-glucosidase potential activity was two times greater than in the 10–15 cm layer (Fig. 2A). Acetate esterase activity was not significantly affected by the slope exposure (Table 4); whereas a decrease was found with increasing soil depth, being two times higher in the 0–5 cm soil layer compared to 10–15 cm layer (Fig. 2B). Likewise, slope exposure did not significantly influence the chitinase activity (Table 4). A significant decrease with soil depth was however measured (Fig. 2C). The effect of slope exposure on the leucine-aminopeptidase activity was altitude-dependent (Table 4), thereby giving significant differences only at 1400 m a.s.l. (Fig. 2D). A significant decrease was found with soil depth on both slopes (Fig. 2D; Table 4). The acid phosphomonoesterase potential activity was significantly higher at N-facing sites, irrespective of the altitudinal gradient (Fig. 3A; Table 4). At both slopes the three soil depths were different from each other with the highest activity in the uppermost soil layer (0–5 cm; Fig. 3A). Slope exposure also affected significantly the alkaline phosphomonoesterase activity (Table 4); however, this activity was two-times higher atS-facing sites along the altitudinal gradient (Fig. 3B). The highest potential activities were recorded in the uppermost soil layer (Fig. 3A, B). The pyrophosphate-phosphodiesterase potential activity followed the same trend as that shown for the alkaline phosphomonoesterase (Table 4), with higher values at the S- than at the N-facing sites (Fig. 3C). A significant decrease was observed with soil depth at both slopes (Fig. 3C). Significant changes with exposure were recorded for arylsulphatase activity (Table 4), being one to two times higher at S-facing sites along the altitudinal gradient (Fig. 3D). Also here, a significant decrease with increasing soil depth was found on both slopes (Fig. 3D).

3.3. Microbial community abundance, activity and composition

An overview of the microbial biomass, activity and abundance of the three domains (bacteria, fungi and archaea) as a function of the slope

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Table 2
Physico-chemical properties of the soils collected at the ten study sites at north-and south-facing areas (N_{1-5} and S_{6-10}, respectively). The results are shown pairwise, i.e., the couples of north- and south-facing sites at the same elevation (N_1-S_6; N_2-S_7; N_3-S_8; N_4-S_9; N_5-S_{10}), so as to evaluate the exposure-effect. Values are means (n = 3) with the standard deviations in brackets. Data are expressed on a dry weight basis. Different font of letters discriminate among the three soil depths (0–5 cm, bold type; 5–10 cm, gray; 10–15 cm, italics) and different letters, in each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) as a function of the exposure-effect.

Sites	Soil depth (cm)	Volatile solids (%)	Bulk density (g/cm^3)	Sand (%)	Silt (%)	Clay (%)	pH	Electrical conductivity (μS cm^{-1})	Total C (%)	Total N (%)	C/N	NH_4^+ (mg kg^{-1} dw)	NO_3^- (mg kg^{-1} dw)
N_1	0–5	47.7 (5.7) **bc**	0.25 (0.1) **bc**	59 (5.9) **ab**	26 (5.0) **a**	15 (1.1) **bcd**	4.8 (0.4) **ab**	81.3 (22.3) **bc**	24.6 (2.4) **b**	0.9 (0.2) **bc**	26.9 (3.9) **a**	45.3 (12.7) **bc**	24.3 (4.3) **bc**
	5–10	22.2 (11.5) bc	0.91 (0.2) b	45 (3.5) ab	34 (1.2) ab	20 (3.5) ab	4.8 (0.4) bcd	39.2 (5.2) bcd	10.6 (6.2) bc	0.5 (0.3) cd	20.6 (2.2) ab	29.4 (5.0) bcd	11.7 (7.5) b
	10–15	11.0 (2.1) *b*	0.97 (0.1) *bc*	51 (7.2) *abc*	35 (3.9) *abc*	14 (5.1) *ab*	4.8 (0.3) *bcd*	27.8 (4.5) *bc*	4.4 (1.9) *b*	0.3 (0.1) *b*	18.1 (3.7) *abc*	17.0 (6.8) *b*	2.2 (0.8) *b*
S_6	0–5	21.4 (7.2) **d**	0.70 (0.1) **a**	56 (4.2) **ab**	30 (2.1) **a**	14 (2.1) **bcd**	6.0 (0.5) **a**	84.1 (2.5) **bc**	10.2 (3.7) **c**	0.5 (0.1) **c**	19.9 (3.3) **abc**	25.3 (5.2) **c**	7.3 (1.0) **c**
	5–10	9.3 (1.3) c	1.37 (0.1) a	56 (5.1) ab	30 (3.3) ab	13 (1.8) ab	5.7 (0.6) a	40.5 (27.5) bcd	4.0 (0.5) c	0.2 (0.04) d	17.4 (2.8) bc	13.7 (7.0) d	3.2 (0.8) b
	10–15	6.5 (1.7) *b*	1.52 (0.1) *a*	57 (6.5) *ab*	30 (4.9) *bc*	13 (1.6) *ab*	5.6 (0.5) *ab*	19.8 (11.6) *bc*	2.6 (0.8) *b*	0.2 (0.03) *b*	15.4 (1.9) *abc*	6.7 (1.6) *b*	3.1 (0.2) *b*
N_2	0–5	86.8 (5.2) **a**	0.15 (0.04) **c**	46 (12.3) **ab**	33 (9.0) **a**	21 (4.4) **abc**	4.7 (0.8) **b**	169.3 (10.8) **ab**	42.8 (9.8) **a**	1.8 (0.2) **ab**	23.8 (4.2) **ab**	49.5 (8.2) **bc**	51.5 (19.7) **ab**
	5–10	58.2 (16.7) a	0.78 (0.3) bc	45 (6.5) ab	33 (8.7) ab	22 (3.1) ab	4.3 (0.6) cd	87.9 (15.5) a	33.1 (13.5) a	1.3 (0.4) ab	24.8 (4.0) a	61.5 (31.7) bc	12.1 (8.6) b
	10–15	25.5 (17.3) *ab*	0.93 (0.2) *bc*	38 (1.6) *d*	43 (0.6) *ab*	19 (1.6) *ab*	4.5 (0.6) *cd*	45.3 (26.5) *ab*	11.3 (8.4) *ab*	0.5 (0.4) *ab*	20.0 (1.8) *ab*	24.1 (5.9) *ab*	4.4 (0.8) *b*
S_7	0–5	51.0 (0.1) **bc**	0.31 (0.2) **bc**	57 (10.0) **ab**	34 (10.7) **a**	13 (0.7) **cd**	5.7 (0.2) **ab**	178.0 (110.0) **a**	23.1 (1.0) **b**	1.3 (0.1) **abc**	18.1 (2.0) **bc**	65.5 (33.6) **bc**	26.9 (15.3) **bc**
	5–10	21.0 (1.9) bc	0.61 (0.05) cd	58 (3.9) ab	29 (4.8) ab	13 (0.9) ab	5.8 (0.2) a	46.9 (21.2) b	9.0 (2.3) bc	0.6 (0.2) cd	15.9 (1.5) bc	17.4 (2.8) cd	5.9 (4.2) b
	10–15	14.6 (4.3) *ab*	1.18 (0.05) *ab*	59 (1.7) *a*	32 (5.6) *abc*	8 (4.6) *b*	5.8 (0.3) *a*	28.8 (8.1) *bc*	5.6 (1.6) *b*	0.4 (0.1) *ab*	14.9 (2.0) *abc*	9.5 (1.2) *b*	3.4 (0.2) *b*
N_3	0–5	83.8 (3.9) **a**	0.17 (0.1) **c**	51 (13.8) **ab**	31 (4.8) **a**	18 (14.8) **abcd**	4.6 (0.3) **b**	184.9 (36.4) **a**	46.3 (2.3) **a**	2.1 (0.2) **a**	22.5 (2.2) **abc**	170.1 (22.3) **a**	38.5 (13.1) **bc**
	5–10	69.7 (22.1) a	0.33 (0.2) d	37 (13.2) b	45 (12.7) a	18 (7.3) ab	4.2 (0.2) d	90.3 (26.8) a	38.7 (12.9) a	1.8 (0.7) a	22.0 (2.3) ab	113.9 (21.5) a	7.1 (4.6) b
	10–15	33.5 (7.4) *a*	0.84 (0.07) *bc*	41 (11.2) *cd*	46 (15.0) *a*	13 (4.2) *ab*	4.2 (0.3) *d*	64.7 (12.9) *a*	18.8 (8.1) *a*	0.9 (0.4) *a*	21.1 (1.8) *a*	53.1 (17.5) *a*	1.5 (0.05) *b*
S_8	0–5	60.5 (16.6) **b**	0.41 (0.1) **abc**	60 (3.9) **ab**	21 (11.1) **a**	19 (8.5) **abcd**	5.4 (0.4) **ab**	164.1 (108.3) **ab**	24.0 (11.4) **b**	1.1 (0.5) **bc**	21.0 (0.8) **ab**c	53.9 (29.3) **bc**	20.2 (5.7) **bc**
	5–10	19.8 (6.7) bc	0.69 (0.1) bc	48 (8.9) ab	36 (6.4) ab	16 (3.1) ab	5.4 (0.2) ab	31.3 (15.3) bcd	10.1 (5.9) bc	0.6 (0.4) cd	16.7 (2.0) bc	23.2 (11.3) cd	2.2 (2.3) b
	10–15	17.3 (4.7) *ab*	0.89 (0.2) *bc*	35 (10.4) *d*	46 (12.0) *ab*	20 (1.6) *ab*	5.4 (0.3) *abc*	25.5 (8.5) *bc*	6.1 (0.5) *b*	0.4 (0.1) *ab*	14.2 (1.7) *abc*	10.1 (1.0) *b*	1.1 (0.05) *b*
N_4	0–5	44.9 (19.3) **bc**	0.39 (0.09) **bc**	68 (7.2) **a**	22 (3.2) **a**	10 (4.4) **d**	5.2 (0.4) **ab**	110.4 (33.8) **abc**	20.7 (9.4) **bc**	1.3 (0.6) **abc**	15.9 (4.4) **bc**	142.4 (53.7) **a**	79.4 (23.3) **a**
	5–10	22.7 (11.1) bc	0.91 (0.07) b	67 (12.7) a	23 (6.8) b	10 (6.4) b	4.8 (0.2) bcd	43.0 (2.8) bc	11.6 (7.1) bc	0.9 (0.5) bc	12.4 (1.8) c	71.2 (11.6) ab	42.6 (14.9) a
	10–15	15.5 (5.6) *ab*	0.92 (0.09) *bc*	61 (9.6) *a*	25 (2.7) *c*	14 (11.7) *ab*	4.8 (0.2) *bcd*	34.9 (6.6) *abc*	6.9 (3.1) *b*	0.5 (0.2) *ab*	12.7 (2.8) *c*	25.6 (11.3) *ab*	36.0 (9.6) *a*
S_9	0–5	36.6 (1.3) **cd**	0.48 (0.05) **ab**	37 (8.7) **b**	37 (6.7) **a**	26 (2.0) **a**	5.4 (0.1) **ab**	51.2 (8.1) **c**	19.2 (1.0) **bc**	1.3 (0.1) **abc**	14.8 (0.7) **c**	36.9 (4.9) **c**	9.5 (2.2) **c**
	5–10	21.3 (1.6) bc	0.60 (0.05) cd	39 (7.5) b	36 (6.7) ab	24 (1.8) a	5.1 (0.1) abc	20.1 (1.2) bcd	10.7 (1.2) bc	0.8 (0.1) bcd	14.0 (0.4) c	39.0 (19.2) bcd	2.5 (0.8) b
	10–15	16.0 (2.3) *ab*	0.84 (0.04) *bc*	45 (6.5) *bcd*	26 (16.6) *c*	29 (12.1) *a*	5.3 (0.1) *abc*	14.4 (1.7) *bc*	7.1 (0.8) *ab*	0.5 (0.1) *ab*	13.7 (1.4) *bc*	32.0 (9.4) *ab*	1.6 (0.03) *b*
N_5	0–5	49.8 (13.1) **bc**	0.35 (0.1) **bc**	50 (10.7) **ab**	28 (8.8) **a**	21 (2.3) **abc**	4.9 (0.2) **ab**	37.2 (8.8) **c**	27.3 (8.0) **b**	1.5 (0.3) **ab**	18.4 (1.8) **bc**	50.5 (19.6) **bc**	12.6 (6.4) **c**
	5–10	29.9 (8.3) b	0.74 (0.2) bc	53 (2.4) ab	29 (1.6) ab	18 (1.3) ab	4.9 (0.1) abcd	15.3 (6.6) d	16.3 (5.6) b	0.8 (0.2) bcd	20.7 (1.4) ab	32.3 (20.1) bcd	4.2 (1.6) b
	10–15	19.7 (3.0) *ab*	0.65 (0.2) *c*	57 (6.6) *ab*	26 (4.1) *c*	17 (3.2) *ab*	4.9 (0.1) *abcd*	9.2 (1.2) *c*	10.8 (1.5) *ab*	0.5 (0.04) *ab*	20.0 (2.4) *ab*	13.6 (5.3) *b*	3.3 (0.1) *b*
S_{10}	0–5	36.5 (5.1) **cd**	0.40 (0.1) **abc**	49 (11.6) **ab**	28 (3.9) **a**	24 (7.7) **ab**	5.1 (0.2) **ab**	45.5 (7.3) **c**	20.7 (2.3) **bc**	1.3 (0.2) **abc**	16.3 (0.4) **bc**	80.5 (17.7) **b**	15.3 (1.0) **c**
	5–10	23.4 (0.4) bc	0.52 (0.08) cd	51 (5.6) ab	27 (1.4) ab	21 (4.9) ab	5.0 (0.1) abcd	19.4 (7.5) cd	11.7 (0.4) bc	0.7 (0.03) bcd	16.0 (0.7) bc	33.1 (8.2) bcd	5.5 (1.8) b
	10–15	15.4 (0.1) *ab*	0.68 (0.04) *c*	54 (2.1) *ab*	27 (0.7) *c*	19 (2.5) *ab*	5.1 (0.1) *abcd*	12.0 (1.2) *bc*	6.5 (0.6) *b*	0.4 (0.1) *ab*	15.7 (1.6) *abc*	23.8 (9.2) *ab*	4.8 (0.8) *b*

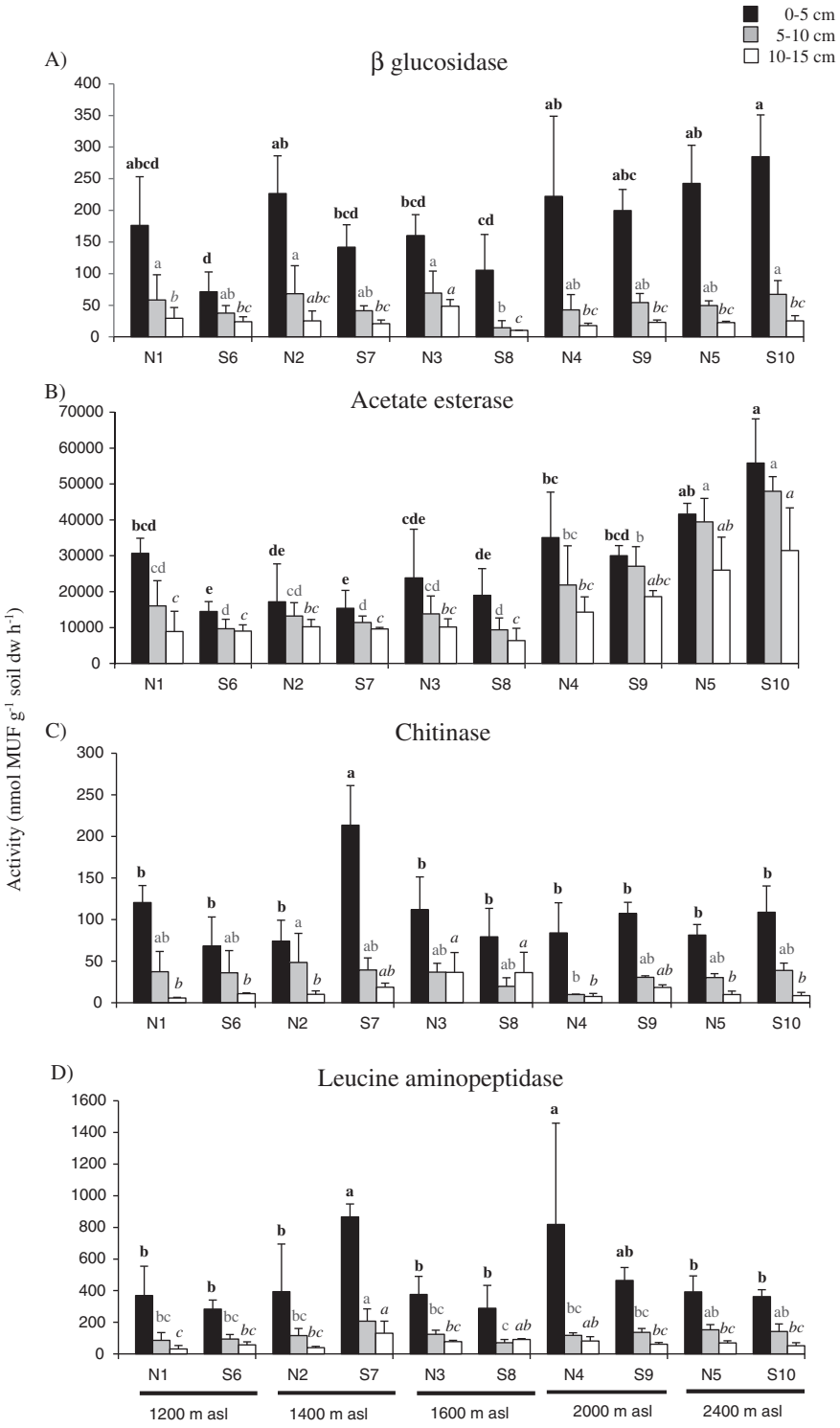

Fig. 2. Potential activities of β-glucosidase (A), acetate esterase (B), chitinase (C), leucine aminopeptidase (D) in the soils from the ten study sites at north- and south-facing areas (N_{1-5} and S_{6-10}, respectively). The results are shown pairwise, i.e. the couples of north- and south-facing sites (N_1-S_6; N_2-S_7; N_3-S_8; N_4-S_9; N_5-S_{10}) at the same elevation (1200 m; 1400 m; 1600 m; 2000 m; 2400 m a.s.l.). Values are mean values ± standard deviation. Different font of letters discriminates among the three soil depths (0–5 cm, bold type; 5–10 cm, gray; 10–15 cm, italics) and different letters, in each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) as a function of slope exposure.

exposure and soil depth along the climosequence is given in Table 3. The output of factorial ANOVA with regard to the different experimental factors is shown in Table 4. Soil microbial biomass assessed as double-strand DNA (dsDNA) was not significantly affected by the slope exposure (Table 4). However, a significant reduction in dsDNA was observed with soil depth (Table 4), being two to three times higher in the 0–5 cm soil layer compared to the 10–15 cm layer (Table 3). In contrast, a higher amount of microbial biomass (determined by SIR) was detected at the N-facing slopes (two times higher; Tables 3 and 4) regardless of the altitude. Moreover, the highest SIR values were found in the 0–5 cm soil layer (Tables 3 and 4), being two to four times greater than in the 5–10 and 10–15 cm layers, respectively (Table 3). Although both methods (dsDNA vs. SIR) are culture-independent, the latter method probably stimulates the activity/reactivity of predominant and fast growing soil

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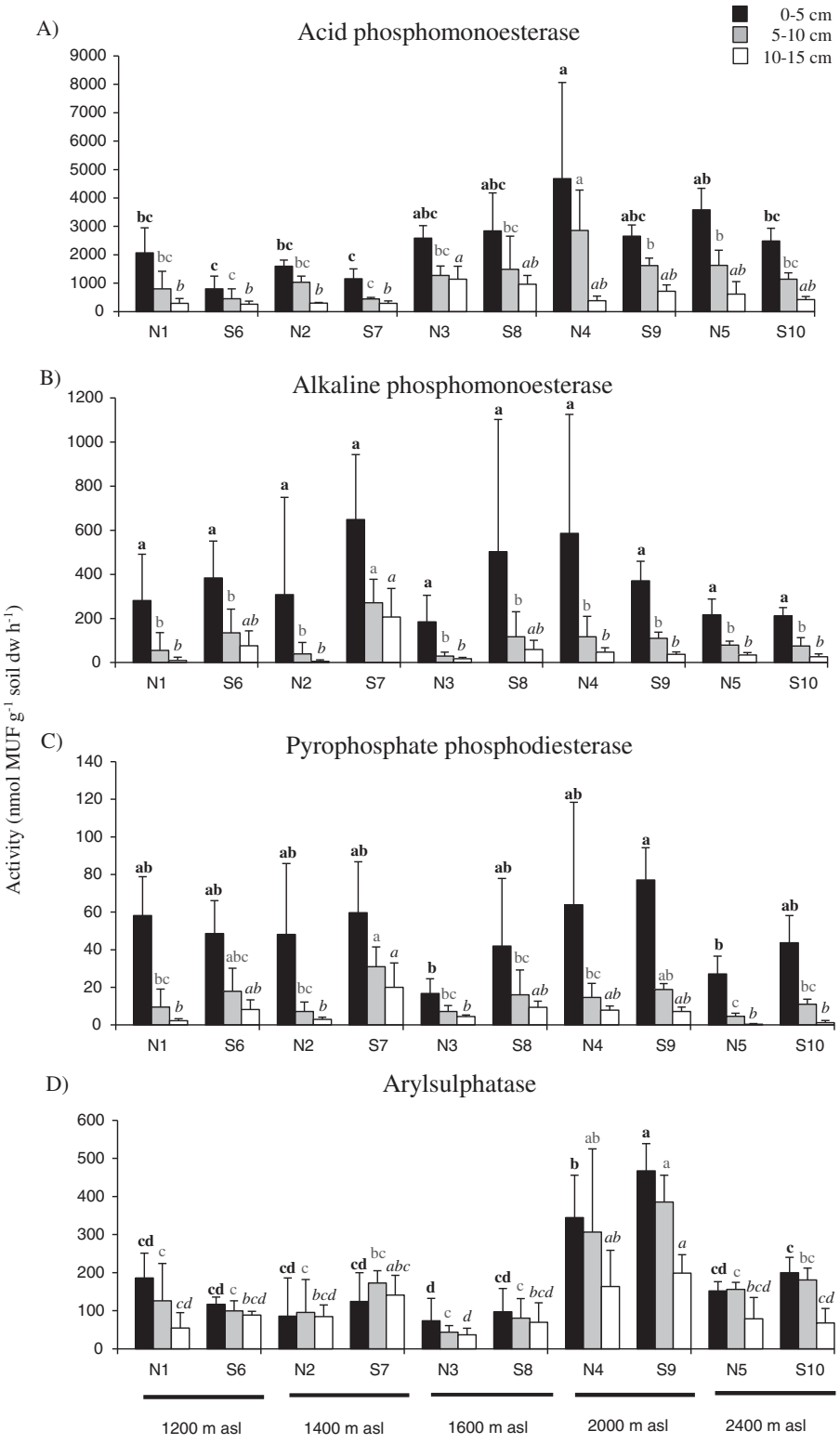

Fig. 3. Potential activities of acid-phosphomonoesterase (A), alkaline phosphomonoesterase (B), pyrophosphate phosphodiesterase (C), arylsulphatase (D) in the soils collected at the ten study sites at north- and south-facing areas (N_{1-5} and S_{6-10}, respectively). The results are shown pairwise, i.e. the couples of north- and south-facing sites (N_1-S_6; N_2-S_7; N_3-S_8; N_4-S_9; N_5-S_{10}) at the same elevation (1200 m; 1400 m; 1600 m; 2000 m; 2400 m a.s.l.). Values are mean values ± standard deviation. Different font of letters discriminates among the three soil depths (0–5 cm, bold type; 5–10 cm, gray; 10–15 cm, italics) and different letters, in each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) as a function of slope exposure.

microorganisms (Lin and Brookes, 1999); whereas the dsDNA-approach proposed by Fornasier et al. (2014) directly measures the extractable DNA of the overall autochthonous soil microbiota, independent of their growth strategy (r- *vs.* k-strategists). Furthermore, the aforementioned dsDNA-method, based on the direct quantification of crude (not purified) DNA, bypasses an unavoidable DNA loss when PCR-compatible DNA is required for downstream analyses and thus, the method yields a reliable quantitative estimator of the soil microbial biomass.

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Table 3
Microbiological properties of the soils collected at the ten study sites at north-and south-facing areas (N_{1-5} and S_{6-10}, respectively). The results are shown pairwise, i.e., the couples of north- and south-facing sites at the same elevation (N_1-S_6; N_2-S_7; N_3-S_8; N_4-S_9; N_5-S_{10}), so as to evaluate the exposure-effect. Values are means (n = 3) with the standard deviations in brackets. Data are expressed on a dry weight basis. Different font of letters discriminate among the three soil depths (0–5 cm, bold type; 5–10 cm, gray; 10–15 cm, italics) and different letters, in each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) as a function of the exposure-effect.

Sites	Soil depth (cm)	Microbial biomass index (μg dsDNA g^{-1} soil)	Substrate-induced respiration (μg C g^{-1} h^{-1})	Basal respiration (μg C g^{-1} h^{-1})	Bacteria (gene copy number g^{-1} soil)	Fungi (gene copy number g^{-1} soil)	Archaea (gene copy number g^{-1} soil)
N_1	0–5	170.45 (23.8) **ab**	46.78 (6.5) **ab**	26.69 (3.3) **ab**	1.23E + 09 (7.22E + 08) **b**	3.39E + 09 (4.60E + 09) **a**	1.40E + 08 (7.54E + 07) **ab**
	5–10	90.03 (33.1) bc	27.74 (5.6) a	16.45 (3.6) ab	6.64E + 08 (8.35E + 07) b	2.78E + 08 (1.56E + 08) b	1.25E + 08 (5.79E + 07) abcd
	10–15	64.65 (19.0) *bcd*	18.89 (3.6) *a*	8.04 (2.7) *a*	4.44E + 08 (6.10E + 07) *a*	4.03E + 08 (1.19E + 08) *bc*	2.69E + 08 (4.47E + 07) *ab*
S_6	0–5	104.43 (8.2) **b**	49.56 (18.5) **a**	10.86 (7.8) **ab**	3.38E + 09 (1.38E + 09) **ab**	5.13E + 08 (6.27E + 08) **b**	1.25E + 08 (9.32E + 07) **abc**
	5–10	63.94 (16.8) c	15.26 (4.7) bc	13.33 (10.2) abcd	3.32E + 09 (1.92E + 07) ab	3.76E + 08 (3.23E + 08) b	1.71E + 08 (5.13E + 07) abc
	10–15	50.79 (10.9) *d*	6.98 (1.9) *bc*	7.74 (5.6) *a*	1.15E + 09 (7.28E + 08) *a*	1.09E + 08 (2.62E + 07) *c*	1.27E + 08 (2.60E + 07) *ab*
N_2	0–5	194.46 (73.5) **ab**	33.15 (5.9) **abc**	30.88 (9.9) **a**	6.17E + 09 (4.92E + 09) **a**	5.92E + 08 (4.23E + 08) **b**	7.16E + 07 (5.80E + 07) **abc**
	5–10	148.75 (44.9) a	20.08 (2.1) ab	17.54 (3.3) ab	4.39E + 09 (3.74E + 09) a	3.62E + 09 (5.30E + 09) a	1.47E + 08 (1.03E + 08) abcd
	10–15	85.55 (19.4) *ab*	8.52 (4.5) *bc*	8.39 (3.0) *a*	2.72E + 09 (4.00E + 09) *a*	1.49E + 09 (1.03E + 09) *ab*	2.20E + 08 (3.76E + 07) *a*
S_7	0–5	178.27 (99.3) **ab**	15.20 (0.8) **bc**	7.32 (4.7) **b**	7.92E + 09 (5.46E + 09) **a**	3.62E + 08 (3.07E + 08) **b**	1.66E + 07 (1.22E + 07) **c**
	5–10	108.42 (41.5) abc	5.52 (0.6) c	7.22 (5.9) cd	3.47E + 09 (2.15E + 09) ab	3.13E + 09 (2.12E + 09) ab	4.64E + 07 (3.74E + 07) d
	10–15	97.92 (21.2) *a*	4.00 (1.4) *c*	6.71 (4.2) *a*	1.74E + 09 (1.99E + 09) *a*	2.50E + 09 (7.23E + 08) *a*	9.69E + 07 (3.74E + 07) *b*
N_3	0–5	144.46 (44.5) **ab**	46.62 (10.0) **ab**	31.10 (1.0) **a**	3.46E + 09 (2.50E + 09) **ab**	2.67E + 08 (2.22E + 08) **b**	9.18E + 07 (8.62E + 07) **abc**
	5–10	101.68 (14.9) abc	22.50 (11.2) ab	18.60 (8.2) a	2.75E + 09 (2.01E + 09) ab	3.25E + 08 (1.57E + 08) b	1.04E + 08 (5.21E + 07) bcd
	10–15	63.28 (25.4) *bcd*	8.75 (1.8) *bc*	7.41 (2.4) *a*	1.76E + 09 (1.94E + 09) *a*	3.67E + 08 (1.84E + 08) *bc*	1.49E + 08 (9.04E + 07) *ab*
S_8	0–5	161.37 (86.9) **ab**	26.18 (6.4) **abc**	22.49 (5.6) **ab**	4.33E + 09 (7.75E + 08) **ab**	2.96E + 08 (2.00E + 08) **b**	1.34E + 08 (9.45E + 07) **ab**
	5–10	93.67 (57.3) abc	6.29 (2.5) c	5.44 (1.8) cd	4.01E + 09 (7.75E + 08) a	5.49E + 08 (1.64E + 08) ab	2.11E + 08 (2.51E + 07) ab
	10–15	56.09 (23.0) *cd*	4.49 (0.6) *bc*	4.31 (0.5) *a*	2.45E + 09 (2.28E + 09) *a*	4.41E + 08 (1.08E + 08) *bc*	1.69E + 08 (6.93E + 07) *ab*
N_4	0–5	222.32 (107.5) **a**	39.25 (21.1) **abc**	31.49 (18.2) **a**	4.65E + 09 (1.32E + 09) **ab**	3.48E + 08 (3.18E + 08) **b**	1.61E + 08 (6.51E + 07) **a**
	5–10	104.49 (49.1) abc	13.90 (7.8) bc	10.92 (5.3) abcd	3.39E + 09 (2.13E + 09) ab	1.76E + 08 (8.94E + 07) b	2.29E + 08 (1.77E + 08) a
	10–15	76.41 (27.9) *abcd*	7.75 (1.8) *bc*	6.96 (1.5) *a*	3.72E + 09 (2.24E + 09) *a*	7.53E + 07 (5.98E + 07) *c*	2.33E + 08 (1.47E + 08) *a*
S_9	0–5	167.91 (6.9) **ab**	12.91 (4.6) **c**	8.93 (1.6) **ab**	5.39E + 09 (2.25E + 09) **ab**	1.94E + 08 (2.05E + 08) **b**	3.76E + 07 (2.34E + 07) **bc**
	5–10	123.34 (22.1) ab	5.73 (0.9) c	4.68 (0.5) d	5.04E + 09 (3.68E + 08) a	2.93E + 08 (1.57E + 08) b	6.47E + 07 (2.53E + 07) cd
	10–15	78.94 (11.2) *abc*	6.63 (1.3) *bc*	4.53 (0.5) *a*	4.93E + 09 (5.03E + 08) *a*	4.02E + 08 (2.24E + 08) *bc*	1.42E + 08 (3.27E + 07) *ab*
N_5	0–5	109.22 (11.1) **b**	39.27 (10.9) **abc**	25.70 (7.0) **ab**	5.44E + 09 (1.30E + 09) **ab**	4.15E + 08 (1.37E + 08) **b**	1.28E + 08 (8.85E + 07) **abc**
	5–10	80.01 (5.4) bc	20.34 (9.6) ab	13.86 (4.0) abc	4.40E + 09 (1.03E + 09) a	1.68E + 08 (5.49E + 07) b	1.20E + 08 (6.82E + 06) abcd
	10–15	67.84 (15.1) *bcd*	13.75 (5.9) *ab*	9.28 (2.8) *a*	2.78E + 09 (1.90E + 09) *a*	1.93E + 08 (6.67E + 07) *c*	3.09E + 08 (1.17E + 07) *a*
S_{10}	0–5	164.84 (48.8) **ab**	19.34 (7.4) **abc**	15.53 (5.6) **ab**	4.73E + 09 (1.21E + 09) **ab**	1.53E + 08 (5.27E + 07) **b**	6.73E + 07 (2.03E + 07) **abc**
	5–10	103.82 (16.1) abc	13.12 (2.4) bc	9.13 (2.6) bcd	2.66E + 09 (1.31E + 09) ab	9.57E + 07 (5.60E + 07) b	6.72E + 07 (1.39E + 07) cd
	10–15	75.61 (21.9) *abcd*	9.06 (2.1) *bc*	7.41 (0.9) *a*	3.84E + 09 (7.55E + 08) *a*	1.06E + 08 (3.85E + 07) *c*	2.80E + 08 (8.36E + 07) *ab*

The range of standards (gene copies μL^{-1}) used for quantifying each group (bacteria, fungi, archaea) by quantitative real-time PCR, along with the details of the calibration curves were the following:
Bacteria: range = 10^2–10^7; $R^2 = 0.99756$; slope = −3.365; intercept = 31.890; efficiency = 0.98.
Fungi: range = 10^2–107; $R^2 = 0.99802$; slope = −3.382; intercept = 32.707; efficiency = 0.85.
Archaea: range = 10^2–10^7; $R^2 = 0.99739$; slope = −4.010; intercept = 35.192; efficiency = 0.78.

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Table 4
Factorial ANOVA for the physico-chemical and microbiological parameters included in the present study.

Parameters	Exposure		Altitude		Soil depth		Exposure × altitude		Exposure × soil depth		Altitude × soil depth	
	F	p	F	p	F	p	F	p	F	p	F	p
Volatile solids	**125.05**	***	**58.15**	***	**29.55**	***	**3.74**	**	1.66	ns	**2.42**	*
Bulk density	**19.04**	***	**24.90**	***	**135.35**	***	**12.70**	***	**3.27**	*	**5.40**	***
Sand	0.23	ns	**3.22**	**	0.85	ns	**13.82**	***	0.24	ns	**2.28**	*
Silt	0.23	ns	**5.04**	***	1.77	ns	**2.79**	*	0.49	ns	**2.38**	*
Clay	2.04	ns	**3.15**	*	0.73	ns	**13.15**	***	0.11	ns	0.88	ns
pH	**107.30**	***	**3.63**	*	1.70	ns	**8.24**	***	0.26	ns	0.26	ns
EC	**26.29**	***	**43.28**	***	**124.61**	***	**7.02**	***	2.20	ns	0.52	ns
Total C	**51.37**	***	**17.96**	***	**78.38**	***	**6.22**	***	1.03	ns	0.75	ns
Total N	**30.57**	***	**17.55**	***	**66.28**	***	**4.52**	**	1.47	ns	0.43	ns
C/N	**46.86**	***	**22.81**	***	**13.42**	***	**5.74**	***	0.26	ns	1.79	ns
NH_4^+	**54.13**	***	**16.30**	***	**73.61**	***	**12.88**	***	1.83	ns	0.40	ns
NO_3^-	**66.39**	***	**11.83**	***	**123.26**	***	**22.73**	***	**3.57**	*	**4.29**	***
gluc	**12.16**	***	**3.61**	**	**161.67**	***	**8.25**	***	0.08	ns	1.58	ns
ester	1.26	ns	**40.64**	***	**31.06**	***	**2.85**	*	0.46	ns	0.89	ns
chit	1.81	ns	2.10	ns	**71.03**	***	0.43	ns	0.68	ns	1.09	ns
leu	1.82	ns	**5.25**	**	**153.71**	***	**5.75**	***	0.84	ns	1.43	ns
acP	**7.79**	**	**19.87**	***	**82.70**	***	1.01	ns	2.45	ns	1.67	ns
alkP	**4.02**	*	1.44	ns	**23.45**	***	0.39	ns	0.13	ns	0.45	ns
piroP	**30.18**	***	**9.50**	***	**115.06**	***	1.85	ns	1.28	ns	**2.80**	**
aryS	**7.04**	**	**16.91**	***	**7.68**	***	0.88	ns	0.11	ns	1.47	ns
dstDNA	0.66	ns	**4.41**	**	**38.88**	***	1.83	ns	0.08	ns	0.35	ns
SIR	**94.58**	***	**14.99**	***	**117.62**	***	1.36	ns	1.48	ns	1.70	ns
BR	**55.18**	***	2.05	ns	**40.18**	***	1.26	ns	**4.02**	*	1.51	ns
Bacteria	**4.33**	*	**5.39**	***	**7.66**	***	1.00	ns	0.01	ns	0.72	ns
Fungi	0.60	ns	**7.42**	***	0.33	ns	1.73	ns	2.69	ns	**2.15**	*
Archaea	**11.24**	***	**3.26**	**	**17.88**	***	**4.93**	***	0.21	ns	1.52	ns

EC (electrical conductivity); *gluc* (β- glucosidase), *ester* (acetate-esterase), *chit* (chitinase), *leu* (leucine-aminopeptidase), *acP* (acid phosphomonoesterase), *alkP* (alkaline phosphomonoesterase), *piroP* (pirophosphate phosphodiesterase), *aryS* (arylsulphatase); dstDNA (soil microbial biomass index), SIR (substrate induced respiration), BR (basal respiration), bacteria (16S rRNA gene copy number); fungi (18S rRNA gene copy number), archaea (16S rRNA gene copy number).
ns (no significant).
Values in bold indicate significant differences according to ANOVA.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Exposure also affected significantly soil microbial activity assessed as BR (Table 4) and, in general, higher values were recorded at the N- than at the S-facing sites along the altitudinal gradient (Table 3). A significant decrease in BR was observed with soil depth (Tables 3 and 4). The bacterial 16S rRNA gene copy number was significantly influenced by the slope exposure (Table 4), with higher levels at south exposure (Table 3). A decrease in bacterial abundance was found with soil depth at both slopes (Tables 3 and 4). In addition, the NMDS ordination plots and the cluster analyses based on DGGE fingerprints revealed shifts in the bacterial community diversity as a function of the slope exposure (Fig. 4A; Fig. S1). Specifically, a clear separation between N- and S-facing sites was observed along the NMDS axis 1 for the three soil depths (Fig. 4A; Table 5). Moreover, for each soil depth the ten study sites grouped distinctly along the NMDS axis 2, which indicates changes in bacterial community diversity in response to the altitude (Fig. 4A; Table 5). Among the studied physico-chemical parameters, total C content appeared to be among the most determinant factors for structuring bacterial communities in all of the three soil depths (Table 5). Soil pH and NO_3^- content also influenced bacterial communities in both the 5–10 and 10–15 cm layers (Table 5). No significant differences were found in the bacterial phylotype richness and diversity with slope exposure, irrespective of the altitudinal gradient (Table S1). However, these two indexes significantly decreased with increasing soil depth ($F_{2,60} = 11.56$, $p \leq 0.0001$; $F_{2,60} = 8.88$, $p \leq 0.0001$ respectively).

A low pH is considered physiologically disadvantageous to bacteria leading to a reduced bacterial competition and thus favouring fungal growth (Rousk et al., 2010). Bearing this in mind we could expect that the more acidic conditions at north-exposure could have promoted the fungal growth leading to a higher abundance of this group in comparison to the S-facing slopes. However, exposure did not have a significant impact on the fungal 18S rRNA gene copy number for any of the study sites as revealed by the qPCR approach (Tables 3 and 4). This suggests that fungi were less sensitive than bacteria to variations in pH between the two slope exposures. Pietikäinen et al. (2005) also showed that fungal populations of a forest and an agricultural soil appeared to be less negatively affected by low temperatures than bacteria. This could also explain why no differences in the fungal abundance were found. Fungal NMDS ordination plots and the dbRDA model analysis (Fig. 4B; Table 5) also revealed that only the fungal community inhabiting the 5–10 cm soil layer was significantly affected by the slope exposure. This is in agreement with the fungal DGGE patterns (Fig. S2). Total C significantly shaped the fungal communities in the upper-soil layer (Table 5), while the NH_4^+ content, the C/N ratio and pH were identified as the abiotic factors responsible for the fungal community changes in the 5–10 and 10–15 cm soil layers (Table 5). Neither the fungal phylotype richness nor the fungal diversity were significantly influenced by the slope exposure (Table S1). A significant increase of both parameters was, however, recorded with soil depth ($F_{2,60} = 100.46$, $p \leq 0.0001$; $F_{1,60} = 32.61$; $p \leq 0.0001$, respectively).

Exposure had a significant impact on the archaeal 16S rRNA gene copy number, although this effect varied along the altitudinal gradient (Table 4). The abundance of archaea was three times greater in soils of N-facing sites located at an altitude of 1400 m a.s.l. and 2000 m a.s.l., while no differences in this parameter with exposure were found in the remaining sites (Table 3). Indeed, at these specific altitudes, the archaeal abundance was negatively correlated with soil pH (N_2-S_7: $R = -0.559$, $p \leq 0.05$; N_4-S_9: $R = -0.718$, $p \leq 0.01$). Accordingly, Bengtson et al. (2012) observed a reduction in the archaeal abundance, assessed by qPCR, with increasing soil pH (pH range 4.0 to 5.1) in an arable soil. This and other previous studies (Nicol et al., 2008; Lehtovirta et

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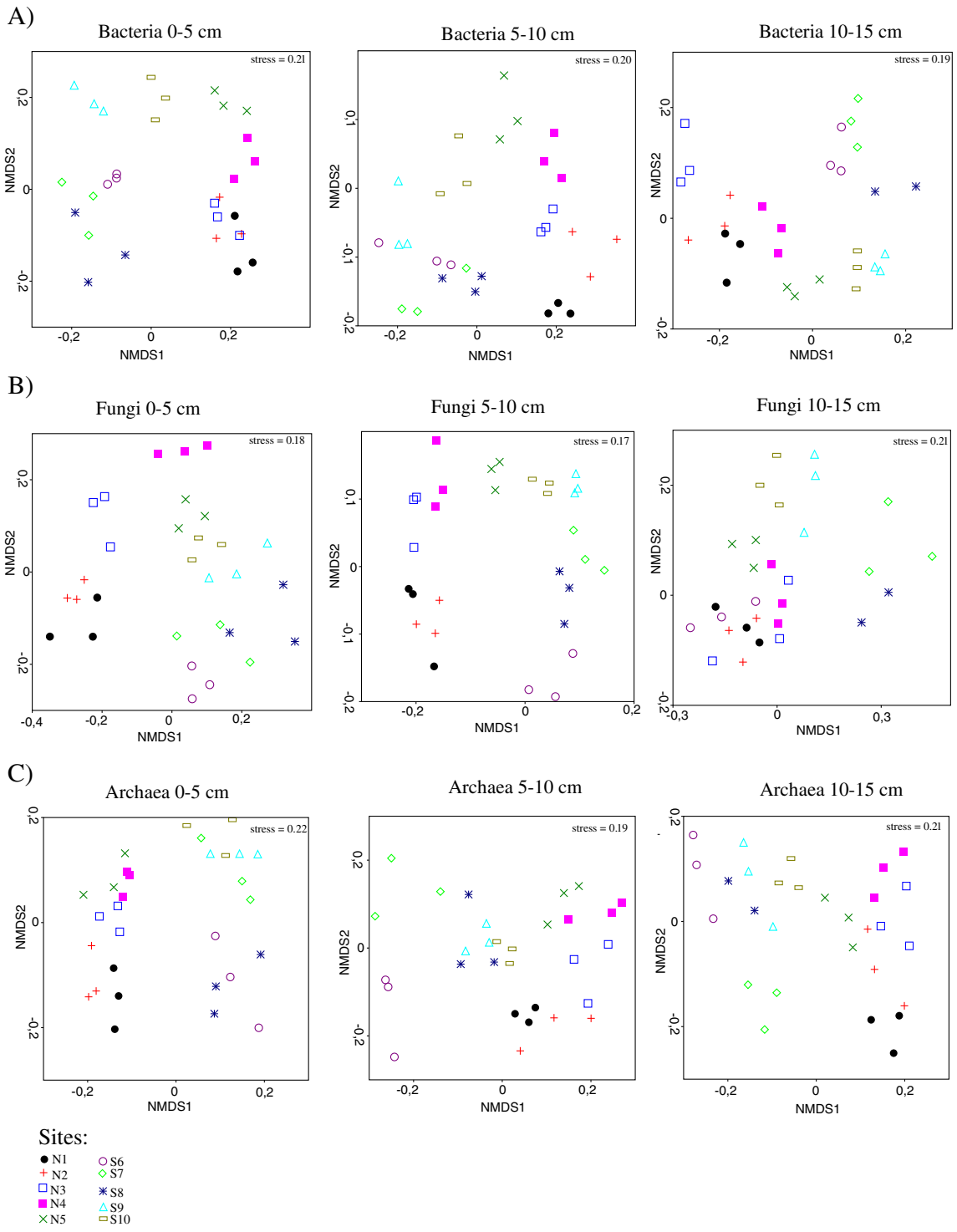

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination describing (A) bacterial, (B) fungal and (C) archaeal communities at different soil depths (0–5 cm, 5–10 cm, 10–15 cm) for the ten sites located at north- (N_{1-5}) and south-facing (S_{6-10}) slopes.

al., 2009; Bates et al., 2011) underpinned that soil pH together with C/N ratio are important parameters affecting the archaeal populations. Archaeal community diversity was also significantly affected by slope exposure (Fig. 4C; Fig. S3C). The discrimination between the N- and the S-facing sites along the NMDS axis 1 was more pronounced for the 0–5 cm soil layer (Fig. 4C). Moreover, for the top 5 cm a clear shift in archaeal community with the altitude was recorded along the NMDS axis 2 (Fig. 4C). In accordance with Bates et al. (2011), the C/N ratio was the main factor shaping the archaeal community composition in the top 5 cm (Table 5). Additionally, no significant exposure effects were found for the archaeal phylotype richness and diversity along the studied climosequence, except for the altitude of 1200 m a.s.l. (Table S1), where we measured a higher phylotype richness at north exposure ($F_{4,60} = 4.68$, $p = 0.002$). Furthermore, both the phylotype richness and diversity increased with increasing soil depth ($F_{2,60} = 43.65$, $p \leq 0.0001$; $F_{2,60} = 12.57$, $p \leq 0.0001$, respectively).

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Table 5
Relationships between the predictor variables and the microbial community structure of the three domains assessed as PCR-DGGE at different soil depths (0–5, 5–10, 10–15 cm).

	Soil depth (cm)	Altitude			Exposure			pH			% C			NO_3^-			NH_4^+			C/N ratio		
		var	F	p	var	F	p	var	F	p	var	F	p	var	F	p	var	F	p	var	F	p
Bacteria	0–5	**3.38**	**3.61**	***	**2.42**	**2.59**	***	–	–	–	**2.77**	**2.96**	**	–	–	–	–	–	–	–	–	–
Bacteria	5–10	**17.79**	**6.53**	***	**8.00**	**2.94**	***	**6.34**	**2.33**	**	**4.56**	**1.68**	*	**5.09**	**1.87**	*	–	–	–	–	–	–
Bacteria	10–15	**2.92**	**1.91**	*	**5.77**	**3.76**	***	**3.91**	**2.55**	**	**4.09**	**2.67**	*	**3.83**	**2.50**	**	–	–	–	–	–	–
Fungi	0–5	**7.41**	**3.75**	**	–	–	–	–	–	–	**4.75**	**2.40**	*	–	–	–	–	–	–	–	–	–
Fungi	5–10	**29.02**	**7.12**	***	**23.07**	**5.66**	**	–	–	–	–	–	–	–	–	–	**16.82**	**4.13**	**	**15.01**	**3.68**	**
Fungi	10–15	**11.87**	**2.96**	**	–	–	–	**11.97**	**2.99**	**	–	–	–	–	–	–	–	–	–	–	–	–
Archaea	0–5	**6.60**	**2.43**	***	**11.35**	**4.18**	***	–	–	–	–	–	–	–	–	–	–	–	–	**13.42**	**2.05**	**
Archaea	5–10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Archaea	10–15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Results show marginal tests using the dbRDA model, where var. indicates the proportion of microbial community variation (Var) explained by the predictor variable.
Values in bold indicate significant differences according to the dbRDA model.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

4. Discussion

4.1. Changes of the soil physico-chemical and microbiological properties along the climosequence

As confirmed by the present study, altitudinal-defined climate conditions provide specific vegetation types and soil environments thereby influencing the diversity, abundance and activity of soil microbial communities (Djukic et al., 2010; Zhang et al., 2013). On the one hand, we found that both microbial biomass and activity assessed by SIR and basal respiration were more expressed at the N- than at the S-facing sites. Along these lines, Ascher et al. (2012) reported higher levels of microbial biomass, determined by the total amount of phospholipid fatty acids (PLFAs) and the yields of intracellular DNA, on northern slopes in the Trentino area, in the vicinity of the study sites of the present work. Nahidan et al. (2015) also detected higher values of microbial biomass carbon at N-facing slopes (using the fumigation–incubation method). Additionally, Kang et al. (2003) measured an increased soil respiration, determined by infrared gas analyser, at N-facing sites when compared to the S-facing slope irrespective of the altitude (220–1100 m a.s.l.) in three mountain forests in Korea. However, an opposite trend was observed by Carletti et al. (2009), who found higher levels of soil microbial biomass carbon at the S-facing sites. The disparity between our results and those reported by Carletti et al. (2009) must also be interpreted in the light of the 'method-result effect' (Nannipieri et al., 2003). Indeed, within our study we also observed a different effect of exposure on microbial biomass depending on the methodology used (dsDNA *vs.* SIR).

Furthermore, altitude- and enzyme-specific exposure effects were detected along our climosequence. This supports the importance of performing a multiple enzyme assay to avoid misinterpretation of environmental changes induced effects by considering single enzyme activities as indicators of specific nutrient cycles (Nannipieri et al., 2012). Two enzyme activities involved in the C cycle were checked in the present study, namely the β-glucosidase and the acetate esterase. The first one has a relevant role in soils because it catalyses the hydrolysis of cellulose, one of the main components in the plant debris (Nannipieri et al., 2012). Its potential activity was greater in soils from the N- than those from the S-facing slopes primarily at the altitude of 1600 m a.s.l., which is in accordance with the positive correlation found between the OM levels (assessed as volatile solids) and β-glucosidase activity at this study site (N_3-S_8, $R = 0.850$, $p \le 0.001$). Nonetheless, no exposure effect was observed for β-glucosidase activity at the higher sites (2000 and 2400 m a.s.l.). This might be due to the higher proportion of grassland and a colder climate compared to the other sites surveyed, thus influencing the enzymatic processes involved in the plant debris decomposition, and hence the β-glucosidase activity (Blagodatskaya et al., 2016). It is also known that fungi are the predominant source of certain enzymes like glucosidases in soil (Hayano and Tubaki, 1985). No significant differences in the fungal abundance were, however, recorded between N- and S-facing slopes.

Moreover, no exposure effect was detected for both acetate esterase and chitinase potential activities regardless of the altitude. This could be due the fact that these two latter enzymes are involved in the degradation of SOM components with slower turnover times (acetic acid esters and chitin, respectively) than those degraded by β-glucosidase. The differences in the SOM content with respect to slope exposure might have affected to a lesser extent the potential activities of these two enzymes. Additionally, this could indicate that the turnover of high molecular compounds (like chitin) is less sensitive to the temperature differences between both slope exposures (Table 1). Significant differences in the leucine-aminopeptidase potential activity with slope exposure were only recorded for the altitude of 1400 m a.s.l., registering a higher activity at south exposure. Previous studies (Sinsabaugh et al., 2005; Ramirez et al., 2012) suggested that higher N concentrations could limit the related enzyme activities in soil, which could partly explain why a greater activity was measured at the S-facing site that are characterised by a lower inorganic N pool. Sinsabaugh et al. (2008) also observed a higher leucine-aminopeptidase activity responsible for the release of amino acids from polypeptides with increasing soil pH levels (pH 4 to 8.5). Although both slope exposures were generally acidic, we found an increase in pH at S-facing sites. Moreover, it has been reported that bacteria play an important role in the production of leucine-aminopeptidases (Burke et al., 2011) and, accordingly, we detected higher bacterial gene copy numbers at the S-facing slope.

Acid and alkaline phosphomonoesterases are responsible for the mineralisation of organic P into phosphate by hydrolysing phosphoric (mono) ester bonds under acid and alkaline conditions, respectively (Nannipieri et al., 2012). They have an important role for the P cycling in forest ecosystems, particularly where P availability may limit plant productivity (Nannipieri et al., 2003). In our study, we found a greater acid phosphomonoesterase activity at the N-facing slopes. Although plants exude acid phosphomonoesterases, especially under P deficiency, the majority of phosphatase enzymes in soil probably originate from microorganisms (Bünemann, 2015). Indeed, we found a positive correlation between acid phosphomonoesterase activity and soil microbial biomass (dsDNA) for each pairs of sites (N_1-S_6, $R = 0.848$, $p \le 0.001$; N_2-S_7, $R = 0.721$, $p \le 0.001$; N_3-S_8, $R = 0.871$, $p \le 0.001$; N_4-S_9, $R = 0.754$, $p \le 0.001$; N_5-S_{10}, $R = 0.567$, $p \le 0.05$).

Tabatabai (1994) stated that bacterial phosphatases have a higher pH optimum than fungal phosphatases and as such, soils dominated by alkaline phosphatases are expected to have larger bacterial populations. Accordingly, a greater alkaline phosphomonoesterase activity was recorded at the south-facing slopes characterised by less acidic conditions. Arylsulphatase activity, which is involved in the hydrolysis of organic sulfate esters into inorganic S (Turner, 2010), was also more pronounced at the south-facing slopes, where we found a higher bacterial abundance. According to Makoi and Ndakidemi (2008) bacteria

secrete arylsulphatases into the environment as a response to S limitation. In disagreement with our findings, Nahidan et al. (2015) observed higher alkaline phosphomonoesterase and arysulphatase activities at the N-facing slopes in a rangeland ecosystem of west central Iran.

Although microbial biomass and activity were more pronounced at the N-facing slopes, the higher bacterial abundance was recorded at the S-facing slope along the altitudinal gradient. It has been shown that bacterial growth is more favoured at neutral or slightly alkaline conditions (Rousk et al., 2010). Even small variations in the soil pH range (Pennanen et al., 1998; Siles and Margesin, 2016) such as those observed in the present study, in which soil pH values differed by 1 unit between both slope exposures, may induce changes in the bacterial abundance and diversity. Soil acidity was also one of the most important driving factors for the community structure of bacteria inhabiting the 5–10 and 10–15 cm soil layers as indicated by the dbRDA model analysis (Table 5). This was not the case for the bacterial DGGE profiles from the uppermost topsoil (0–5 cm); indicating that other abiotic factors like total C affected the bacterial community diversity to a larger extent than soil pH. However, fungi were not affected by exposure, indicating that they were less affected than bacteria to variations in soil pH with exposure. It should also be mentioned that we found a high variation among replicates with regard to qPCR quantification, probably due to the variation inherent to the soil heterogeneity in the study area. Compared to fungi and bacteria, archaea are not among the primary decomposers of SOM (Singh et al., 2012a, 2012b). They are nonetheless expected to have an important role in alpine settings as they are known to be well adapted to the harsh conditions of these environments (Singh et al., 2012b; Xu et al., 2014). We found that the impact of exposure on the archaeal abundance was altitude-dependent, registering a higher copy number at the N- than at the S-facing slopes for the altitudes of 1400 and 2000 m a.s.l. Both soil pH and the C/N ratio were the main drivers affecting the abundance and diversity of archaeal communities in these study sites.

4.2. Shifts in the soil physico-chemical and microbiological properties with soil depth

We hypothesised that exposure and altitudinal effects on the studied microbiological parameters would be more evident in the uppermost topsoil layer (0–5 cm), because this soil layer is more exposed to the changing environmental conditions such as shifts in temperature and/or OM input. This hypothesis seems to be confirmed for microbial activity (BR). Furthermore, a reduced microbial biomass and activity was measured in the soil layer 10–15 cm. Similarly, Fierer et al. (2003) detected a reduced soil microbial biomass with increasing soil depth using different approaches such as SIR, total PLFA content and chloroform fumigation-extraction. Accordingly, we found that the hydrolytic enzyme activities were also reduced with soil depth. Such a reduction occurred in concert with a decline in soil moisture, total C and N, and inorganic N contents. It is known that the availability of substrate for enzymatic breakdown decreases with depth, which may result in a spatial disconnection of enzyme and substrate (Holden and Fierer, 2005). No changes in soil pH, one of the factors often associated with enzyme activities, were recorded within the three topsoil layers.

The organic matter in the deeper layers is due to decomposition products from aboveground or rhizo-deposition (Lejon et al., 2005; Schmidt et al., 2011). The decreasing availability of fresh organic material with increasing depth is likely to affect the autochthonous microbiota in terms of abundance, richness and diversity (Schnecker et al., 2015; Xu et al., 2015). Accordingly, a reduced bacterial abundance and diversity was found in the 10–15 cm soil layer in comparison with the top 5 cm. Although no changes were observed for fungi in terms of abundance we measured an increase in fungal richness and diversity with soil depth. This could be attributed to mycorrhizal associations and rooting distribution in these deeper layers (Lejon et al., 2005). Furthermore, the higher archaeal abundance, richness and diversity with increasing soil depth underline the capacity of this microbial group to adapt to less favourable environmental conditions. In accordance with our findings, Kemnitz et al. (2007) found an increase in archaeal abundance (gene copy numbers) along a 20 cm soil profile in an acidic forest soil in Marburg (Germany).

On the whole, a clear discrimination between north- and south-facing slopes was detectable for bacterial communities (DGGE-genetic fingerprinting) for each of the three soil depths; whereas it was soil layer-dependent for fungal and archaeal communities. These specific microbial responses could be due to the different soil physico-chemical characteristics ascribed to each soil depth (Egli et al., 2009, 2010; Ascher et al., 2012).

5. Conclusions

Our complex climosequence approach provided evidences that thermal conditions (exposure and altitude) and consequently the state factor *climate,* shape edaphic properties and soil microbiota in terms of biomass, activity and composition, supporting and deepening previous findings from the Trentino area (Ascher et al., 2012). Our results showed (1) a higher microbial biomass (SIR) and activity (BR) in the north-facing slopes irrespective of the altitude, contradicting our initial hypothesis. The discrepancy to findings in literature with respect to the microbial biomass results might be ascribed to the different methodology used (dsDNA *vs.* SIR). (2) Altitude- and enzyme-specific exposure effects were detected along the climosequence. (3) The three microbial domains responded differently to exposure in terms of abundance: bacteria (S > N; altitude-independent), fungi (N ~ S), archaea (N > S; altitude-dependent). (3) The NMDS ordination plots enabled a new view on the exposure effects on the microbial community structures, whereas changes in both phylotype richness and diversity in function of the slope exposure were only detected for the archaeal community (N > S; dependent on the altitude). (4) The effect of climate was most pronounced in the uppermost topsoil-layer, however only for the microbial activity. On the whole, our findings encourage the application of a similar climosequence approach in different Alpine environments, so as to further discriminate among the complex multiple-factors shaping soil biota in the context of global warming.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.09.176.

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