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Soil microbial biomass, activity and community composition along altitudinal gradients in the High Arctic (Billefjorden, Svalbard)

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Abstract The unique and fragile High Arctic ecosystems are vulnerable to proceeding global climate warming. Elucidation of factors driving microbial distribution and activity in Arctic soils is essential for comprehensive understanding of the ecosystem functioning and its response to environmental change. The goals of this study were to investigate the microbial biomass, activity, microbial community structure (MCS) and its abiotic controls in soils along three elevational gradients in coastal mountains of Billefjorden, Central Svalbard. Soils from four different altitudes (25, 275, 525, and 765 m above sea level) were analysed for a suite of characteristics including temperature regimes, organic matter content, base cation availability, moisture, pH, basal respiration, and microbial biomass and community structure using phospholipid fatty acids (PLFA). We observed significant altitudinal zonation of most edaphic characteristics reflected by soil microbial properties. The microbial biomass and activity normalized per unit of organic carbon significantly increased with elevation. The two dominant microbial groups, fungi and bacteria, had different habitat preferences, resulting in high fungi to bacteria (F/B) ratios at the most elevated sites. The changes in MCS were mainly governed by the bedrock chemistry, soil pH, organic carbon content and soil moisture. While the direct impact of summer soil temperature regimes on soil microbes was likely negligible, it´s influence on plant distribution along the gradients have strong implications for edaphic conditions and consequently also for soil microbes. Our results highlight the need to consider unvegetated high elevation areas as hotspots of microbial activity and important habitats within the High Arctic ecosystem.

1 Introduction

Knowledge about the distribution and activity patterns of soil microbial communities is essential to understand ecosystem functioning as the soil microbes play fundamental role in biogeochemical cycling and drive productivity in terrestrial

ecosystems (van de Heijden et al., 2008). The altitudinal gradients offer great opportunity to study distribution of microbial communities well adapted to local environment and explain this patterning by natural gradients of soil conditions and climate regimes over short spatial distances (e.g. Ma et al., 2004; Margesin et al., 2009). Since the proceeding climate change will strongly affect environmental conditions in the Arctic (Collins et al., 2013), our understanding of microbial distribution and activity patterns along the arctic alpine gradients together with their controlling factors can help us to better understand current functioning and predict future development of ecosystems in this region.

Despite the fact that Arctic tundra comprises 5% of the land on Earth (Nemergut et al., 2005) and most coastal areas in the northern circumpolar region have mountainous character, our knowledge about activity and structure of soil microbial communities along mountainside gradients in the Arctic is very limited. The research on spatial variation in microbial community composition and activity in polar regions was conducted mainly at narrow elevation range (Oberbauer et al., 2007; Trevors et al., 2007; Björk et al., 2008; Van Horn et al., 2013; Blaud et al. 2015; Tytgat et al., 2016) or focused on initial soil development following glacier retreat (Bekku et al., 2004; Yoshitake et al., 2007; Schűtte et al., 2010). As such, these studies could not cover the effect of edaphic gradients associated with increasing altitude and changing microclimate (Cebon et al., 1999). In contrary, the research on elevational patterns of microbial community structure (MCS) and activity has been conducted mainly in mountain regions of lower latitudes from tropics to temperate zone. Several studies have shown that microbial activity generally decreases as the elevation increases (Schinner, 1982; Margesin et al., 2009; Xu et al., 2014). However, the studies aiming to describe the soil microbial diversity and community structure indicated that there are no general altitudinal trends. For example, the microbial community composition did not differ along elevational gradients in Swiss Alps (Lazzaro et al., 2015), while other studies have documented that microbial biomass (Ma et al., 2004; Giri et al., 2007; Margesin et al., 2009), bacterial (Ma et al., 2004; Lipson, 2007; Shen et al., 2013) and fungal (Schinner and Gstraunthaler, 1981) diversity decreases as altitude increases, and several studies reported the mid–altitudinal peak in microbial diversity (Fierer et al., 2011; Singh et al., 2012; Meng et al., 2013). Beside differences in fungal and bacterial diversity, the relative abundance of these main microbial functional groups is also variable. For example, Siles and Margesin (2016), Djukic et al. (2010), and Xu et al. (2014) found decreasing fungi to bacteria (F/B) ratio as elevation increases, while Margesin et al. (2009) reported increasing relative abundance of fungi with altitude in Central Alps, resulting in higher F/B ratio at more elevated sites.

The research focusing on environmental controls over microbial communities in polar and alpine regions recognized many significant factors, including vegetation, litter C : N stoichiometry, organic carbon content, soil pH, nutrient availability, microclimatic conditions, and bedrock chemistry. However, the effect of these variables was site specific, disabling any generalization of these findings at global scale. The studies linking edaphic conditions and soil microbial properties along elevational gradients directly in the Arctic are thus fundamental to understand functioning of unique and fragile Arctic ecosystems and predict their future development. So far, only few studies assessing altitudinal trends in soil microbial properties were conducted in the Scandinavian Arctic (Löffler et al., 2008; Männistö et al. 2007). To

extend our knowledge about microbial ecology and soil functioning in the arctic alpine ecosystems, we conducted study aiming to assess the activity, biomass and structure of soil microbial communities and determine their controlling environmental factors along three altitudinal gradients in Billefjorden, Central Svalbard. The specific objectives of this study were (i) to describe gradients of microclimatic and geochemical soil properties; (ii) to assess microbial activity (soil basal respiration) and abundance of main microbial groups (fungi, Gram–negative and Gram–positive bacteria, Actinobacteria, phototrophic microorganisms) using phospholipid fatty acid (PLFA) analysis; and (iii) to identify environmental factors explaining the trends in soil microbial parameters along these altitudinal gradients.

2 Materials and methods

2.1 Study site and soil sampling

In August 2012, we collected soils from three altitudinal gradients located on the east coast of Petunia bay, Billefjorden, Central Svalbard (78° 40´ N, 16° 35´ E). The annual mean, minimum and maximum air temperatures recorded in the area at the sea level in the period of 2011–2013 were –3.8, –28.7 and 16.2 °C, respectively, and the temperatures stayed permanently below 0 °C for eight months a year. The mean annual precipitation in the Central Svalbard area is only 191 mm (Svalbard Airport, Longyearbyen, 1981–2010) and is equally distributed throughout the year (Førland et al. 2010).

The three altitudinal gradients (Gr1–3) had similar exposition (Gr1 W–E, Gr2 WNW–ESE, Gr3 WSW–ENE; Fig. 1). Four sampling sites at different altitudes: 25, 275, 525 and 765 m (± 5 m) above sea level (a.s.l.) were selected along each gradient in geomorphologically stable areas with a similar slope (20±5°), representing climosequences from high Arctic tundra to unvegetated barren soils. Vegetation of two lowest sites was characterized by *Dryas octopetala* dominated communities, with significant contribution of *Cassiopetetragona*, *Saxifragaoppositifolia* and variable contribution of grasses (C*arexnardina, C. rupestris, C. misandra*; Prach et al., 2012). The vascular plants species formed scattered vegetation patches at the altitude 525 m a.s.l. with *Salix polaris*, *Saxifraga oppositifolia* and *Papaver dahlianum* being the most abundant species, while the soils at the most elevated sites were covered by biological soil crusts. The soils were classified as Leptic Cryosols (Jones et al., 2010) with loamy texture, considerable amount of gravel and clay content increasing with altitude (Table 2). The soils were particularly shallow, from 0.1–0.2 m depth at 25 m a.s.l. to only few cm of soil overlaying a rocky horizon at the most elevated site.

On each sampling site, nine soil cores (4 cm deep, 5.6 cm diameter) were collected and mixed into three representative samples. Each representative sample was mixed from one soil core taken from the edge of the vegetation tussocks (if vegetation was present) and two other cores taken in increasing distance from the vegetation to maintain the consistency with respect to heterogeneity of vegetation cover and soil surface. The triplicates were collected approximately 5 m apart from each other. Immediately after sampling, the soil was sieved (2mm) to remove larger rocks and roots, sealed in

plastic bags and kept frozen till further processing. Soil subsamples for biomarker analysis were later freeze–dried and stored at –80°C until extraction.

2.2 Monitoring of microclimatic characteristics

To describe the soil microclimatic conditions along the altitudinal gradients, we measured soil temperature at –5 cm directly at the sampling sites of Gr1 using dataloggers (Minikin Ti Slim, EMS Brno, CZ), and determined the soil water content in soil subsamples by drying to constant weight at 105°C. The temperature regimes at particular altitudinal levels were characterized by 10 climatic variables (Table 1).The period of above–zero daily mean ground temperatures is referred to as summer season throughout the text. We also considered number of days with daily mean ground temperatures above 5 °C, characterizing a period with conditions suitable for growth of vascular plants (Kleidon and Mooney, 2000). The positive soil surface energy balance was calculated as a sum of daily mean summer temperatures. The records from three years (2011–2013) continuous measurements at two automated weather stations located at 25 and 495 m a.s.l. approximately 3 km apart from the observed gradients (hereafter referred as AWS_{25} and AWS_{495}, respectively; Fig. 1) were used to evaluate seasonal variation of soil temperature and moisture regimes (Figs. S1, S2, respectively), and coupling of soil and atmospheric temperatures (measured at –5 cm and 2 m above terrain, respectively; Fig. S1). Even though we were not able to continuously measure soil moisture directly at the sampling sites, we regarded data from both AWS locations as representative for the evaluation of seasonal moisture regimes at our sampling sites due to their similar character.

2.3 Soil characteristics

The particle size distribution was assessed using aerometric method (Lovelland and Whalley, 2001), the soil type was classified according to U.S. Department of Agriculture. The soil pH was determined in soil–water mixture (1:5, w/v) using glass electrode. The cation exchange capacity (CEC) was considered to be equal to the sum of soil exchangeable base cations Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} extracted with 1M NH_4Cl (Richter et al., 1992). The amount of H^{+} and Al^{3+} ions was neglected due to high soil pH. Base cations accessible for plant and microbial growth (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) were extracted by the Mehlich 3 reagent (Zbíral and Němec, 2000). Cations were measured by atomic absorption spectroscopy (AA240FS instrument, Agilent Technologies, USA). Total soil organic carbon (TOC) and nitrogen (TN) contents were measured in HCl fumigated samples (Harris et al. 2001) using elemental analyser (vario MICRO cube, Elementar, Germany). The soil NO_{3-} and soluble reactive phosphorus (SRP) contents were determined in water extracts from 10 g field–moist soil subsamples (4:1, v/w) using Flow Injection Analyzer (FIA Lachat QC8500, Lachat Instruments, USA).

2.4 Basal and specific microbial respiration

The potential microbial activity was characterized by basal soil respiration (BR) in the incubation experiment. Soil subsamples (10 g of slowly melted field–moist soil) were pre–incubated in 100 mL flasks at 6 °C. After 14 days, the flasks were thoroughly ventilated, sealed and BR was measured as the CO_2 production in 24 h using Agilent 6850 GC system (Agilent technologies, CA, USA). The specific respiration (SR) per unit of microbial biomass was calculated as BR/$PLFA_{tot}$ ratio.

2.5 Microbial community structure

The soil microbial community structure was defined by an analysis of PLFA according to modified protocol of Frostegård et al. (1993). Briefly, 1–3 g (according to TOC content) of freeze–dried soil samples was extracted twice with single–phase extraction mixture consisting of chloroform, methanol and citrate buffer. After overnight phase separation achieved by adding more chloroform and buffer, the organic phase was purified on silica columns (SPE–SI Supelclean 250mg/3 mL; Supelco®, PA, USA) using chloroform, acetone and methanol. The polar fraction was trans–esterified to the fatty acid methyl esters (FAME) (Bossio and Scow, 1998). All FAMEs were quantified by an internal standard calibration procedure using methyl–nonadecanoate (19:0) as an internal standard. To identify the FAMEs, retention times and mass spectra were compared with those obtained from standards (Bacterial Acid Methyl Esters standard, the 37–component FAME Mix, PUFA–2, and PUFA–3; Supelco, USA). The ISQ mass spectrometer (MS) equipped with Focus gas chromatograph (GC) (Thermo Fisher Scientific, USA) was used for chromatographic separation and detection.

Only specific PLFAs were used to assess the microbial community structure: a14:0, i15:0, a15:0, i16:0, i17:0, a17:0 were used as markers of Gram–positive bacteria (G+); 16:1ω9, 16:1ω5, cy17:0, 18:1ω11, 18:1ω7, cy19:0 as markers of Gram–negative bacteria (G–); 10Me16:0 and 10Me18:0 as markers of Actinobacteria (Kroppenstedt, 1985), 18:1ω9, 18:2ω6,9 as markers of fungi (Frostegård and Bååth, 1996) and polyunsaturated fatty acids 18:4ω3, 20:5ω3 were used as markers of phototrophic microorganisms (Hardison et al., 2013; Khotimchenko et al., 2002). A sum of Actinobacterial markers, PLFAs specific to G+ and G– bacteria and general bacterial markers 15:0, 17:0 and 18:1ω5 was used to calculate bacterial biomass and fungi to bacteria (F/B) ratio. The sum of all lipid markers mentioned above and nonspecific PLFAs 14:0, 16:0, 18:0 and 16:1ω7 was used as proxy for microbial biomass ($PLFA_{tot}$).

2.6 Sterol analyses

Ergosterol, β–sitosterol and brassicasterol were used as biomarkers of fungal biomass (Gessner et al., 1991), plant (Sinsabaugh et al., 1997) and algal (Volkman, 1986; 2003) residues in organic matter (OM), respectively. Sterols were simultaneously determined using microwave assisted extraction adapted from Montgomery et al. (2000) and GC/MS (ISQ MS equipped with Focus GC, Thermo Fisher Scientific, USA) analysis. Briefly, 0.5 g of freeze–dried soil was treated with 6 mL of methanol and 2 mL of 2 M NaOH. Vials were heated twice at the centre of a microwave oven (2450 MHz and 540 W output) for 25 s. After cooling, the contents were neutralized with 1 M HCl, treated with 3 mL of methanol and extracted with hexane (3×4 mL). Extracts were spiked by an internal standard (cholesterol), evaporated and derivatized by adding of pyridine and 1 % BSTFA at 60 °C for 30 min prior analysis. Sterols were quantified by an internal standard calibration procedure.

2.7 Statistical analyses

All data were checked for normality and log–transformed if necessary. The significance of environmental gradients and corresponding shifts in MCS (relative abundances of summed PLFA specific for fungi, G– and G+ bacteria, Actinobacteria and soil phototrophic microorganisms) in horizontal direction (ie. effect of gradient) and vertical direction (ie. effect of altitude) were tested using the partial redundancy analyses (RDA) with covariates. Variation partitioning was subsequently performed to quantify the unique and shared effects of altitude and gradient on spatial variability of MCS. Forward selection procedure was used to identify the soil geochemical parameters best explaining the shifts in MCS. Only *P* values adjusted by Holms correction were considered. In next step, we tested the significance of remaining MCS patterns related to gradient or altitude using the previously selected edaphic properties as covariates. The multivariate tests were performed without standardization by samples, but with centering and standardization by variables (because the variables were not always measured at the same scale, see Šmilauer and Lepš 2014) and Monte Carlo test with 1999 permutations. Only adjusted explained variation was referred throughout the text. Since the soil characteristics of the sample triplicates characterizing each sampling site are likely auto–correlated due to low inter–sample distance, only the whole–plots (ie. sampling sites) were freely permuted, while the split–plots were exchangeable only within the whole–plots. Altitudinal differences in particular soil and microbial parameters along respective gradients were addressed by ANOVA and Tukey–HSD post hoc test. All statistical tests were considered significant at $P < 0.05$. Multivariate statistical analyses were performed with CANOCO for Windows version 5.0 (Ter Braak and Šmilauer 2012), for ANOVA, Tukey HSD test and correlations between soil and/or microbial parameters, Statistica 13 was used (StatSoft, USA).

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3 Results

3.1 Altitudinal changes in soil microclimate

The soil microclimate at the studied sites was characterized by two distinct periods, respecting the air temperature dynamics (Fig. S1). The longer winter period lasted typically from the middle of September to early June. The winter soil temperatures were strongly stratified according to the elevation and the temperature means decreased from –4 °C at 25 m a.s.l. to –10 °C at 765 m a.s.l. (Table 1, Fig. 2). In contrast, a short summer period was characterized by significant diurnal fluctuation of soil temperatures and weak altitudinal temperature stratification (Fig. 2). The length of the summer season more than doubled at the lowest elevations compared to the most elevated study sites, while the period with daily mean soil temperatures above 5 °C shortened almost four times along the gradient. Correspondingly, the positive surface energy balance gradually decreased with altitude (Table 1). The maximum daily mean temperatures and diurnal temperature fluctuation were highest at the mid–elevated sites, with the highest mean summer soil temperature at 275 m a.s.l. In contrary, the least and most elevated sites experienced lower summer maximum daily means and lower soil temperature amplitudes (Table 1). The effect of altitude on soil moisture content was significant along Gr1 and Gr3 ($P < 0.001$ and 0.01, F = 22.76 and 7.39, respectively) with the lowest sites being driest and the highest sites moistest, but nonsignificant along Gr2. Continual volumetric measurements of soil water content at AWS_{25} and AWS_{495} in 2011–2013 showed that the soil moisture apparently fluctuated only in June and the beginning of October as a result of freeze–thaw cycles when temperature oscillated around zero (for more information, see Fig. S2**).** The desiccation events did not occur during the summer periods 2011–2013.

3.2 Gradients of soil geochemical properties

Gradient and elevation significantly affected soil geochemical properties (partial RDA, pseudo–F = 8.1, $P < 0.001$). Both factors explained 58 % of the total variation in soil characteristics and most of the explained variance (72 %) was by the RDA ascribed to the vertical zonation (Fig. S3a). The effect of elevation was significantly reflected in all soil properties listed in Tables 2 and 3, but the altitudinal trends were in most cases specific for particular gradients, as indicated by significant interactive effect between gradient and altitude (Tables 2, 3). The CEC and availabilities of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} were spatially variable and reflected complicated geology of the Petunia bay area. The apparent patterns were higher contents of available Mg^{2+} and K^{+} in soils from Gr1 and significantly increasing Mg^{2+} availability along this gradient (Table 2). The soil moisture content decreased with elevation along Gr1 and Gr3, but was stable at all sites along Gr2 (Table 2). The mean soil pH ranged from 7.8 to 9.0 and increased with altitude (Table 2, Fig. S3a) while the soil TOC and TN contents declined from lower to upper elevations along all gradients. The only exception was the lowest site at Gr2, where we found low soil OM content compared to respective sites from other two gradients. The C : N ratio, sitosterol content and the ratio between plant–derived sitosterol and brassicasterol of algal origin were the only soil characteristics affected solely by site

elevation. Their values uniformly decreased with elevation irrespective of soil OM content (Table 3), thus indicating altitudinal shift in OM quality and origin. The soils from the most elevated site on Gr1 had the lowest OM content and the highest pH and Mg^{2+} availability. This site differed pronouncedly also in microbial properties (see below) from respective sites on Gr2 and Gr3 (Tables 2 and 3; Figs. 3a, c).

3.3 Microbial biomass and activity along the altitudinal gradients

The soil PLFA content did not show any consistent trends related to gradient or elevation. The microbial biomass significantly decreased along Gr1 due to very low biomass at the most elevated site, while the highest PLFA amounts along Gr2 were found at 275 and 765 m a.s.l. and sites along Gr3 contained stable and high PLFA amounts (Fig. 3a, Table 3). The soil PLFA content was most strongly correlated with soil TOC and TN contents (r = 0.773 and 0.719, respectively; both P < 0.0001), soil moisture (r = 0.772; P < 0.0001) and Mg^{2+} availability (r = –0.775; P < 0.0001). The BR in soils along Gr1 was significantly lower in the two most elevated sites compared to respective sites along Gr2 and Gr3 and differed also in the elevational trend – the microbial activity along Gr1 decreased from lower to upper elevations (Fig. 3c). The BR was significantly correlated only with Ca^{2+} and Mg^{2+} availability (r = 0.489 and –0.545; P = 0.003 and 0.001, respectively). The SR did not differ between gradients and showed relatively uniform elevational pattern, being significantly higher at the most elevated sites compared to lower altitudes (Fig. 3e). With respect to significant correlation of microbial biomass with the soil OM as a substrate for growth, and spatial heterogeneity of soil OM content (Table 3), we normalized the microbial biomass and activity per unit of organic carbon content. These data revealed significantly increasing normalized soil microbial biomass and BR with altitude along Gr2 and Gr3. Along Gr1, however, the soils from both mid–elevated sites had higher normalized microbial biomass and activity compared to the highest and lowest sites (Fig. 3b, d).

3.4 MCS along the altitudinal gradients and its controlling factors

The partial RDA revealed significant interactive effect of elevation and gradient on MCS (pseudo–F = 4.8, P < 0.001). Both factors explained 51 % of the total variation in the MCS – the model ascribed 66 % of explained variability to altitude, 26 % to gradient, and 8 % of explained variability was shared by both factors. The changes between and along gradients were significantly driven by the soil geochemical properties (pseudo–F = 7.1; P < 0.001), which explained 72 % of the variation in the MCS. The test was significant also for the whole PLFA profile (pseudo–F = 7.3; P < 0.001, 76 % of explained variability) and the projection of all soil geochemical properties and microbial PLFAs to the ordination space revealed very similar elevational patterns (see Fig. S3). The forward selection of explanatory variables retained four geochemical parameters – Mg^{2+} availability, pH, moisture and TOC content, all together accounting for 55 % of variation in the data (pseudo–F = 11.6, P < 0.001). The subsequent reanalyzes of the gradient effect using selected soil properties as covariates

was non–significant (pseudo–F = 1.3, P = 0.533), while altitude still accounted for 31 % of variation in the MCS and its effect remained significant (pseudo–F = 5.7, P = 0.001).

The most pronounced shift in the MCS was given by different altitudinal preferences of fungi and bacteria. While the bacterial PLFAs were consistently more abundant in soils from lower elevations, the fungal contribution to total PLFA content increased with elevation (Figs. 4, S3c). These changes resulted in gradual increase of F/B ratio along all three gradients (Fig. 6b).The bacteria preferred soils with lower pH and higher TOC and moisture contents, while the fungal importance within the microbial community increased in opposite conditions towards to higher elevations (Fig. 4). The PLFAs of Actinobacteria as well as phototrophic microorganisms had higher relative abundance in soils from more elevated sites (Figs. S3 b,c). Our data have shown decreasing abundance of G– bacteria relative to Actinobacteria with altitude (Fig. 6C). Similar significant trend was also found for the ratio between G– and G+ bacteria along Gr 1, but not along Gr2 and Gr3 (Fig. 6d). In general, PLFA specific to G– bacteria were more abundant than PLFAs of G+ bacteria (Fig. 6d; mean G–/G+ ratio ± SD = 1.76 ± 0.17; n = 36). The most discrepant site in terms of MCS was the most elevated locality along Gr1 (Fig. 5), typical by high abundance of PLFAs specific to Actinobacteria, while the fungal PLFAs were less abundant compared to respective sites along Gr2 and Gr3 (Fig. 6a).

4 Discussion

4.1 Edaphic conditions along the altitudinal gradients

The study sites along elevation gradients faced up different microclimatic regimes. The altitudinal soil temperature stratification in winter did not correspond to the course of atmospheric temperatures (compare Fig. S1a and S1b). This was caused by a weak or absent snow cover insulation due to generally low precipitations (Førland et al., 2010) complemented by the effect of wind reducing snow cover at open areas of higher altitudes. The harsh winter soil microclimate at high elevations contrasted to temperature regimes in most temperate alpine ecosystems in Europe or North America, experiencing temperatures slightly below zero due to significant insulation from heavier snowpack (Ley et al., 2004; Nemergut et al., 2005; Djukic et al., 2013). The pronounced altitudinal stratification of soil temperatures controls microbial activity during winter (Nikrad et al., 2016), but may also impact microbial properties during the consecutive summer period (Welker et al., 2000; Ley et al., 2004), as well as seasonal dynamics of microbial community structure (Lipson et al., 2002; Lipson and Schmidt, 2004; Nemergut et al., 2005).

As summer season became longer at lower elevations, the increasing positive surface energy balance (Table 1) had strong positive impact on the occurrence of vascular plants in lower elevated sites (Kleidon and Mooney, 2000; Klimeš and

Doležal, 2010). The comparison of mean summer temperatures and diurnal temperature fluctuations along the Gr1 (Table 1) imply that the lowest and highest sites experienced on average colder, but more stable soil microclimate during the summer period compared to the mid–elevated sites. The soil water content in the study area was according to the long–term volumetric measurements from AWS_{25} and AWS_{495} relatively stable in time (Figure S2). It could be due to maritime summer climatic conditions with high cloudiness and fog occurrence, maintaining the soil moisture levels (Sawaske and Freyberg, 2015). However, soil moisture content was significantly variable in space (Table 2), which could be ascribed to differences in soil OM content and soil texture. In summary, our data suggested that summer soil microclimatic regimes did not correspond with increasing altitude in terms of mean temperatures and temperature stability. It is thus questionable, whether the summer soil microclimate could directly affect the significant altitudinal trends in soil microbial properties. However, the effect of altitude on MCS remained significant when we subtracted the effect of soil geochemical properties from the multivariate statistics, while the effect of gradient did not. We thus suggest that the remaining portion of variability in the MCS was related with microclimatic gradients, but rather indirectly through its effect on plant distribution. Further, the soil microorganisms along the investigated gradients did not suffer from desiccation, commonly identified among the most stressing events in polar and alpine ecosystems (Ley et al., 2004; Van Horn et al., 2013; Tytgat et al., 2016).

The increasing occurrence and biomass of vascular plants down the slope resulted in growing soil OM content. The only exception was the lowest site of Gr2 with relatively low OM content, comparable with more elevated sites along this gradient. We explain this discrepancy by the proximity of glacier stream, which could wash away the upper soil organic layer during abnormal spring–melt events in the past. The presence of vascular plants influenced also the OM quality. The increasing content of sitosterol documented growing contribution of undecomposed plant–derived material to soil OM with decreasing altitude. This growing plant litter inputs at low elevations led to higher C : N stoichiometry of OM and increased the capacity of the soils to retain moisture. Together with increasing OM content and activity of plant roots, the soils become less alkaline, had higher phosphorus availability and contained less Ca^{2+}. The sitosterol was present even in the highest sites, where no vascular plants were present. This indicated a contribution of the plant–derived material to the soil OM likely through a wind transport (Seastedt et al. 2004; Ley et al., 2004). However, the soil sitosterol to brassicasterol ratios gradually decreasing with increasing elevation (Table 3) pointed to growing importance of autochthonous cyanobacterial and algal sources of OM in high elevation habitats. This corroborates with findings of high cyanobacterial abundance during initial stages of soil development (Hodkinson et al., 2003) and in the soil crusts (Pushkareva et al., 2015) on Svalbard, similarly as in high elevation soils in Himalayas (Řeháková et al., 2011; Lazzaro et al., 2015). The elevational gradients in the occurrence of vascular plants and corresponding changes in soil properties and OM quality had important implications for MCS and activity.

4.2 Altitudinal changes in soil microbial biomass and activity

We have found no consistent altitudinal effect either on soil microbial biomass or on its basal respiration. Moreover, each of the microbial characteristic was related to different abiotic factors. While the soil microbial biomass increased with soil OM content and soil moisture similarly as previously documented by Margesin et al., (2009) and Van Horn et al., (2013), basal microbial respiration followed the trends in the availability of double charged base cations. The microbial activity thus revealed contrasting altitudinal trend along Gr1, where it decreased with altitude similarly to other studies (Schinner, 1982; Väre et al., 1997; Niklińska and Klimek, 2007), and along Gr2 and Gr3, where it was higher at the most elevated sites. Interestingly, Mg^{2+} availability was negatively related to both the soil microbial biomass and the basal respiration, which can be explained by the fact that higher Mg^{2+} concentrations decreased the amount of growth and cell divisions in many bacterial species (Webb, 1949a). Additionally, Mg^{2+}availability together with pH also significantly shifted MCS (discussed below). These relations highlight the importance of soil parent material in shaping microbial environment in extreme arctic ecosystems, consistently with other studies from polar region (Van Horn et al., 2013; Tytgat et al., 2016).

The significant altitudinal effect, closely connected with gradual change in soil pH and OM quality, became evident when we normalized soil microbial biomass and activity per unit of soil organic C content. Both normalized characteristics gradually increased with an increasing altitude as a presence of vascular plants and their contribution to soil OM decreased, pH increased and a character of the bedrock became more important. At the same time, fungi to bacteria ratio within the microbial communities increased as well. Such changes imply potentially very different functioning of the microbial communities in the most elevated versus lower sites along the studied altitudinal gradients.

4.3 Exceptionality of the bare soils on the most elevated sites on the gradients

The most elevated, least moist and most alkaline sites were characterized by the highest ratio of living microbial biomass to soil TOC content, with the highest specific activity of the present microbial communities, which were dominated by fungi. We suggest that high normalized microbial biomass, reaching 8 to 10 μmol PLFA g^{-1} TOC and largely exceeding values commonly reported for mountainside gradients (Djukic et al., 2010; Xu et al., 2014), forests (Hackl et al., 2005) and arable soils (Frostegård et al., 1996), related to increasing contribution of microbial primary producers to OM formation in these bare soils. The algal biomass lack lignin–like structures, is poor in complex compounds and lead to formation of OM with low C : N ratio, which implies fast nutrient turnover in the soil (Swift et al., 1979) and corroborates with a high specific microbial activity (Fig. 3e). The shift in the primary sources of organic substrates thus resulted in slow accumulation of low quality OM, which resulted in high portion of living microbial biomass per soil TOC content.

The high normalized microbial activity could relate to shift in the MCS, since the F/B ratio was correlated with soil respiration ($r = 0.649$; $P < 0.001$) more strongly than abundance of particular microbial groups or the soil PLFA content. The

fungal proliferation in the microbial communities at the most elevated sites (the F/B ratios of 0.8–1.4; Fig. 6b) could be ascribed to higher competitiveness of fungi compared to bacteria due to the lower temperature growth optima (Margesin et al., 2003), and corroborates with importance of fungi in alpine soils reported previously (Ley and Schmidt, 2002; Margesin et al., 2009). However, the exceptionally high F/B ratios in our soils point that fungi may play even more important role in arctic than in alpine habitats, where F/B ratios of 0.05–0.2 were commonly reported (Björk et al., 2008; Margesin et al., 2009; Djukic et al., 2010; Hu et al., 2016). Interestingly, we reported increasing F/B ratio with increasing soil alkalinity. As fungi have wider pH–growth optimum than bacteria (Wheeler et al., 1991) and their dominance usually increases with soil acidity (Högberg et al., 2007; Joergensen and Wichern, 2008; Rousk et al., 2009), our data suggest that similar patterns can occur also with increasing soil alkalinity. However, the effect of pH gradients could be confounded by other environmental factors related with vegetation occurrence and its consequences for soil moisture, OM content and quality.

Among the most elevated sites, we should note different character of the highest site on Gr1. This site had most distinct edaphic conditions compared to all other sites such as exceptionally low OM content and soil moisture but very high Mg^{2+} availability, with strong negative effects on both soil microbial biomass and activity. Accordingly, it was occupied by microbial community of the most distinct structure (Figs. 5, 6). Namely, there was the lowest proportion of G– bacteria relative to G+ bacteria and the highest abundance of Actinobacteria among all the sites. This proliferation of G+ microorganisms could be related to their tolerance to high Mg^{2+} availability, which inhibited growth of G– species (Webb, 1949b). Our data further indicated that Actinobacteria partly replaced fungi in Mg^{2+} rich and OM poor soils. Unlike other bacteria and similarly to fungi, Actinobacteria form branching hyphae, play important role in mineralization of complex OM (Prescott et al., 2005), and could thus substitute the fungal role in functioning of microbiome in these high altitude barren soils rich in Mg^{2+} content. Despite these differences, the soil of the most elevated site on Gr1 showed a high specific respiration and proliferation of fungi in the community similarly to respective sites along Gr2 and Gr3.

4.4 The effect of vegetation occurrence on microbial community structure and dynamics

The increasing plant productivity on other sampling sites down the slope resulted in increasing inputs of low quality litter with higher C : N stoichiometry and larger content of structural compounds into the soil. Plant occurrence was further connected with competition for available nutrients and their immobilization in permanent structures (Lipson and Monson et al., 1998). Together, it reduces the proportion of microbial biomass per unit of soil C as well as its normalized and specific respiration. At the same time, the F/B ratio of the microbial community decreased. We suggest that an increasing bacterial contribution to soil microbial community followed growing occurrence of vascular plants, which released easily assimilable organic compounds from their roots during vegetation season (Lipson et al., 1999; Bardgett et al., 2005). The increasing fungal dominance in high elevation habitats indicate that absence of plants due to climatic constraints have inverse consequences for soil MCS compared to development of microbial communities in young soils during succession. Here, the

358 microbial communities switch from being bacterial dominated during early stages of succession to being fungal dominated at
359 the maximal plant biomass phase (Ohtonen et al., 1999). It is interesting to note that the F/B ratios in the most vegetated, but
360 the least elevated sites (this study) corresponded to F/B ratios at the latest stages of soil succession following glacier retreat
361 (Ohtonen et al., 1999).

362 The gradual disappearance of vascular plants along the altitudinal gradients also affected seasonal microbial
363 dynamics in soils. We can support this assumption by closer correlation between fungal PLFAs and soil ergosterol content in
364 soils from the two upper elevations (r = 0.934; P < 0.001; n = 18) than from the two lower elevations (r = 0.587; P = 0.01; n
365 = 18), having also higher ergosterol to fungal PLFA ratio (0.11 ± 0.03compared to 0.21 ± 0,07). Unlike PLFA, the sterols are
366 not cleaved shortly after cell death and remain in soil for longer period (Mille–Lindblom et al., 2004). These trends thus
367 indicated that the actual presence of fungi (PLFAs) coincides better with long–term fungal abundance (ergosterol) in the
368 sites with absence/rare presence of plants than in lower and vegetated tundra sites, where it can be more temporarily variable
369 (lower in summer/higher in winter). The seasonal changes in MCS in the Arctic tundra thus likely corresponded with
370 previously described microbial dynamics in alpine ecosystems, where fast–growing organisms (Ley et al., 2004) that feed on
371 root exudates (Lipson et al., 1999) thrive in the top vegetation season, and communities dominated by fungi proliferate on
372 plant litter during the rest of the year (Lipson et al., 2002; Lipson and Schmidt, 2004; Nemergut et al., 2005; Bardgett et al.,
373 2005). In contrary, the microbial communities from the barren soils at the most elevated sites might be temporarily more
374 stable in comparison to those in lower elevations. Nevertheless, data supporting this pattern are missing and the problematics
375 of seasonal dynamics in soil microbial activity and community structure in the High Arctic including high elevation habitats
376 deserves future scientific interest.

377

378 5 Conslusions

379 The results of our study have shown that soils were along the Arctic elevational gradients characterized by distinct microbial
380 communities and differed in their size and activity. The soil microbial properties were mainly controlled by soil geochemical
381 properties, while the direct effect of summer microclimatic conditions was likely negligible. However, it indirectly affected
382 the soil microbes through effects on plant distribution and its influence on edaphic conditions. Down along the gradients, the
383 gradually increasing plant productivity and litter inputs were associated with decreasing soil pH, and increasing OM content
384 and soil moisture, which were together with bedrock chemistry recognized as the main factors driving the soil microbial
385 properties. The bedrock chemistry, represented mainly by differences in Mg^{2+} availability, played important role in shaping
386 the soil MCS especially in the high elevation habitats. As the future climate warming will likely cause an upward migration
387 of the vegetation, the increasing plant litter inputs to currently unvegetated soil at higher altitudes will overreach the
388 influence of parent material and entail an increasing abundance of bacteria and decreasing F/B ratio in the summer microbial

assemblages. These changes will diminish the variability of microbial properties among differently elevated habitats and could have negative implications for microbial diversity in the High Arctic. The closer coupling of organic matter and microbial biomass at the most elevated sites highlights the potential of these ostensibly most extreme soil habitats to harbour considerably large and active microbial biomass as long as OM and water are available. However, better prediction of the ecosystem development under proceeding climate change needs further investigation of soil organic matter formation and seasonal patterns in microbial diversity and activity, especially in the high elevation barren soils.

Author contribution

P. Kotas, H. Šantrůčková, J. Elster, and E. Kaštovská contributed to the analysis of the data and preparation of the manuscript. P. Kotas and J. Elster designed the study and performed sampling. The microbial community structure and environmental parameters were assessed by P. Kotas and E. Kaštovská.

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Competing interests

The authors declare that they have no conflict of interest.

References

Bardgett, R. D., Bowman, W. D., Kaufmann, R., and Schmidt, S.K.: A temporal approach to linking aboveground and belowground ecology, Trends Ecol. Evol., 20, 634–641, 2005.

Bekku, Y. S., Kume, A., Masuzawa, T., Kanda, H., Nakatsubo, T., and Koizumi, H.: Soil respiration in a high arctic glacier foreland in Ny-Ålesund, Svalbard, Polar Bioscience, 17, 36–46, 2004.

Björk, R. G., Björkman, M. P., Andersson M. X., and Klemedtsson L.: Temporal variation in soil microbial communities in Alpine tundra, Soil Biol. Biochem., 40, 266–268, 2008.

Blaud, A., Lerch, T. Z., Phoenix, G. K., and Osborn M. A.: Arctic soil microbial diversity in a changing world, Res. Microbiol., 166, 796–813, 2015.

Bossio, D.A. and Scow, K.M.: Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns, Microb. Ecol., 35, 265–278, 1998.

Cebon, P., Dahinden, U., Davies, H. C., Imboden, D., and Jaeger, C. C.: Views from the Alps: regional perspectives on climate change, Massachusetts, MA: The MIT Press., 1999.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J. L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W. J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A. J., and Wehner, M.: Long-term climate change. Projections, commitments and irreversibility. In: Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M. M. B., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (eds.).: Climate Change 2013. The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press. Cambridge. p. 1029-1136, ISBN: 9781107057991, 2013.

Djukic, I., Zehetner, F., Mentler, A., and Gerzabek, M. H.: Microbial community composition and activity in different Alpine vegetation zones, Soil Biol. Biochem., 42, 155–161, 2010.

Djukic, I., Zehetner, F., Watzinger, A., Horacek, M., and Gerzabek, M. H.: In situ carbon turnover dynamics and the role of soil microorganisms therein: a climate warming study in an Alpine ecosystem, FEMS Microbiol. Ecol., 83, 112–124, 2013.

Fierer, N., McCain C. M., Meir, P., Zimmermann, M., Rapp, J. M., Silman, M. R., and Knight, R.: Microbes do not follow the elevational diversity patterns of plants and animals, Ecology, 92, 797–804, 2011.

Førland, E.J., Benestad, R., Hanssen-Bauer, I., Haugen, J.E., and Skaugen, T.: Temperature and Precipitation Development at Svalbard 1900-2100, Advances in Meterology, doi:10.1155/2011/893790, 2010.

Frostegård, A. and Bååth, E.: The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fert. Soils, 22, 59–65, 1996.

Frostegård, Å., Bååth, E., and Tunlid, A.: Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis, Soil Biol. Biochem., 25, 723–730, 1993.

Gessner, M. O., Bauchrowitz, M. A., and Escautier, M.: Extraction and quantification of ergosterol as a measure of fungal biomass in leaf litter. Microb. Ecol., 22, 285– 291, 1991.

Giri, D. D., Shukla, P. N., Kashyap, S., Singh, P., Kashyap, A. K., and Pandey, K. D.: Variation in methanotrophic bacterial population along an altitude gradient at two slopes in tropical dry deciduous forest, Soil Biol. Biochem., 39, 2424–2426, 2007.

Hackl, E., Pfeffer, M., Donat, C., Bachmann, G., and Zechmeister-Boltenstern, S.: Composition of the microbial communities in the mineral soil under different types of natural forest, Soil Biol. Biochem., 37, 661–671, 2005.

Hardison, A. K., Canuel, E. A., Anderson, I. C., Tobias, C. R., Veuger, B., and Waters, M. N.: Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments, Biogeosciences, 10, 5571–5588, 2013.

Hodkinson, I. D., Coulson, S. J., and Webb, N. R.: Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard, J. Ecol., 91, 651–663, 2003.

Högberg, M. N., Högberg, P., and Myrold, D. D.: Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia, 150, 590–601, 2007.

Hu, L., Xiang, Z., Wang, G., Rafique, R., Liu W., and Wang, C.: Changes in soil physicochemical and microbial properties along elevation gradients in two forest soils, Scandinavian Journal of Forest Research, 31, 242–253, 2016.

Joergensen, R. G. and Wichern, F.: Quantitative assessment of the fungal contribution to microbial tissue in soil, Soil Biol. Biochem., 40, 2977–2991, 2008.

Jones, A, Stolbovoy, V., Tarnocai, C., Broll, G., Spaargaren, O., and Montanarella, L. (eds.): Soil Atlas of the Northern Circumpolar Region. European Commission, Publications Office of the European Union, Luxembourg, 144pp., 2010.

Khotimchenko, S. V., Vaskovsky, V. E., and Titlyanova, T. V.: Fatty acids of marine algae from the pacific coast of North California, Bot. Mar., 45, 17–22, 2002.

Kleidon, A. and Mooney, H. A.: A global distribution of biodiversity inferred from climatic constraints: results from a process-based modelling study, Global Change Biol., 6, 507–523, 2000.

Klimeš, L. and Doležal J.: An experimental assessment of the upper elevational limit of flowering plants in the western Himalayas, Ecography, 33, 590–596, 2010.

Kroppenstedt, R. M.: Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow, M., Minikin, D. E., Eds. Chemical methods in bacterial systematics. Academic Press, London, pp. 173–194, 1985.

Lazzaro, A., Hilfiker, D., and Zeyer, J.: Structure of microbial communities in alpine soils: seasonal and elevational effects, Frontiers in Microbiology, doi: 10.3389/fmicb.2015.01330, 2015.

Ley, R. E. and Schmidt, S. K.: Fungal and bacterial responses to phenolic compounds and amino acids in high altitude barren soils, Soil Biol. Biochem., 34, 989–995, 2002.

Ley, R. E., Williams, M. W., and Schmidt, S. K.: Microbial population dynamics in an extreme environment: controlling factors in talus soils at 3750m in the Colorado Rocky Mountains, Biogeochemistry, 68, 313–335, 2004.

Lipson, D. A.: Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients, FEMS Microbiol. Ecol., 59, 418–427, 2007.

Lipson, D. A. and Monson, R. K.: Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events, Oecologia, 113, 406–414, 1998.

Lipson, D. A. and Schmidt, S. K.: Seasonal changes in an Alpine soil bacterial community in the Colorado Rocky Mountains, Appl. Environ. Microb., 70, 2867–2879, 2004.

509 Lipson, D. A., Schmidt, S. K., and Monson, R. K.: Links between microbial population dynamics and nitrogen availability in
510 an alpine ecosystem, Ecology, 80, 1623–1631, 1999.
511
512 Lipson, D. A., Schadt, C. W., and Schmidt, S. K.: Changes in soil microbial community structure and function in an alpine
513 dry meadow following spring snow melt, Microb. Ecol., 43, 307–314, 2002.
514
515 Lovelland P. J. and Whalley W. R.: Particle size analysis. In: Soil and Environmental Analysis Physical Method (Eds Smith,
516 K.A. and Mullins, Ch.E.), Dekker Press, New York, USA, 2001.
517
518 Löffler, U. C. M., Cypionka, H., and Löffler, J.: Soil microbial activity along an arctic-alpine altitudinal gradient from a
519 seasonal perspective, Eur. J. Soil Sci., 59, 842–854, 2008.
520 Ma, X., Chen, T., Zhang, G., and Wang R.: Microbial community structure along an altitude gradient in three different
521 localities, Folia Microbiol., 49, 105–111, 2004.
522
523 Männistö, M., Tiirola, M., and Häggblom, M. M.: Bacterial communities in Arctic fjelds of Finnish Lapland are stable but
524 highly pH-dependent, FEMS Microbiol. Ecol., 59, 452–465, 2007.
525
526 Margesin, R., Gander, S., Zacke, G., Gounot, A. M., and Schinner, F.: Hydrocarbon degradation and enzyme activities of
527 cold-adapted bacteria and yeasts, Extremophiles, 7, 451–458, 2003.
528
529 Margesin, R., Jud, M., Tscherko, D., and Schinner, F.: Microbial communities and activities in alpine and subalpine soils,
530 FEMS Microbiol. Ecol., 67, 208–218, 2009.
531
532 Meng, H., Li, K., Nie, M.,Wan, J.-R., Quan, Z.-X., Fang, C.-M., Chen, J.-K., Gu, J.-D., and Li, B.: Responses of bacterial
533 and fungal communities to an elevation gradient in a subtropical montane forest of China, Appl. Microbiol. Biot., 97, 2219–
534 2230, 2013.
535
536 Mille-Lindblom, C., von Wachenfeldt, E., and Tranvik, L. J.: Ergosterol as a measure of living fungal biomass: persistence
537 in environmental samples after fungal death, J. Microbiol. Meth., 59, 253–262, 2004.
538
539 Montgomery, H. J., Monreal, C. M., Young, J. C., and Seifert, K. A.: 2000. Determination of soil fungal biomass from soil
540 ergosterol analyses, Soil Biol. Biochem., 32, 1207–1217, 2000.
541

Nemergut, D. R., Costello, E. K., Meyer, A. F., Pescador, M. Y., Weintraub, M. N., and Schmidt, S. K.: Structure and function of alpine and arctic soil microbial communities, Res. Microbiol., 156, 775–784, 2005.

Niklińska, M. and Klimek, B.: Effect of temperature on the respiration rate of forest soil organic layer along an elevation gradient in the Polish Carpathians, Biol. Fert. Soils, 43, 511–518, 2007.

Nikrad, M. P., Kerkhof, L. J., and Häggblom, M. M.: The subzero microbiome: microbial activity in frozen and thawing soils, FEMS Microbiol. Ecol., 92, doi:10.1093/femsec/fiw08, 2016.

Oberbauer, S. F., Tweedie, C. E., Welker, J. M., Fahnestock, J. T., Henry, G. H. R., Webber, P. J., Hollister, R. D., Walker, M. D., Kuchy, A., Elmore, E., and Starr, G.: Tundra CO2 fluxes in response to experimental warming across latitudinal and moisture gradients. Ecol. Monogr., 77, 221–238, 2007.

Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., and Trappe, J.: Ecosystem properties and microbial community changes in primary succession on a glacier forefront, Oecologia, 119, 239–246, 1999.

Prach, K., Klimešová, J., Košnar, J., Redčenko O., and Hais M.: Variability of contemporary vegetation around Petuniabukta, central Spitsbergen, Pol. Polar Res., 33, 383–394, 2012.

Prescott, L.M., Harley, J.P., and Klein, D.A.: Microbiology, Sixth edition, McGraw Hill International edition, New York. 2005.

Pushkareva, E., Pessi, I., Wilmotte, A., and Elster, J.: Cyanobacterial community composition in Arctic soil crusts at different stages of development, FEMS Microbiol. Ecol., doi: 10.1093/femsec/fiv143, 2015.

Řeháková, K., Chlumská, J., and Doležal, J.: Soil cyanobacterial and microalgal diversity in dry mountains of Ladakh, NW Himalaya, as related to site, altitude, and vegetation, Microb. Ecol., 62, 337–346, 2011.

Richter, D. D., Johnson, D. W., and Dai, K. H.: Cation exchange reactions in acid forested soils: effects of atmospheric pollutant deposition. In: Johnson, D.W., Lindberg, S.E. (eds.), Atmospheric Deposition and Nutrient Cycling in Forest Ecosystems, Spring-Verlag, New York, pp 339–358, 1992.

Rousk, J., Brookes, P. C., and Bååth, E.: Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization, Appl. Environ. Microb., 75, 1589–1596, 2009.

Sawaske, S. R. and Freyberg, D. L.: Fog, fog drip, and streamflow in the Santa Cruz Mountains of the California Coast Range, Ecohydrology, 8, 695–713, 2015.

Schinner, F.: Soil microbial activities and litter decomposition related to altitude, Plant Soil, 65, 87–94, 1982.

Schinner, F. and Gstraunthaler, G.: Adaptation of microbial activities to the environmental conditions in alpine soils, Oecologia, 50, 113–116, 1981.

Schűtte, U. M. E., Abdo, Z., Foster, J., Ravel, J., Bunge, J., Solheim, B., and Forney, L. J.: Bacterial diversity in a glacier foreland of the high Arctic, Mol. Ecol., 19, 54–56, 2010.

Seastedt, T. R., Bowman, W. D., Caine, T. N., McKnight, D., Townsed, A., and Williams, M. W.: The landscape continuum: A model for high-elevation ecosystems, BioScience, 54, 111–121, 2004.

Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., and Chu, H.: Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain, Soil Biol. Biochem., 57, 204–211, 2013.

Siles, J. S. and Margesin, R.: Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: What are the driving factors? Soil Microbiology, 72, 207–220, 2016.

Singh, D., Takahashi, K., Kim, M., Chun, J., and Adams, J. M.: A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan, Microb. Ecol. 63, 429–437, 2012.

Sinsabaugh, R. L., Antibus, R. K., Jackson, C. R., Karpanty, S., Robinson, M., Liptak, M., and Franchini, P.: A β-sitosterol assay for fine-root mass in soil, Soil Biol. Biochem., 29, 39–44, 1997.

Šmilauer, P. and Lepš, J.: Multivariate analysis of ecological data using CANOCO5. Cambridge University Press, Cambridge, 2014.

Swift, M. J., Heal, O. W., and Anderson, J. M.: Decomposition in terrestrial ecosystems. Blackwell Scientific Publications, Oxford, 1979.

Ter Braak, C. J. F. and Šmilauer, P.: Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA, 496 pp, 2012.

Trevors, J. T., Kevan, P. G., and Tam, L.: Microbial diversity across a Canadian sub-Arctic, isostatically rebounding, soil transect. Polar Science, 4, 81–91, 2010.

Tytgat, B., Verleyen, E., Sweetlove, M., D'hondt, S., Clercx, P., Van Ranst, E., Peeters, K., Roberts, S., Namsaraev, Z., Wilmotte, A., Vyverman, W., and Willems, A.: Bacterial community composition in relation to bedrock type and macrobiota in soils from the Sør Rondane Mountains, East Antarctica, FEMS Microbiol. Ecol., 92, doi: 10.1093/femsec/fiw126, 2016.

van de Heijden, M. G. A., Bardgett, R. D., and van Straalen, N. M.: The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems, Ecol. Lett., 11, 296–310, 2008.

Van Horn, D. J., Van Horn, M. L., Barrett, J. E., Gooseff, M. N., Altrichter, A. E., Geyer, K. M., Zeglin, L. H., and Takacs-Vesbach, K.: Factors controlling soil microbial biomass and bacterial diversity and community composition in a cold desert ecosystem: Role of geographic scale, PLoS One, 8, doi: 10.1371/journal.pone.0066103, 2013.

Väre, H., Vestberg, M., and Ohtonen, R.: Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in northern Fennoscandia, Arctic Alpine Res., 29, 93–104, 1997.

Volkman, J. K.: Sterols in microorganisms, Appl. Microbiol. Biot., 60, 495–506, 2003.

Volkman, J. K.: A review of sterol markers for marine and terrigenous organic matter, Org. Geochem., 9, 83–99, 1986.

Webb, M.: The influence of magnesium on cell division. 2. The effect of magnesium on the growth and cell division of various bacterial species in complex media, Microbiology, 3, 410–417, 1949a.

Webb, M.: The influence of magnesium on cell division. 3. The effect of magnesium on the growth of bacteria in simple chemically defined media, Microbiology, 3, 418–424, 1949b.

Welker, J. M., Fahnestock, J. T., and Jones, M. H.: Annual CO2 flux in dry and moist arctic tundra: Field responses to increases in summer temperatures and winter snow depth, Climatic Change, 44, 139–150, 2000.

Wheeler, K. A., Hurdman, B. F., and Pitt, J. I.: Influence of pH on the growth of some toxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*, Int. J. Food Microbiol., 12, 141–150, 1991.

Xu, M., Li, X., Cai, X., Gai, J., Li, X., Christie, P., and Zhang, J.: Soil microbial community structure and activity along a montane elevational gradient on the Tibetan Plateau, Eur. J Soil Biol., 64, 6–14, 2014.

Yoshitake, S., Uchida, M., Koizumi, H., and Nakatsubo, T.: Carbon and nitrogen limitation of soil microbial respiration in a High Arctic successional glacier foreland near Ny-Ålesund, Svalbard, Pol. Res., 26, 22–30, 2007.

Zbíral, J. and Němec, P.: Data presentation, interpretation, and communication: Integrating of Mehlich 3 extractant into the Czech soil testing scheme, Communications in Soil Science and Plant Analysis, 31, 2171–2182, 2000.

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Tables

Table 1. Climatic variables; temperatures given in °C

Sites	Altitudes [m a.s.l.]	Means Summer	Means Winter	Means Year	Min daily means Winter	Max daily means Summer	Mean daily amplitude Summer	Max daily amplitude Summer	Number of days with daily mean > 0 °C	Number of days with daily mean > 5 °C	Positive soil surface energy balance
Alt 1	25	5.8	-3.6	-0.8	-7.0	11.2	5.2	10.9	110	62	615
Alt 2	275	7.1	-5.7	-2.7	-10.3	14.5	8.5	18.2	96	54	571
Alt 3	525	5.8	-8.9	-4.9	-15.8	14.7	8.1	17.7	91	40	480
Alt 4	765	5.3	-9.5	-6.6	-17.1	11.6	5.5	14.0	51	11	290

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Table 2. Geochemical characteristics of soils along studied altitudinal gradients. Means ± SE (n = 3) are given in the upper part of the table. Results of two–way ANOVAs (F–values) of the effects of gradient (Gr), altitude (Alt) and their interaction (Gr x Alt) are presented in the lower part of the table.

gradient	altitude [m a.s.l]	soil type	soil moisture [%]	pH	CEC [meq/100g^{-1}]	Ca^{2+} [mg g^{-1}]	Mg^{2+} [mg g^{-1}]	K^{+} [µg g^{-1}]	Na^{+} [µg g^{-1}]	NO_3^{-} [ng g^{-1}]	SRP [ng g^{-1}]
Gr1	35	sandy loam	**c** 28.4 ± 2.5	**a** 7.8 ± 0.1	**b** 35.8 ± 0.4	**a** 4.9 ± 0.2	**a** 0.50 ± 0.03	**b** 104 ± 2.3	**c** 16.0 ± 1.4	**b** 783 ± 145	**b** 103 ± 27
	280	sandy loam-loam	**b** 18.0 ± 0.5	**a** 7.9 ± 0.2	**a** 27.4 ± 2.3	**a** 5.2 ± 0.6	**a** 0.55 ± 0.08	**b** 81 ± 8.8	**ab** 8.4 ± 1.3	**a** 139 ± 38	**a** 25 ± 6.1
	520	loam	**b** 18.6 ± 2.5	**a** 8.1 ± 0.1	**a** 30.3 ± 0.7	**a** 4.3 ± 0.4	**b** 0.85 ± 0.04	**c** 160 ± 18.1	**b** 11.3 ± 1.1	**a** 134 ± 10	**a** 41 ± 3.1
	765	clay-loam	**a** 12.1 ± 1.8	**b** 9 ± 0.0	**a** 26.8 ± 2.3	**b** 19.8 ± 1.0	**c** 1.25 ± 0.06	**a** 11 ± 2.7	**a** 7.3 ± 0.0	**a** 120 ± 49	**a** 24 ± 4.5
Gr2	30	sandy loam	**a** 21.1 ± 2.4	**a** 7.8 ± 0.1	**a** 25.6 ± 2.7	**b** 14.7 ± 2.6	**a** 0.19 ± 0.01	**ab** 52 ± 4.0	**b** 13.2 ± 1.7	**a** 128 ± 10	**c** 287 ± 32
	275	sandy loam-loam	**a** 21.1 ± 2.4	**a** 7.9 ± 0.1	**a** 30.3 ± 1.7	**bc** 16.5 ± 1.1	**b** 0.26 ± 0.01	**b** 59 ± 4.3	**ab** 10.1 ± 1.7	**a** 128 ± 10	**b** 98 ± 15
	520	sandy loam-loam	**a** 21.7 ± 5.3	**b** 8.4 ± 0.1	**a** 30.8 ± 1.1	**a** 7.8 ± 1.6	**c** 0.34 ± 0.01	**b** 69 ± 3.3	**ab** 9.6 ± 1.8	**a** 234 ± 98	**ab** 57 ± 25
	765	loam	**a** 22.5 ± 1.7	**c** 8.8 ± 0.1	**b** 45.1 ± 0.5	**c** 27.9 ± 9.3	**b** 0.25 ± 0.01	**a** 41 ± 8.8	**a** 8.1 ± 1.4	**a** 166 ± 35	**a** 25 ± 4.0
Gr3	25	sandy loam	**b** 39.5 ± 1.4	**a** 8.1 ± 0.1	**b** 49.4 ± 2.1	**a** 7.7 ± 0.3	**b** 0.20 ± 0.03	**a** 52 ± 5.3	**b** 17.1 ± 1.1	**b** 313 ± 98	**b** 76 ± 23
	275	sandy loam-loam	**ab** 31.9 ± 2.9	**a** 8.1 ± 0.1	**a** 39.2 ± 5.4	**b** 10.8 ± 0.6	**b** 0.21 ± 0.01	**ab** 59 ± 1.9	**b** 18.5 ± 0.5	**ab** 179 ± 49	**b** 116 ± 27
	525	loam	**ab** 28.2 ± 6.5	**a** 8 ± 0.1	**a** 34.9 ± 3.0	**b** 13.0 ± 4.6	**b** 0.22 ± 0.00	**b** 66 ± 6.6	**b** 18.4 ± 3.1	**a** 131 ± 4	**b** 87 ± 28
	770	loam	**a** 22.5 ± 1.7	**b** 8.8 ± 0.1	**a** 30.6 ± 3.9	**bc** 14.2 ± 0.1	**a** 0.16 ± 0.00	**a** 52 ± 1.6	**a** 9.9 ± 0.2	**ab** 197 ± 31	**a** 31 ± 1.6
		d.f.									
Gr		2	**31.4 *****	0.10	**22.1 *****	**6.43 ****	**634 *****	**51.7 *****	**36.2 *****	1.43	**18.5 *****
Alt		3	**11.1 *****	**98 *****	**4.61 ***	**14.1 *****	**66.9 *****	**74.9 *****	**18.7 *****	**4.37 ***	**44.1 *****
Gr x Alt		6	**5.07 ****	**5.6 *****	**20.5 *****	0.83	**60.6 *****	**31.6 *****	**3.94 ****	**5.34 ****	**9.86 *****

Different letters indicate significant differences between sampling sites along particular gradients ($P < 0.05$; upper part of the table). Statistically significant differences are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (lower part of the table).

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Table 3. Total soil carbon (TOC) and nitrogen (TN) contents, their molar ratios, contents of sitosterol and ergosterol, sitosterol/brassicasterol ratios and soil PLFA contents in soils along the altitudinal gradients. Means ± SE (n = 3) are given in the upper part of the table. Results of two–way ANOVAs (F–values) of the effects of gradient (Gr), altitude (Alt) and their interaction (Gr x Alt) are presented in the lower part of the table.

gradient	altitude [m a.s.l.]	TOC [mg g^{-1}]	TN [mg g^{-1}]	TOC/TN	Sitosterol [µg g^{-1} TOC]	Ergosterol [µg g^{-1} TOC]	Sitosterol / Brassicasterol	PLFAtot [µmol PLFA g^{-1} TOC]
Gr1	35	**c** 70.6 ± 13.4	**b** 5.0 ± 1.01	**b** 12.1 ± 0.2	**c** 534 ± 62.8	**b** 225 ± 21.7	**b** 5.5 ± 0.4	**a** 2.9 ± 0.5
	280	**b** 21.1 ± 1.9	**a** 2.0 ± 0.29	**ab** 9.0 ± 0.7	**bc** 521 ± 140	**b** 286 ± 48.0	**b** 5.3 ± 0.8	**b** 8.1 ± 1.1
	520	**b** 18.5 ± 4.2	**a** 1.8 ± 0.31	**ab** 8.8 ± 0.7	**ab** 293 ± 66.5	**b** 258 ± 38.4	**b** 4.7 ± 1.0	**b** 7.9 ± 0.1
	765	**a** 4.4 ± 1.5	**a** 0.5 ± 0.07	**a** 7.9 ± 2.6	**a** 81.1 ± 2.7	**a** 57 ± 27.1	**a** 2.3 ± 0.4	**a** 3.3 ± 0.7
Gr2	30	**ab** 30.6 ± 4.8	**a** 1.9 ± 0.40	**c** 13.7 ± 0.9	**bc** 515 ± 44.9	**a** 199 ± 9.4	**b** 6.7 ± 0.7	**a** 5.4 ± 0.7
	275	**b** 37.2 ± 5.0	**a** 3.0 ± 0.26	**b** 10.7 ± 0.7	**c** 616 ± 143	**bc** 321 ± 30.2	**b** 5.6 ± 1.2	**a** 6.4 ± 0.4
	520	**a** 24.4 ± 7.8	**a** 1.9 ± 0.64	**b** 9.8 ± 1.2	**ab** 299 ± 73.3	**b** 241 ± 39.2	**a** 2.9 ± 0.4	**a** 6.8 ± 1.7
	765	**a** 21.6 ± 3.6	**a** 2.8 ± 0.20	**a** 6.7 ± 0.6	**a** 161 ± 36.9	**c** 451 ± 34.1	**a** 2.7 ± 0.7	**b** 10.4 ± 0.4
Gr3	25	**c** 81.1 ± 8.7	**b** 6.1 ± 0.38	**b** 11.5 ± 0.7	**b** 587 ± 144	**a** 194 ± 37.3	**b** 6.4 ± 2.1	**a** 3.5 ± 0.2
	275	**b** 62.2 ± 9.1	**ab** 4.8 ± 0.32	**b** 11 ± 0.7	**ab** 370 ± 42.9	**a** 209 ± 33.0	**a** 4.2 ± 0.7	**a** 4.5 ± 0.4
	525	**ab** 39.6 ± 11.4	**a** 4.8 ± 0.32	**b** 10.6 ± 0.6	**a** 270 ± 112	**a** 261 ± 44.3	**a** 3.3 ± 1.0	**b** 6.7 ± 1.1
	770	**a** 23.1 ± 3.9	**a** 2.5 ± 0.37	**a** 7.9 ± 0.2	**a** 151 ± 37.8	**b** 447 ± 22.1	**a** 3.1 ± 0.9	**c** 10.2 ± 0.5
	d.f.							
Gr	2	**27.8 *****	**31.5 *****	1.57	0.79	**17.7 *****	1.04	**8.91 *****
Alt	3	**42.4 *****	**26.4 *****	**23.6 *****	**28.4 *****	**3.80 ***	**14.4 *****	**29.1 *****
Gr x Alt	6	**8.33 *****	**11.3 *****	1.96	1.34	**30.3 *****	2.17	**19.9 *****

Different letters indicate significant differences between sampling sites along particular gradients ($P < 0.05$; upper part of the table). Statistically significant differences are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (lower part of the table).

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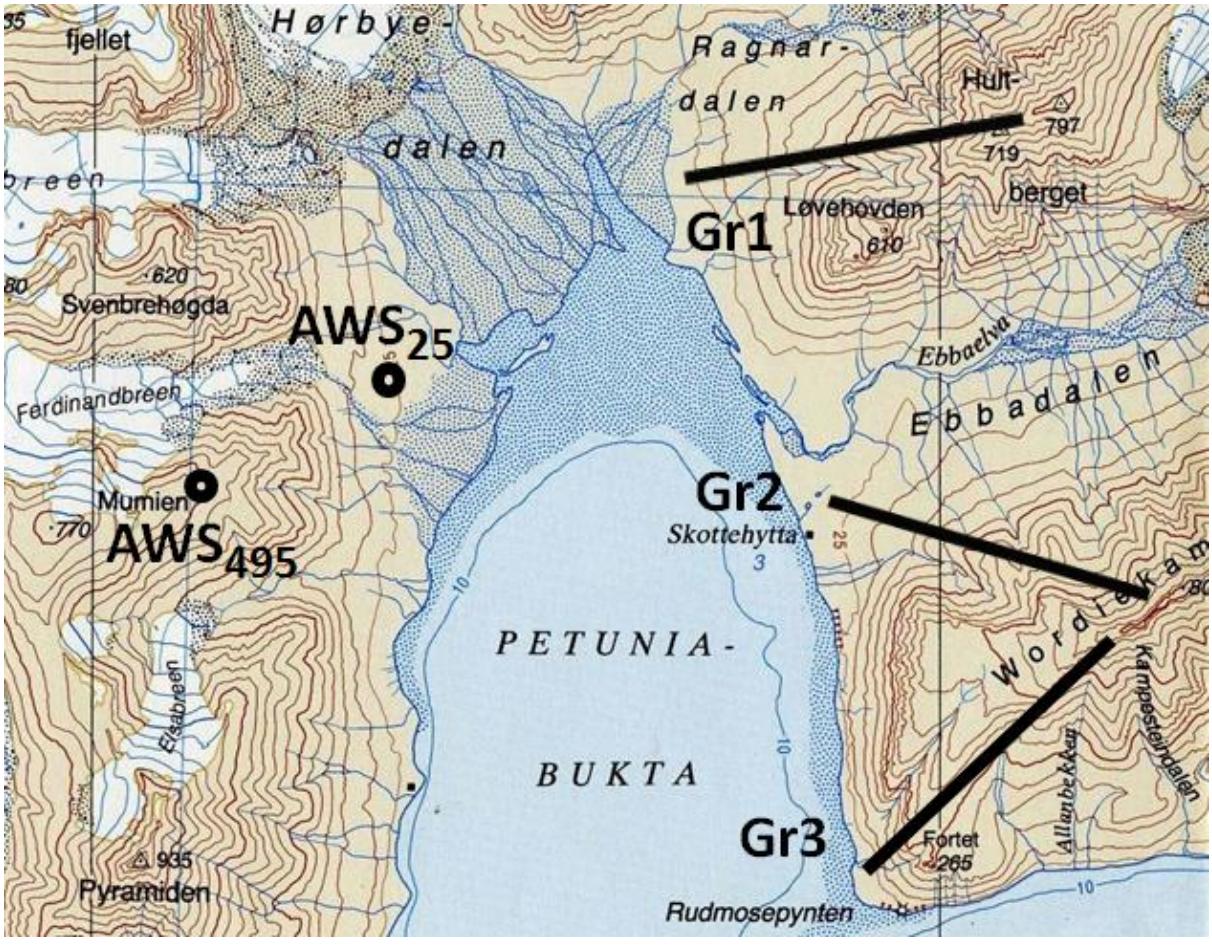
724 **Figures**

725



726

727 **Figure 1. Location of the three investigated gradientsGr1–3 and automated weather stations (AWS) in Petunia bay, Billefjorden,**
728 **Central Spitsbergen. Map source: map sheet C7, Svalbard 1:100 000, Norwegian Polar Institute 2008.**

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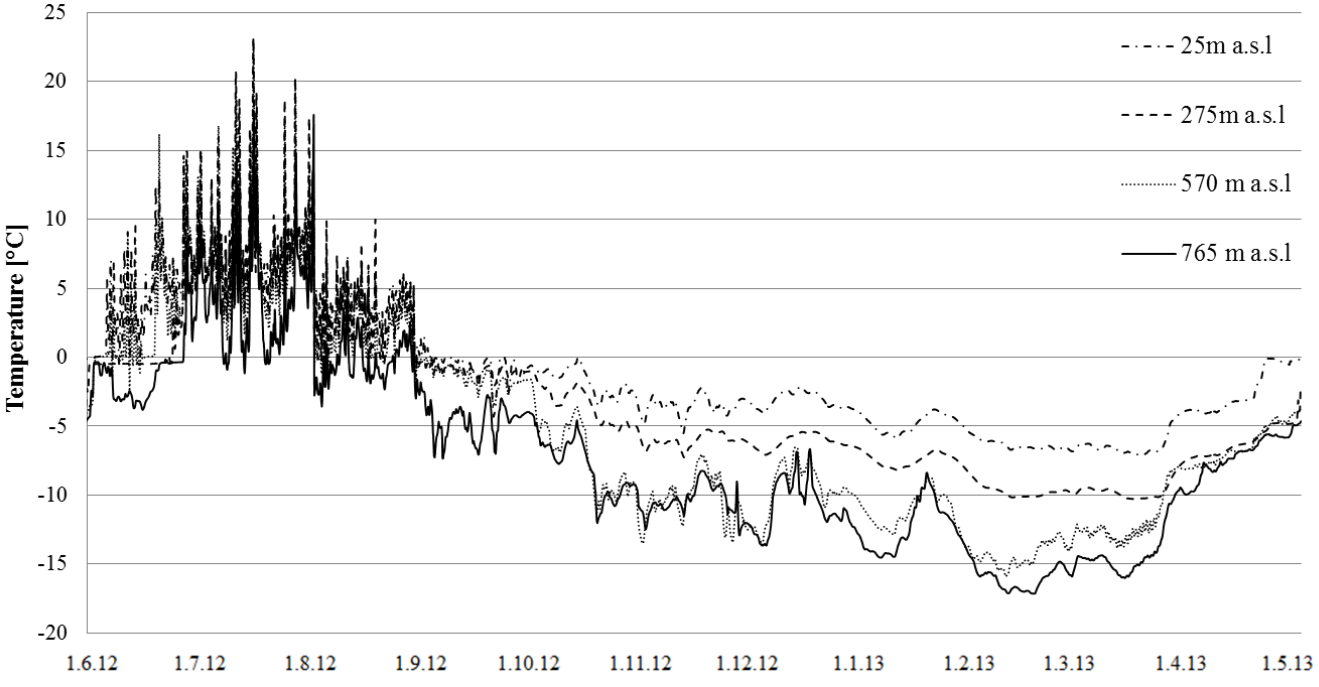

730

731 **Figure 2. Time series of soil temperatures at –5 cm from sampling sites located along Gr1 in the period June 2012 – May 2013**

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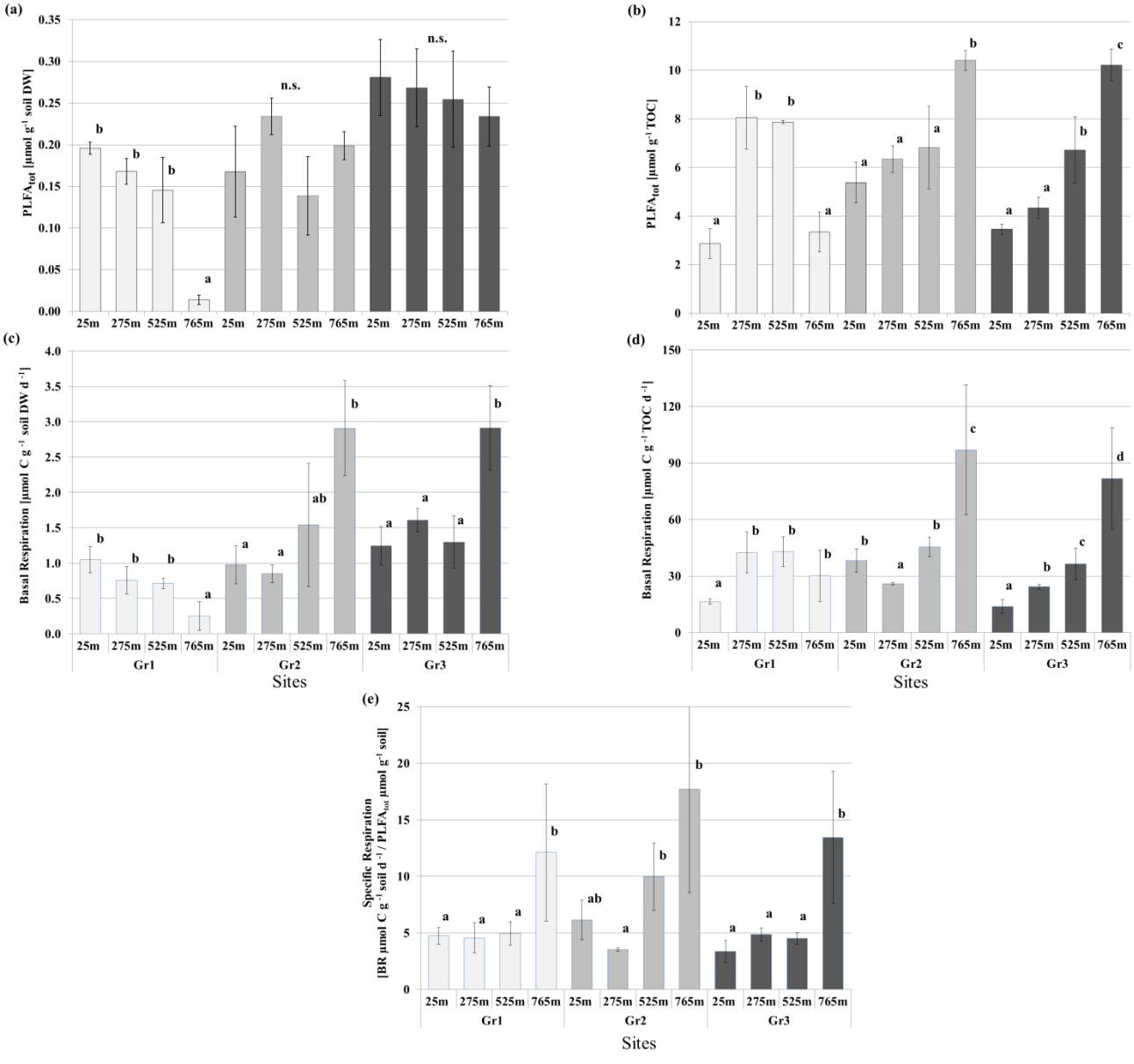

Figure 3. The soil PLFA contents and basal respiration rates in the soil (a, c) and normalized per soil TOC content (b, d), respectively, together with specific respiration (e). Error bars indicate mean ± S.D. (n = 3). Small case letters denote significant differences among altitudes within particular gradients ($P < 0.05$; One–way ANOVA combined with Tukey post hoc test); n.s. – effect of altitude not significant along the particular gradient.

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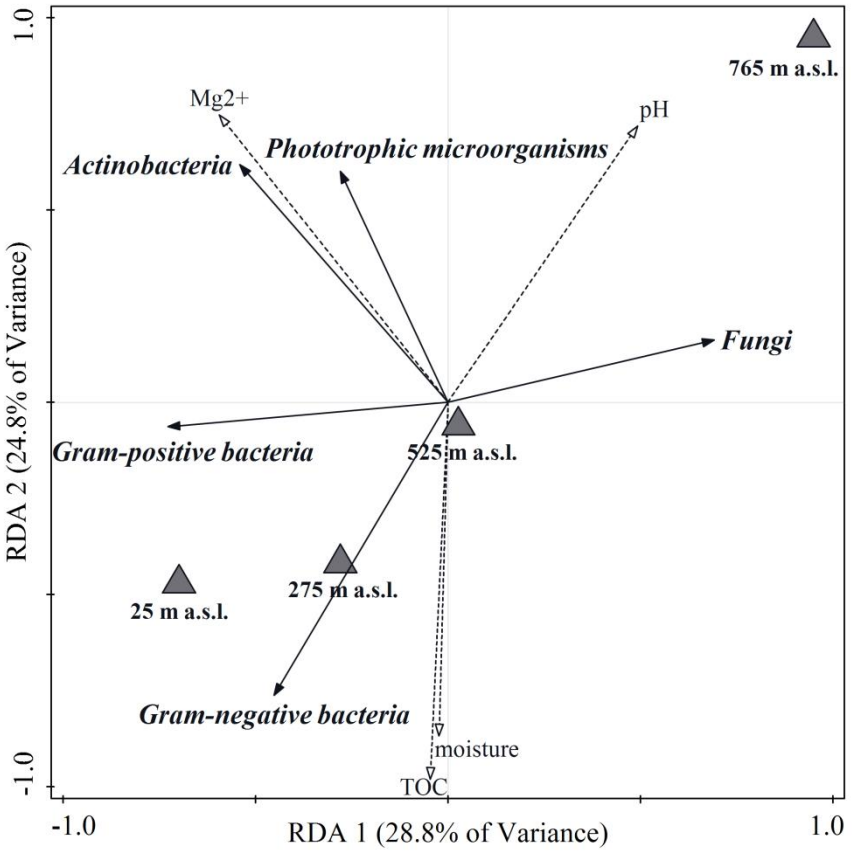

737

738 **Figure 4. The correlation between abundance of main microbial groups (bold italic) and soil geochemical parameters retained by**
739 **forward selection of explanatory variables. Results of RDA. Altitude of sampling sites was used as supplementary variable. *Arrows***
740 **indicate the direction in which the respective parameter value increases, *solid* lines indicate microbial groups, *dotted* lines indicate**
741 **selected environmental variables,. *Triangles* are centroids (n = 9) of the sites with corresponding elevation (n = 9). The numbers in**
742 **parentheses are the portions of the variation explained by each axis.**

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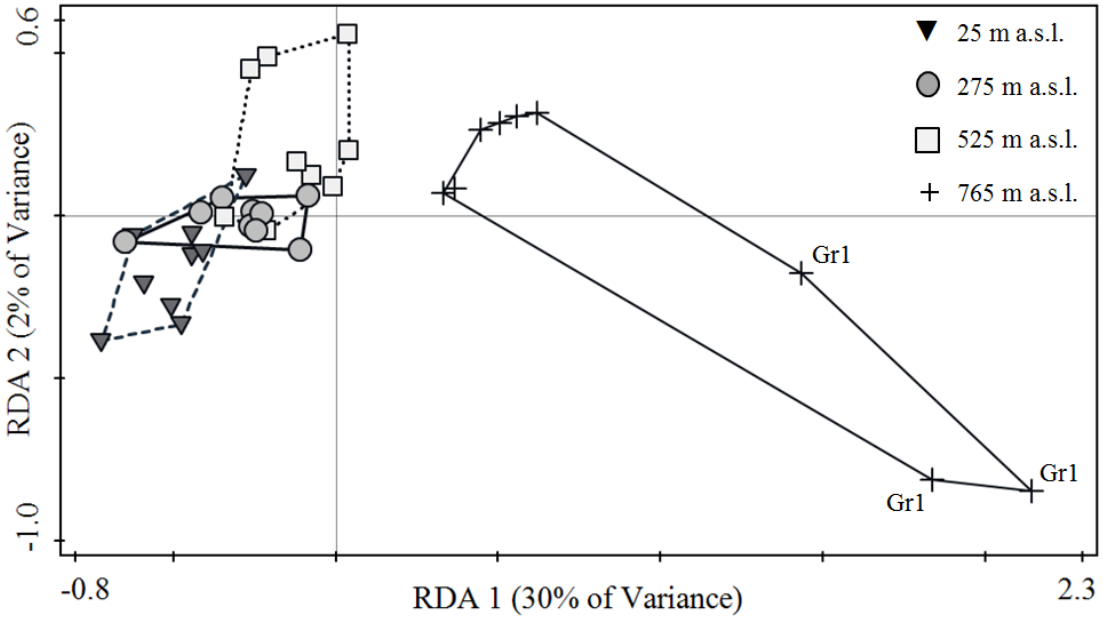

Figure 5. Sample scores on first and second axes from a partial RDA on the microbial PLFA composition. The thin solid line encases the most elevated sites, the dotted line encases sites at 525 m a.s.l., the bold solid line encases sites at 275 m a.s.l., and the dashed line encases sites at 25 m a.s.l., respectively (n = 9). Within the most elevated sites, the sites belonging to Gr1 are marked. The numbers in parentheses are the portions of the variation explained by each axis.

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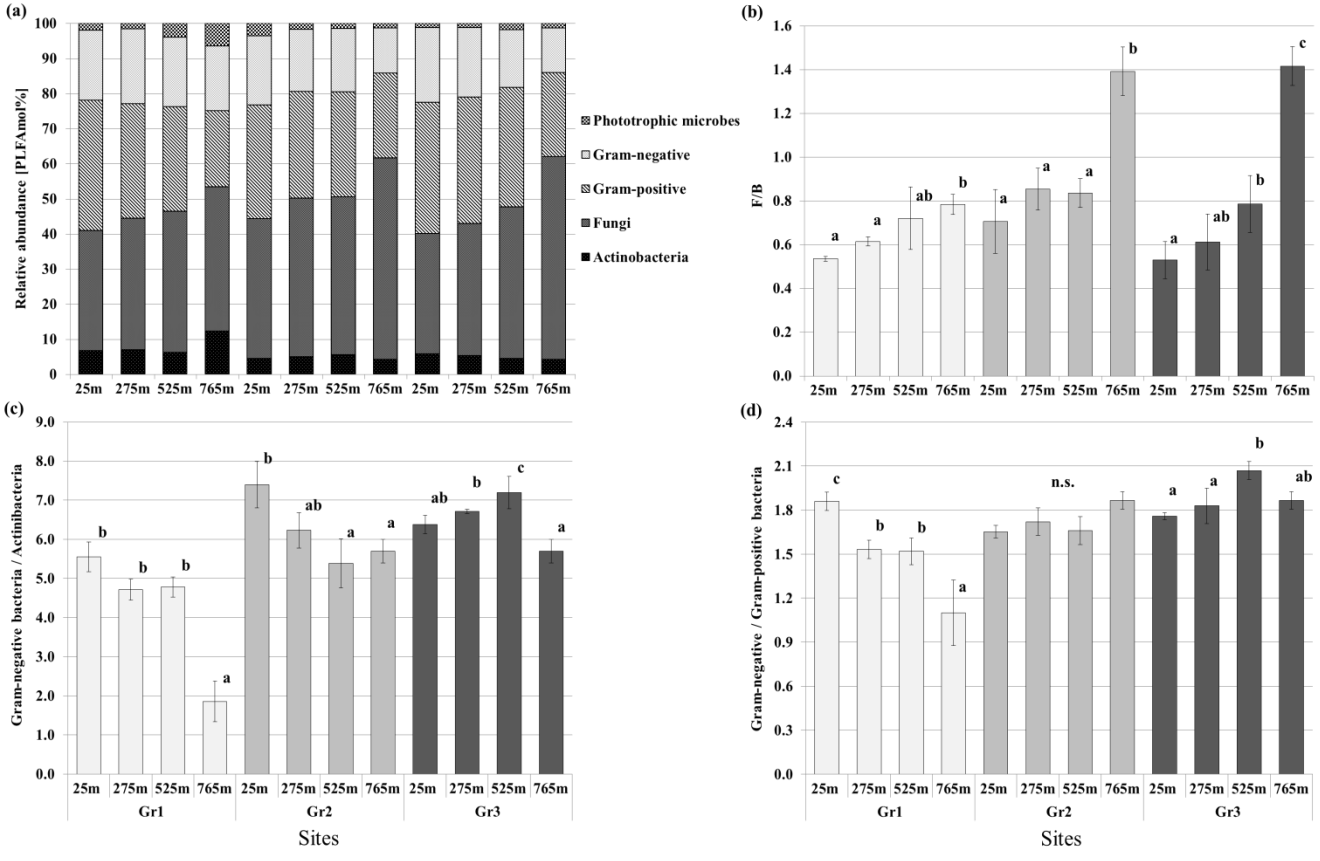

Figure 6. Relative abundances of specific PLFAs within the microbial community (a), fungi to bacteria ratios (b), G– bacteria to Actinobacteria ratios (c), and G– bacteria to G+ bacteria ratios (d) along the altitudinal gradients. Error bars indicate mean ± S.D. (n = 3). Small case letters denote significant differences between altitudes within particular gradients (*P* < 0.05; One–way ANOVA combined with Tukey post hoc test); n.s. – effect of altitude not significant.