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Effects of climate change on fungal community structure and organic matter turnover in soil profiles along elevation gradients in alpine ecosystems

Dissertation

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by

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Preface

This thesis was prepared within the framework of Junior Research Group Project "Effects of climate change on fungal community structure and organic matter turnover in soil profiles along elevation gradients in alpine ecosystems" (Project number: 9523405) funded by the University of Kassel. The thesis is submitted to the Faculty of Organic Agricultural Sciences to fulfil the requirement for the degree of "Doktor der Agrarwissenschaften" (Dr. agr.). The dissertation is based on three papers as first author, that are either published, submitted or in preparation for submission to the international refereed journals. The manuscripts are included in chapters 2, 3 and 4. The general introduction of research topic including objectives are given in Chapter 1. General discussion comprising the work in three manuscripts, general conclusions and outlook are given in Chapter 5, 6 and 7 respectively. The following manuscripts are included in the thesis:

Chapter 2:

Bhople, P., Djukic, I., Keiblinger, K., Zehetner, F., Liu, D., Bierbaumer, M., Zechmeister-Boltenstern, S., Joergensen, R.G., Murugan, R. 2019. Variations in soil and microbial biomass C, N and fungal biomass ergosterol along elevation and depth gradients in Alpine ecosystems. Geoderma 345, 93-103.

Chapter 3:

Bhople, P., Keiblinger, K., Djukic, I., Liu, D., Zehetner, F., Zechmeister-Boltenstern, S., Joergensen, R.G., Murugan, R. 2019. Microbial necromass formation and enzyme activities reflect retardation of plant residue decomposition at an alkaline Alpine elevation gradient. Soil Biology and Biochemistry (submitted).

Chapter 4:

Bhople, P., Samad, A., Šišić, A., Antonielli, L., Sessitsch, A., Djukic, I., Keiblinger, K., Zehetner, F., Zechmeister-Boltenstern, S., Joergensen, R.G., Murugan, R. 2019. Variations in fungal community structure along elevation gradients in contrasting Austrian Alpine ecosystems. (in preparation for submission).

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List of abbreviations

ACE	Abundance based coverage estimator
AMC	L-leucine-7-amido-4-methyl-coumarin
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
BD	Bulk density
BGL	β-glucosidase
C	Carbon
°C	Degree Celsius
Cu	Copper
$CHCl_3$	Chloroform
CIPRA	International Commission for the Protection of the Alps
CO_2	Carbon dioxide
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DOPA	L-3,4-dihydroxyphenylalanine
ECH	Exochitinase
EGL	Exoglucanase
EMF	Ectomycorrhizal fungi
Fig.	Figure
gm	Gramm
G+	Gram positive bacteria
G-	Gram negative bacteria
GalN	Galactosamine
GlcN	Glucosamine
H_2O	Water
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IPCC	Intergovernmental Panel on Climate Change
ITS region	Internal Transcribed Spacer region
IUSS	International Union of Soil Science
K_2SO_4	Potassium sulphate

k_{EC}, k_{EN}	Extractable portions of total C and N from microbial biomass
m asl	Meters above sea level
mg	Milligram
µg	Microgram
ml	Millilitre
ManN	Mannoseamine
MAT	Mean annual temperature
MAP	Mean annual precipitation
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
Mn	Manganese
MUF	4-methylumbelliferyl
MurN	Muramic acid
nmol	Nanomole
N	Total nitrogen
NMDS	Non-metric multidimensional scaling
OTU	Operational taxonomic unit
P	Phosphorous
PCA	Principal component analysis
PCR	Polymerase chain reaction
permANOVA	Permutational analysis of variance
PHO	Phosphatase
PLFA	Phospholipid fatty acid
POD	Peroxidase
POX	Phenoloxidase
PRO	Protease
RDA	Redundancy analysis
SAP	Saprotrophic fungi
SOC	Soil organic carbon
SOM	Soil organic matter
VPA	Variation partitioning analysis
ZAMG	Zentralansalt für Meteorologie und Geodynamik

Summary

Although elevation patterns of diversity for plants and animals are well known, understanding of variations in fungal community structure along elevation gradients is limited. Soil fungi play an important role in soil microbial communities, where they improve soil organic matter (SOM) turnover and nutrient circulation. Hence, any change in fungal community structure will have strong effects on soil and ecosystem functioning. The main purpose of this thesis is therefore to investigate how climate induced shifts in community structure, biomass and residue of soil fungi could explain organic matter turnover as well as carbon (C) and nitrogen (N) dynamics along elevation and soil depth gradients in contrasting alpine ecosystem sites in Austria, that differ only in bedrock and vegetation but have similar climate along elevation levels. The objective was to further investigate fungal community structure and analyse the influencing factors. Thus, in this thesis, various approaches such as Illumina-platform based Next Generation Sequencing of standard marker for fungi, internal transcribed spacer (ITS2) region, and chemical and biochemical methods, were combined. This helped to characterize fungal diversity, soil and microbial nutrient pools of C and N, soil microbial biomass, residues, and activities as well as factors affecting microbial properties in alpine elevation gradient soils.

1. Briefly, simultaneous elevation and soil depth gradients study at two contrasting alpine ecosystem sites having differences in vegetation and bedrock material offered unique opportunity to understand adaptation of microbial communities to local habitat and their pattern through natural gradients of soil conditions with comparison perspective. The results of our first study indicate increasing stocks of MBC with increasing elevations in alkaline and acidic sites and, of MBN following changes in SOC and total N stocks except at low elevation in alkaline site. Also, the alkaline site had higher stocks of fungal biomass ergosterol at all elevation levels compared to those in acidic site. Whereas the contents of SOC, total N, MBC, MBN and ergosterol decreased with depths at all elevations in both sites. The study further demonstrated variations in soil microbial properties driven more strongly by soil chemical properties than environmental variables, suggesting profound control of soil abiotic factors on variations in microbial community structure in two different elevation gradient sites in the Alps. The accumulation of SOC in presence of higher microbial biomass especially of saprotrophic fungi reflect

astonishing phenomenon of retarded plant residue decomposition in alkaline site Hochschwab especially at low and mid elevation levels than at acidic site Rauris.

2. In the second study, in alkaline site Hochschwab, microbial necromass formation was reduced in comparison to acidic site Rauris, which is another strong indication of retarded microbial plant residue decomposition. The other reason for such decomposition retardation can also be the drastically decreased biomass-specific phenoloxidase and peroxidase enzyme activities and lower levels of phosphatase activities in alkaline compared to those in the acidic site. However, in both sites, fungi dominated microbial biomass and necromass, but the dominance was stronger in the alkaline site. This study presented that climatic effects along alpine elevation gradients depend on vegetation induced changes in soil properties, which interact with bedrock properties. Thus, the differences in physical breakdown of parent material and C input *via* root and litter from dominating tree species in acidic and alkaline sites respectively might lead to differences in fungal community structure between the two sites which needed further investigation at genomic level.

3. Consequently, our third study concerning metagenomic sequencing of fungal ITS2 biomarker gene showed that the diversity of soil fungi at both sites, was in the order of phylum Ascomycota > Basidiomycota > Zygomycota. In terms of functional guilds, saprotrophism (Ascomycota) and symbiotrophism (Basidiomycota) dominated in both alkaline site (Hochschwab) and acidic site (Rauris). The correlation analyses further indicated significant effects of environmental factors (elevations, mean annual temperature (MAT) and mean annual precipitation (MAP)) on overall fungal community structure followed by edaphic factors (soil pH, SOC) at both sites. Additionally, only at alkaline site, soil C/N ratio significantly correlated with fungal community structure. The variation in main fungal classes and their driving factors at the two contrasting alpine sites suggest that their shifts may be driven by complex microbial interaction with elevation related environmental factors.

The work in current thesis presents important role of fungal communities' in ecosystem processes such as SOM turnover and C sequestration in high mountain alpine sites, under changing climatic conditions. In next steps it will be helpful to extend the

research to more elevation levels and deeper soil depths and at more alpine sites containing different bedrock material and vegetation, but similar climate. This will further help to discern the combined effects of soil mineral characteristics and different litter and C input under climate change scenario. Also, knowledge on functional diversity of soil fungi and on transition in fungal communities might be strengthened using metatranscriptomic or metaproteomic techniques of quantifying the decomposer gene. Nevertheless, the important data generated in my thesis can be used for model development in order to understand soil microbial ecology and ecosystem management and to establish better links between them. Finally, in line with the earlier studies, the work in this thesis furthers our understanding that effects of climate warming in the Alps are impossible to overlook.

Zusammenfassung

Die floristische und faunistische Biodiversität der Höhenstufen in Gebirgen wie den Alpen ist gut erforscht, hingegen ist das Wissen darüber, wie die pilzliche Gemeinschaftsstruktur entlang von Höhengradienten variiert, noch gering. Bodenpilze spielen eine essentielle Rolle innerhalb der mikrobiellen Gemeinschaft des Bodens, in welchem sie am Umsatz der organischen Bodensubstanz und am Nährstoffkreislauf beteiligt sind. Somit wirkt sich jede Änderung der pilzlichen Gemeinschaftsstruktur auf die Boden- und Ökosystemfunktionen aus. Oberstes Ziel der vorliegenden Arbeit war es deshalb zu untersuchen wie die Klima-induzierten Änderungen in der Gemeinschaftsstruktur der Pilze sowie deren Bio- und Residualmasse den Umsatz der organischen Bodensubstanz sowie die Kohlenstoff- und Stickstoff- Dynamiken entlang von Höhenstufen- und Bodentiefengradienten in zwei unterschiedlichen Ökosystem-Standorten in den österreichischen Alpen erklären können. Die beiden ausgewählten Standorte unterscheiden sich nur bezüglich ihres Ausgangsgesteins und der Vegetation, sind aber vergleichbaren Klimabedingungen entlang der drei untersuchten Höhenstufen ausgesetzt. Ein weiteres Ziel war es die Gemeinschaftsstruktur der Pilze und die Faktoren, von welchen die Zusammensetzung dieser Gemeinschaft am meisten abhängt, zu untersuchen. Deshalb wurden in dieser Arbeit verschiedene methodische Ansätze wie die Illumina-Plattform-basierte Next Generation Sequenzierung eines Standard-Markers für Pilze, der ITS2-Region (internal transcribed spacer region), mit chemischen und biochemischen Methoden kombiniert. Dies erlaubte die Charakterisierung der pilzlichen Diversität, die Quantifizierung der C- und N-Pools der organischen Bodensubstanz und die der mikrobiellen Biomasse, die Analyse der mikrobiellen Biomasse mit unterschiedlichen Markern, die Quantifizierung der mikrobiellen Residuen und die der mikrobiellen Aktivitäten sowie die Analyse der Faktoren, welche die bodenmikrobiologischen Eigenschaften der alpinen Höhengradienten kontrollieren.

1. Die Untersuchung von zwei Standorten in unterschiedlichen alpinen Ökosystemen, die sich in ihrem Ausgangsgestein und ihrer Vegetation voneinander unterscheiden, und in jeweils drei Höhenstufen mit Probenahme in unterschiedlichen Bodentiefen ermöglichte es, die Adaptation der mikrobiellen Gemeinschaften und ihrer taxonomischen Gruppen an den lokalen Lebensraum besser zu verstehen. Die Ergebnisse

der ersten Studie zeigen, dass die Vorräte des in der mikrobiellen Biomasse gebundenen Kohlenstoffs und Stickstoffs mit ansteigender Höhenstufe sowohl am alkalischen als auch am sauren Standort zunehmen und somit den Änderungen der C- und N-Vorräte der organischen Bodensubstanz folgen mit Ausnahme der niedrigsten Höhenstufe des alkalischen Standorts. Die Vorräte an Ergosterol, einem Biomarker für saprotrophe Pilze, waren in allen untersuchten Höhenstufen des alkalischen Standortes größer als im Vergleich zu denen des sauren Standortes. Die Gehalte des Kohlenstoffs der organischen Bodensubstanz, des Gesamtstickstoffs, des Kohlenstoffs und Stickstoffs der mikrobiellen Biomasse und des Ergosterols nahmen auf allen untersuchten Höhenstufen beider Standorte mit der Bodentiefe ab. Außerdem konnte durch diese Forschungsarbeit gezeigt werden, dass die mikrobiellen Eigenschaften stärker von bodenchemischen Eigenschaften abhängt als von den Umweltfaktoren. Veränderungen in der mikrobiellen Gemeinschaftsstruktur entlang der Höhengradienten beider Standorte werden in hohem Maße von abiotischen Bodenfaktoren kontrolliert. Die Akkumulation organischer Bodensubstanz trotz erhöhter mikrobieller Biomasse, insbesondere die der saprotrophen Pilzen, lässt entgegen den Erwartungen auf einen langsameren Abbau von Pflanzenstreu am alkalischen Standort Hochschwab im Vergleich zum sauren Standort Rauris schließen, was insbesondere in der untersten und mittleren Höhenstufe zum Tragen kommt.

2. In Studie zwei, war am alkalischen Standort die mikrobielle Nekromasse im Vergleich zum sauren Standort geringer, was ein weiterer Indikator für den verzögerten mikrobiellen Abbau von Pflanzenstreu ist. Zusätzliche Gründe für einen langsameren Streuabbau können auch die weit geringeren Biomasse-spezifischen Phenoloxidase- und Peroxidase-Enzymaktivitäten und die verringerte Phosphatase –Aktivität am alkalischen Standort im Vergleich zum sauren sein. In den Böden beider Standorte dominierte die Gruppe der Pilze sowohl die mikrobielle Bio- als auch die Nekromasse. Der Anteil der Pilze war am alkalischen Standort im Vergleich zum sauren höher. Klimatische Effekte entlang von alpinen Höhengradienten beeinflussen maßgeblich die Vegetation, die wiederum die Bodeneigenschaften prägt; letztere hängt zudem sehr stark vom Bodenausgangsgestein ab. Die Unterschiede der physikalischen Verwitterung des Ausgangsgesteins und des C-Eintrag durch Wurzeln und Streu der dominierenden

Baumarten am sauren und alkalischen Standort könnte zu Unterschieden in der Zusammensetzung der pilzlichen Gemeinschaft führen, was einer vertieften Analyse auf Genom-Ebene bedarf.

3. In Studie drei, zur metagenomischen Sequenzierung des Pilz-ITS2-Biomarker-Gene wurde gezeigt, dass die Biodiversität der Bodenpilze an beiden Standorten in folgender Größenordnung zueinander standen: Ascomycota > Basidiomycota > Zygomycota. Somit waren saprotrophe Pilze (Ascomycota) und symbiotische Pilze (Basidiomycota) die dominierenden Organismengruppen dieser Standorte. Mittels Korrelationsanalysen konnte erörtert werden, dass die Umweltfaktoren Höhenlage, mittlere Jahrestemperatur und mittlerer Jahresniederschlag die Gesamtstruktur der pilzlichen Gemeinschaft signifikant beeinflussen; des Weiteren zeigten die edaphischen Faktoren (Boden-pH, organische Bodensubstanz) einen signifikanten Effekt auf die Pilz-Gemeinschaft an beiden Standorten. Das C/N-Verhältnis des Bodens korrelierte nur auf dem alkalischen Standort signifikant mit der pilzlichen Gemeinschaftsstruktur. Aufgrund der Variabilität innerhalb der größten Organismengruppen der Pilze an zwei unterschiedlichen alpinen Standorten und der Faktoren, von welchen sie abhängen, kann gefolgert werden, dass diese Veränderungen durch komplexe Interaktionen zwischen mikrobiellen Eigenschaften und höhenspezifischen Umweltfaktoren versursacht werden.

Die vorliegende Arbeit zeigt die Rolle von pilzlichen Gemeinschaften in wichtigen Ökosystemprozessen wie der Umsatz der organischen Bodensubstanz und der C-Sequestrierung an alpinen Standorten unter geänderten Klimabedingungen. Zukünftige Forschungsvorhaben sollten auf zusätzliche Höhenstufen, weitere Bodentiefen und weitere Standorte im Alpenraum, die sich im Ausgangsgestein und der Vegetation voneinander unterscheiden, in Bezug auf das Klima aber vergleichbar sind, ausgeweitet werden. Außerdem ist ein besseres Verständnis der funktionellen Vielfalt von Bodenpilzen und der Veränderung der Zusammensetzung der pilzlichen Gemeinschaft durch metatranskriptomische oder metaproteomische Techniken zur Quantifizierung derjenigen Gene, die grundlegend für den enzymatischen Abbau der organischen Bodensubstanz sind, erstrebenswert. Auf Grundlage der im Rahmen dieser Arbeit gewonnenen Daten können ökologische Modelle entwickelt werden und ein tieferes Verständnis der bodenmikrobiologischen Ökologie, des Ökosystemmanagements sowie

deren Dependenzen erlangt werden. Gleich vorherigen Forschungsarbeiten untermauert die vorgelegte Arbeit die Annahme, dass die im Zuge des Klimawandels zu erwartende Temperaturerhöhung drastische Auswirkungen auf das Ökosystem der Alpen haben wird.

1 General Introduction

Soils are intricately linked with atmospheric processes; thus, global climate change can have significant impacts on all soil properties. Climatic parameters such as temperature and moisture can be directly or indirectly linked with these impacts. Among various soil properties, soil organic matter (SOM) and soil microbial diversity are more extensive compared to any other environment on earth. However, within soil microbial consortia, fungi are key players in SOM decomposition, carbon sequestration (Joergensen and Wichern, 2008) and regulating stability of soil physical properties especially in the alpine soils (Murugan et al., 2019). Therefore, any change in fungal community composition may have cascading effects on soil and ecosystem functioning. Further, environmental variability is an integral part of ecosystem dynamics and is subjective to perturbations which in soil environment could lead to shifts in community composition and associated role of soil microorganisms (Murugan et al., 2014). However, it is worth highlighting that much ambiguity exist on how reactive fungal communities are to the climate induced changes in ecosystem properties in temperature sensitive habitats such as the Alps.

1.1 Climate change and high mountain alpine ecosystems

The high mountain alpine ecosystems in the European Alps are of great environmental and social significance (Beniston, 2005; EEA, 2009). Protection of their delicately balanced large biodiversity has laid the foundation of establishment of International Commission for the Protection of the Alps (CIPRA). The important role of climate in every ecosystem is indisputable, especially in the high mountain alpine ecosystems where meteorological conditions often show extreme behaviour. Climate also governs the natural environment of mountains on short timescales (Beniston, 2005) and characterizes the biogeochemical processes therein (Bradley et al., 2015). The sensitivity and response of mountain ecosystems to climate warming is reflected in its biota which apparently is usually controlled by low temperatures.

Often the earthly-scale alterations in temperature, precipitation patterns and concentration of atmospheric CO_2 are associated with global climate change. Especially in the Alps, the mean annual temperature have increased by 2 ˚C in the past century, while by the end of 21st century, the predicted rise in mean surface temperature by 1-4 ˚C may

generate irreversible effects of high magnitude in alpine regions (IPCC, 2014). The consequences of such temperature changes are likely to be significant and may result in reduced cold and increased hot days and more fluctuations in annual rainfall and snow cover in alpine ecosystems (Bradley et al., 2015; Siles et al., 2016). Therefore, the susceptibility of alpine ecosystems to rapid environmental changes make them interesting locations to detect early signals of climate change and its impacts on ecological properties such as soils (Djukic et al., 2010b, 2013; Sundqvist et al., 2013). Meanwhile, it is unclear how climate interacts to impact soils and how soil microbial communities respond to such changes (Djukic et al., 2010a/b; Siles et al., 2016). Hence compiling such information from high mountain alpine ecosystems is important as climate change may intensify effects on soil communities, stretching the limits towards more extreme occurrences and positively feedback climate warming.

1.2 Elevations as special environmental gradients in alpine ecosystems

Ecologists have adopted elevation gradients as 'natural laboratories' to better understand ecosystem functioning on short-term time scale (Körner, 2003; Margesin et al., 2009; Djukic et al., 2010b). The complex topography of high mountain Alps induces sharp gradients in climatic parameters such as the temperature and precipitation over short distances/elevation levels. Consequently, these mountains manifest high biodiversity, usually with sharp transitions from vegetation to soils and snow to ice (Beniston, 2005). The Alps are unique locations for detection of global change and its environmental involvement owing to their exceptional features such as; i) ecological continuity of parent material over all elevations, ii) climate changes sharply at different elevations but so do vegetation (Körner, 2003), and changes of small magnitude in global events may demonstrate large scale effects in short-time over different elevations (Djukic et al., 2010b; Sundqvist et al., 2013), iii) absence of direct or significant human interference in the high mountain Alps (Bensiton, 2005) and, iv) availability of data, monitoring climate and environment in these special regions (IPCC, 2014).

The vegetation differences along elevations generate zones like; montane to subalpine forests at low (foothill) and mid elevations, alpine grasslands at high elevations and snow-capped nival zones at the summit (Figure I). All of these provide important ecosystem services (Körner, 2003). However, ecosystem structure is shaped, and

ecosystem cycles are affected by large scale variations in climatic factors like temperature and moisture, along the slopes of the mountains (Loarie et al., 2009; Sundqvist et al., 2013). The spatial gradient in temperature change is greatest on mountain slopes and may exacerbate upward migration of tree-line and important microbial groups like fungi (Djukic et al, 2010a, Siles et al., 2016; Andrew et al., 2018). Such an increase in temperature increases net primary productivity and accelerates SOM decomposition (Davidson and Janssens, 2006). While fluctuations in precipitation regulate soil moisture conditions and may result in either drought or water stress to plants and soil microorganisms (Manzoni et al., 2012). Complimentary to these changes, differences in plant species and their litter fall characteristics also affect decomposition in the Alps (Djukic et al., 2010b; Berger and Berger, 2012). However, the critical effects of vegetation induced changes in composition of SOM over elevation gradients and consequent changes in microbial community biomass and structure are less studied in alpine soils (Siles et al., 2017). Hence, critically designed elevation gradient experiments will potentially answer important ecological questions based on "space for time substitution" (Körner, 2003; Djukic et al., 2013).

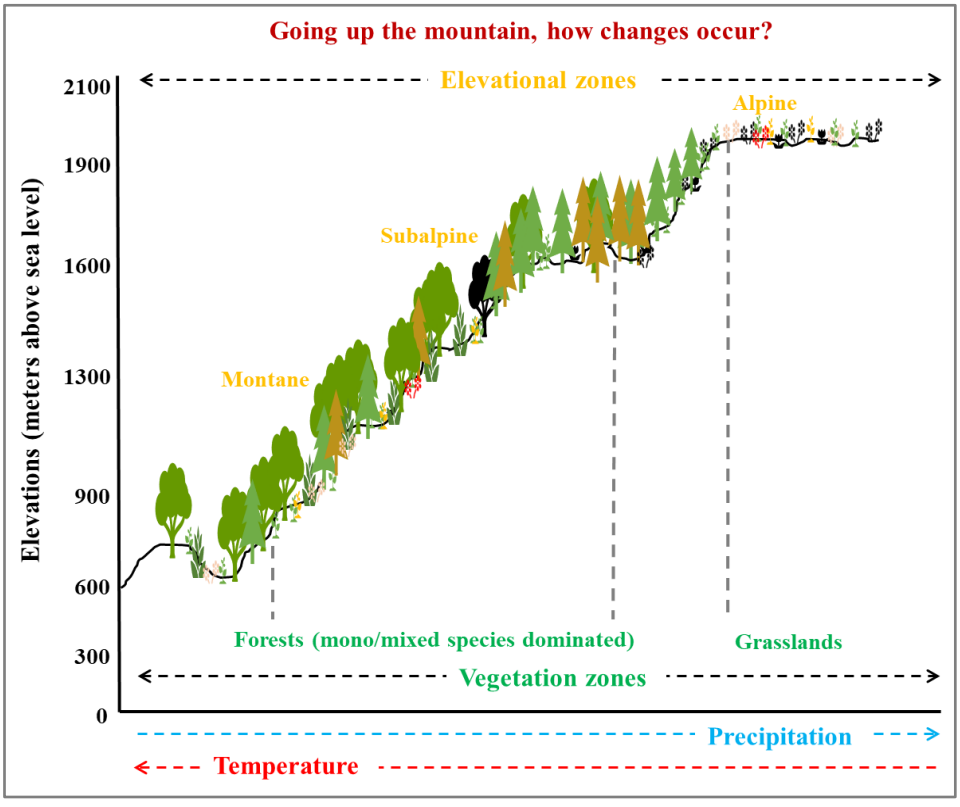

Fig. I. Changes in elevation and vegetation zones in alpine mountain ecosystems.

1.3 Climate change and Soil Organic Matter (SOM) in alpine ecosystems

The soil organic matter (SOM) ranges in size and chemical complexity. It represents a composite mixture of residues of plants and animals at different decomposition stages and, biomass, necromass and products of microbial origin (Lal, 2008; Liang and Blaser, 2011; Rumpel and Kogel-Knabner, 2011). Soil organic carbon (SOC) contributes approximately 60% to SOM (Lal, 2008), therefore understanding SOM dynamics lead to better insights on global carbon cycle (Liang and Balser, 2011). The physical, chemical, and biochemical barriers primarily insure SOM against decomposition (Von Lützow et al., 2006; Jenkinson et al., 2008; Joergensen, 2010). In addition to such mechanisms, SOM in alpine soils is also protected by low temperatures which usually slow down biotic and abiotic degradation of SOM (Von Lützov and Kogel-Knabner, 2009). Low temperature also reduces the oxidation of organic molecules to further decrease organomineral associations in cold soils (Rumpel and Kogel-Knabner, 2011).

The labile C is highly degradable in warmer condition (Joergensen, 2010) and forms the major component of SOM in cold alpine environments (Kogen and Knabner, 2011). Moreover, alpine soils are also characterised by dense microbial communities, exhibiting high growth potential and activities (Margesin et al., 2009; Djukic et al., 2010a, 2013; Bradley et al., 2015). The freezing of soils in alpine environment reduces enzymatic degradation of SOM (Davidson and Janssens, 2006; Wallenstein and Burns, 2011), which in deeper soil layers constitute of easily degradable low-density fractions (Djukic et al., 2010b). Therefore, an apparent increase in temperature will reduce freezing and may stimulate microbial activities, consequently affecting the persistence of SOM (Nannipieri et al., 2003; Sinsabaugh et al., 2008; Joergensen, 2010). Hence, warming of soils in the Alps will expose important C pool to microbial transformation of SOM (Djukic et al., 2010b; Djukic et al., 2013), and generate higher nutrient availability such as of C and N elements in soils. Thus, acknowledging the importance of such nutrient reserves and to achieve ecosystem specific fingerprints, it is legitimate to account information on soil C and N status across different elevations and soil depths beforehand. This will disentangle the role that climate plays on creation of their stocks and factors responsible in the alpine ecosystems.

1.4 Climate change and soil microbial diversity

The complexity of soil microbial communities matches to that of SOM, which changes under changing climatic and plant species composition (Djukic et al., 2010b; Berger and Berger, 2012). In this regard, the implications of direct effects of climate change (i.e. temperature rise) on soil microbial communities will depend on indirect effects of trade off in plant functional groups (Bardgett and Wardle, 2010). Additionally, habitat properties such as warming and alterations in soil moisture conditions (Djukic et al., 2010a; Manzoni et al., 2012) and matrix of pores and soil aggregate sizes (Balser et al., 2010) directly impact soil microorganisms their composition and diversity.

Soil microbial diversity further highlights complexity and variability at different levels of biological configuration. Such as in the genetic inconsistency within taxon (species), their number (richness), relative abundance (evenness) and microbial functional groups (guilds) (Torsvik and Øvreås, 2002). Fungi, bacteria, and archaea predominate in soils. They not only cohabit but also interact *via* competition, predation and parasitism (Torsvik and Øvreås, 2002; Balser et al., 2010). These interactions regulate soil microbial biomass so that the composition of one group affects that of others. In alpine soils, these microbial functional groups respond differently to interactions between climatic changes and other environmental factors (Djukic et al., 2010a, Siles and Margesin, 2016; Siles et al., 2017). Although, recent advances in biochemical and culture-independent techniques have led to significant progress in soil biology and microbiology, little work is done to connect these measures with habitats and ecosystem functions (Djukic et al., 2013; Siles et al., 2016).

1.4.1 Soil microbial biomass, community composition and activities

Soil microorganisms show rapid response to changing environment, at much faster rate than plants and animals (Balser et al., 2010; Sundqvist et al., 2013). In alpine soils, soil microorganisms transform 80-90% SOM (Djukic et al., 2013). Therefore, their biomass is a sensitive indicator of alterations in SOM and soil quality changes (Joergensen, 1996; Joergensen and Mueller, 1996; Zelles, 1999). Two widely used biochemical approaches for general characterization of microbial biomass are the fumigation-extraction method (Vance et al., 1987) and the measurement of total amount

of phospholipid fatty acids (PLFA's) in soils (Frostegård et al., 1993). In the latter, the structural data obtained from lipids is based on the occurrence of fatty acids or quinones in specific microbial taxa. This approach allows inference of metabolic function by identifying presence of microbial group known for that function. Additionally, fungal cell-membrane component ergosterol is highly specific indicator for most higher fungi in soils (Joergensen and Wichern, 2008), but is rarely quantified in the alpine soils. Further, the measurement of enzyme activities is also one of the most important approach in soil microbiology (Nannipieri et al., 2003; Sinsabaugh et al., 2008), as microorganisms produce a suite of extracellular enzymes to degrade SOM and mineralize nutrients. The expressed enzyme activities are closely related to microbial biomass and/or activity (Nannipieri et al., 2003; Wallenstein and Burns, 2011).

Microbial growth, composition and activity are sensitive to temperature and soil moisture changes (Balser et al., 2010; Manzoni et al., 2012; Sinsabaugh et al., 2008). However, growth and activity are individual characteristics of microbial communities and may vary independently (Torsvik and Øvreås, 2002). This means that climatic conditions favouring high level of microbial activity do not necessarily ensure high microbial growth and associated biomass increase. This is also evident from unclear results such as high microbial biomass in alpine grasslands but not their activity (Djukic et al., 2010a), while Margesin et al. (2009) found the opposite in the Central Austrian Alps. Such conspicuousness generated increasing interest in understanding variations in microbial biomass, community structure and activity in elevation gradient soils (Siles et al., 2016, 2017).

1.4.2 Soil microbial residues

Microbial growth is concomitant with microbial senescence leading to the formation of microbial residues that get incorporated in SOM as its stable component. Microbial residue C contribute~40 times higher to the C pool in soil than living microbial biomass C (Liang and Basler, 2011). Hence the catabolic and anabolic activities of soil microorganisms largely influence terrestrial C pool (Liang and Balser, 2011; Rumpel and Kogel-Knabner, 2011). Amino sugars originating from microbial cell walls are considered as microbial residues and are time integrated biomarkers of microbial community composition and contribution to C sequestration (Joergensen, 2018). The

measurement of bacterial muramic acid (MurN) and fungal glucosamine (GlcN) enables estimation of the necromass contribution of the two main microbial functional groups to SOC. However, based on soil conditions, microbial residues are differently formed and accumulated (Rumpel and Kogel-Knabner, 2011). Till to date only few studies have considered to estimate amino sugar residues in the high alpine mountain soils (Murugan et al., 2019). Thus, to strengthen our understanding of climate effects on SOM dynamics in alpine soils, it is needed to consider the effect on concentration, production, and stabilization of microbial derived organic matter in the Alps.

1.5 Fungi as key players in soil ecosystems

Often, fungi exist as free-living in soil matrix and are the only decomposers of lignified cellulose and contribute significantly to the microbial tissue in soils (Joergensen and Wichern, 2008; Peay et al., 2008). They also provide nutrients to plants through mycorrhizal associations (Torsvik and Øvreås, 2002; Bahram et al., 2012). This bequeaths them with unique possibility to showcase immediate responses, both due to direct effects of climate warming and alterations in soil moisture conditions and, indirect responses mediated *via* changes in vegetation. Climate effects on vegetation in the Alps are apparent (Djukic et al., 2010b, 2013), however despite their ubiquity and significance in terrestrial ecosystems (Joergensen and Wichern, 2008), fungal ecological studies along elevation gradients are inadequate. Fungi directly shape the dynamics of plants, animals, and bacteria (Peay et al., 2008; Strickland and Rousk, 2010). Thus, any change in fungal communities' resulting from climate change will have knock-on effects on ecosystem functioning, making their diversity study important in the alpine soils.

1.5.1 Assessing fungal diversity

Unlike bacteria, fungi do not exhibit high frequency of horizontal gene transfer, meaning that their functional traits are stable with reasonably developed and useful species concepts. This maybe because of high recalcitrance (Strickland and Rousk, 2010) and adaptability to extreme environmental conditions (Margesin et al., 2009; Djukic et al., 2010a). Furthermore, they share many ecological similarities with macroorganisms, like plants. Hence much of the ecological theories derived from macroorganisms are relatable to the study of fungi (Peay et al., 2008). Previously, the diversity of fungi was

estimated by examining morphological features of reproductive (fruiting bodies) or vegetative structures (spores and hyphae). However, the consequent results were biased as information on slow growing fungal species and those that do not produce such structures was lacking (Peay et a., 2008). The development of polymerase chain reaction (PCR)-based molecular methods overcame this disparity. Although, drawbacks such as low throughput underrepresented fungal species with low abundance, and that the method is expensive and tedious (Peay et al., 2008; Schmidt et al., 2013). Consequently, the advent of cost-effective Illumina-platform based next generation sequencing (NGS) (Schmidt et al., 2013), enabled DNA surveys with high sample throughput. Also, the advantages of this technique included use of barcodes that can be indexed to DNA molecules of each sample and perform multiple sample sequencing at once. Later to the declaration of internal transcribed spacer (ITS) region as the universal fungal DNA barcode (Schoch et al., 2012) and, formation of community-curated reference database UNITE (Kõljalg et al., 2013), many studies describing fungal communities' documented the structure of specific functional groups across varied ecosystems (Bahram et al., 2012; Tedersoo et al., 2014). However, the use of this technique in elevation gradient studies is at its infancy (Siles et al., 2016, 2017). An advanced use of NGS technology may provide comprehensive information on the distribution of fungal communities in the high alpine ecosystem soils.

1.5.2 Drivers of fungal diversity

At global scale, the fungal metabolism is expected to be positively affected by elevated temperatures, resulting in changes in fungal community structure and composition across different ecosystems (Tedersoo et al., 2014). Whereas, at local scale, soil moisture controls fungal diversity (Manzoni et al., 2012). Bahram et al. (2012) found monotonic decrease in species richness in ectomycorrhizal fungi (EMF) in relation to variation in mean annual temperature and precipitation along increasing elevations in temperate old-growth forest. Similar observations were made by Jarvis et al. (2015) but the shift was related to soil moisture. In the Italian Alps (Siles and Margesin, 2016), soil pH and C/N ratio governed fungal diversity dominated by Ascomycota, Basidiomycota and Zygomycota phyla and, Agricomycetes class. While Gómez-Hernández et al. (2012) found positive relationship between patterns of macromycete community assemblage and

vegetation type along elevation gradients. Under forest vegetation, arbuscular mycorrhizal fungal (AMF) communities significantly differed between the roots of forest specialist plant species and the roots of habitat generalist plant species (Öpik et al., 2009). That said, changes in above ground plant communities affected fungal biomass in the Northern Limestone Alps in Austria (Djukic et al., 2010a) and temperature in the Central Austrian alps (Margesin et al., 2009). Therefore, it is important to undertake surveys that excavate the inner links between environmental variables that impact fungal diversity and showcase shifts in fungal functional guilds especially in the temperature sensitive high alpine ecosystems.

1.6 Theme of the research work

It is from the broad context mentioned above that this dissertation was inspired to investigate how climate induced shifts in community structure, biomass and residue of soil fungi could help to understand the turnover of SOM and carbon (C), nitrogen (N) dynamics in the alpine soils at different elevation and depth gradients. There are many elevation gradient studies investigating SOM characteristics, and microbial aspects in-terms of community composition, activity and diversity in the Alps (Margesin et al., 2009; Djukic et al., 2010a/b, 2013; Siles and Margesin 2016, Siles et al., 2017). However, these studies were conducted only along single elevation gradients and/or in the topsoil layer. In order to further complement the understanding on climate effects on soil and microbial characteristics, information from alpine ecosystems differing only through geology and vegetation, while retaining constancy in climatic parameters such as temperature and precipitation is missing, especially with comparison perspective. Thus, the two sites investigated in the study are Hochschwab in the Northern Limestone Alps *vs*. Rauris in the Central Austrian Alps, referred to as alkaline and acidic sites throughout the thesis, respectively (Figure II). This reference is based on differences in soil chemistry. At Hochschwab, Calcareous Leptic Histosols were formed on alkaline parent material (limestone and dolomites) *vs*. Haplic Podzols formed on acidic parent material (gneiss and schists) at Rauris. At each site three elevation levels were chosen that differed in vegetation composition (Figure III). At low and mid elevations, deciduous spruce-beech forests dominated at alkaline site *vs*. spruce dominated forests at acidic site. In both sites, high elevations had grassland vegetation.

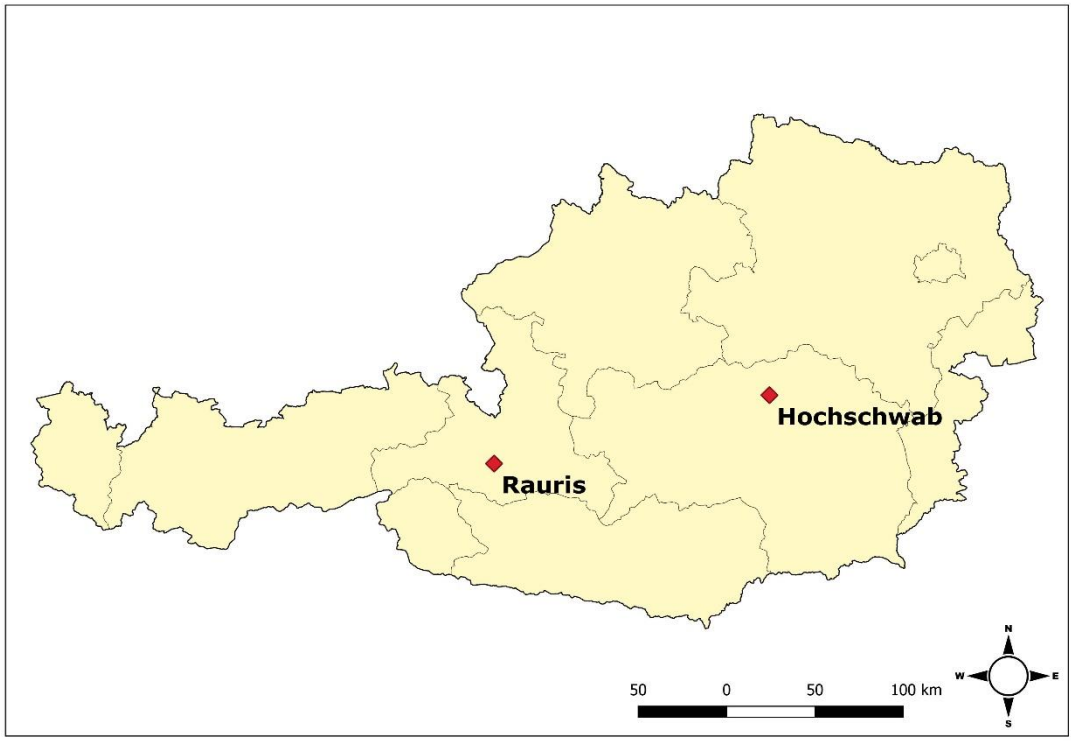

Fig. II. Location of two alpine sites in Austrian Alps; Hochschwab in the Northern Limestone Alps and Rauris in the Central Austrian Alps.

The alkaline site (Hochschwab) is extensively studied, in context of changes in SOM characteristics and microbial aspects (Djukic et al., 2010a/b, 2013). Such information is not available for newly investigated acidic site (Rauris). Therefore, in the current thesis, only the differences in geology and vegetation across the two sites and, different environmental conditions along elevation gradients within each site, offered unique opportunity to study the state factors that are often considered as micro-scale anomaly associated to microorganisms and SOM dynamics. Previous elevation gradient studies (Margesin et al., 2009; Djukic et al., 2010a/b; Siles and Margesin, 2016) assessed microbial community composition or diversity but did not focus exclusively on fungi despite the significant role they play in terrestrial ecosystem processes like decomposition and C sequestration (Joergensen and Wichern, 2008; Joergensen, 2018).

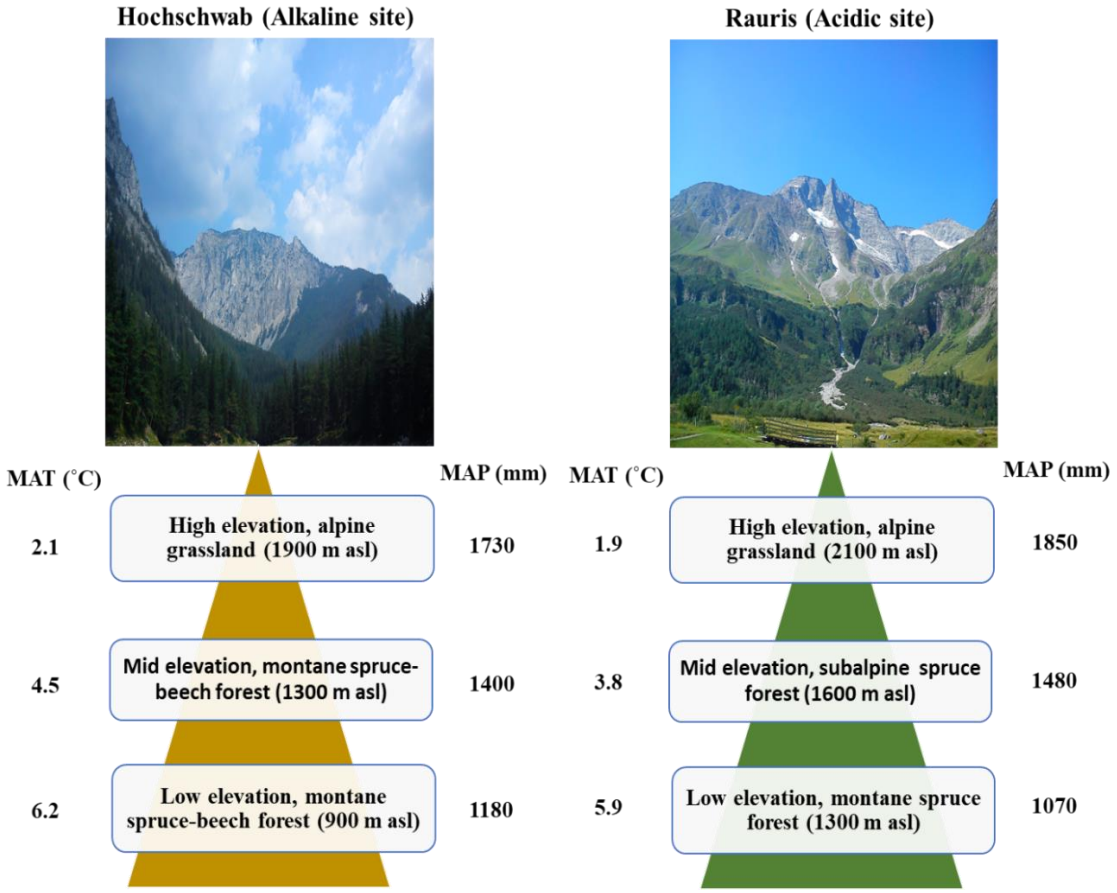

Fig. III. Two alpine sites with elevation gradients showing similar climatic and environmental factors. MAT, mean annual temperature; MAP, mean annual precipitation; m asl, meters above sea level.

1.7 Research objectives

Summarizing the findings above, alpine soils storing enormous amount of C stocks (>92%) (Körner, 2003) might be adversely affected by rising temperature and predicted upward treeline migration (IPCC, 2014; Djukic et al., 2010a). Moreover, large amount of soil organic carbon (SOC) is stored in topsoil (0-5 cm) of high-elevation sites alpine soils mainly due to reduced temperature, increased precipitation and altered litter quality in comparison to the low-elevation sites alpine soils (Djukic et al., 2010b; Siles et al., 2017). The general impacts of elevation induced changes in vegetation and climate on microbial biomass and community structure have rather been inconclusive especially at different soil depths. Hence as most of the studies were conducted only along single elevation gradients and focused on topsoil layer (0-5 cm), we chose to examine whether elevation induced changes in vegetation influence deeper soil layers (5-15 and 15-25 cm) in two

alpine sites that differed in vegetation and bedrock properties. Measurement of microbial biomass C (MBC), microbial biomass N (MBN) and fugal biomass ergosterol stocks using soil depth approach at both sites provide ideal opportunity to study vertical and spatial variability of these sensitive ecological indicators and elaborate understanding of carbon sequestration potential in soils (Murugan et al., 2014). Therefore, to provide this unique set of data on soil and microbial biomass C and N indices at different elevations in alpine sites, the first experiment lead to objective 1.

Objective 1: Exploration of soil and microbial biomass C and N dynamics, and to access relative changes in their proportion at two alpine elevation gradients in acidic and alkaline sites.

At the alkaline site Hochschwab, microbial biomass increased but trend in microbial activity and stocks of SOM was inconsistent with increasing elevations (Djukic et al., 2010a/b). While, in the Central Austrian Alps in Hohe Tauern/Grosslockner area, microbial activity decreased, and biomass increased with increasing elevations (Margesin et al., 2009). In the study of Djukic et al. (2010b) changes in SOM stocks were related to subsequent changes in vegetation, C and litter input. Although, it might also be a result of retarded decomposition processes in presence of high microbial biomass especially at alkaline site Hochschwab. Nevertheless, the elusive results in alpine sites differing in bedrock material (Djukic et al., 2009 in Northern Austrian Alps *vs.* Margesin et al., 2009 in Central Austrian Alps) make simultaneous comparison of elevation gradient sites imperative to explore ecosystem response at regional level. Furthermore, in this relation, microbial residues serve as potential sources of SOM formation and express high stability in soils. They allow identification of specific contribution of fungal and bacterial residues to C sequestration in soils while also integrate environmental effects on soil microorganisms over time (Joergensen and Wichern, 2008). However, microbial residues are rarely quantified in high mountain alpine soils (Murugan et al., 2019). On the other hand, membrane derived PLFA index living microbial biomass (Frostegård et al., 1993) and give useful information on the large functional groups such as Gram-positive (G+), Gram-negative (G-) bacteria, and fungi (Joergensen and Wichern, 2008). Thus, they are an independent control for fungal cell membrane component ergosterol (Weete et al., 2010). In addition to the microbial biomass and necromass formation, plant residues are decomposed by a large variety of extracellular enzymes released by soil microorganisms

which are specifically involved in C and nutrient cycles (Sinsabaugh et al., 2008). Combination of such biochemical markers to understand decomposition process and key factors involved in alpine elevation gradients sites differing in bedrock and vegetation is missing especially with comparison perspective. This lack of information set the stage for second experiment with objective 2.

Objective 2: Investigation of retarded plant residue decomposition at alkaline site Hochschwab in comparison to acidic site Rauris.

Although elevation patterns for plants and animals are known, information about variability in soil fungal communities across elevation gradients is little. The metagenomic approach of extracting soil DNA allows rapid identification of diversity of known and unknown fungi. However, despite the wide geographical range of fungi (Tedersoo et al., 2014) many elevation gradient studies (Gómez-Hernández et al., 2012, Jarvis et al., 2015, Siles et al., 2017) have focused only on specific fungal groups like the ectomycorrhizal (EMF) and/or the arbuscular mycorrhizal fungi (AMF). Also, such studies suffer from basic limitations such as study conducted across single elevation or mountain, or short elevational range, or elevations encompassing same vegetation, keeping the generality of results ambiguous. The quantity and quality of C resources change among plant species (Djukic et al., 2010b) which might strongly determine plant-fungal diversity interactions. Although, relative proportion of fungal biomass increases with elevations (Djukic et al., 2010b; Margesin et al., 2009), exactly which fungal members exhibit such a response to altered climatic conditions in alpine elevation gradients is less studied (Siles and Margesin, 2016; Siles et al., 2017). Plus, the factors most strongly related to fungal diversity change are not clearly known. To gain this knowledge third experiment with objective 3 was decided.

Objective 3: Characterisation of fungal diversity and functional guild attributes, and factors affecting them at two alpine elevation gradients in acidic and alkaline sites.

Overall, the goal of my dissertation was to gain new insights on soil and microbial indices and their stocks, understand microbial community composition, activity, and contribution of microbial residues to SOC sequestration with special reference to fungi at the two Austrian alpine sites, owing to the fact that ecosystem level studies of soil fungal communities are scarce.

2 Variations in soil and microbial biomass C, N and fungal biomass ergosterol along elevation and depth gradients in Alpine ecosystems

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Highlights

- Simultaneous elevation and depth gradients were studied at acidic Rauris and alkaline Hochschwab site.
- The stocks of SOC and total N increased with elevation levels in both sites.
- Microbial C/N ratios were increasing with depth in Hochschwab compared to Rauris.
- Fungal biomass was four times higher in Hochschwab than in Rauris soil horizons.
- No correlation was observed between the ratios of microbial C/N and ergosterol/Microbial biomass C.

Abstract

Changes in soil fungal biomass ergosterol, microbial biomass and their role in soil organic carbon (SOC) dynamics along elevation and depth gradients in the Alps are still poorly explored. Therefore, we investigated changes in stocks of SOC, total N, microbial biomass C (MBC) and N (MBN) and fungal biomass ergosterol at different elevation levels (low, mid and high) in two alpine sites. These two alpine sites represent similar temperature and precipitation regimes, one on alkaline (Hochschwab) the second on acidic (Rauris) bedrock. We found that the stocks of MBC increased with elevations in both sites ranging from 37.6 to 126.1 gm^{-2}, and MBN stocks from 4.1 to 19.8 gm^{-2}, following the changes in SOC and total N stocks, except at alkaline low elevation level. The stocks of MBC and MBN were higher at mid-elevation in alkaline than in acidic site. In contrast, MBN stocks were higher at low and high elevation level in acidic site compared to those in alkaline site. Median ergosterol stocks varied around 0.4 gm^{-2} in alkaline and increased from 0.05 to 0.2 gm^{-2} in acidic site. At all elevation levels, microbial biomass-C/N (MB-C/N) ratio in alkaline site increased with soil depths from 6.0 to 12.3 and remained constant in acidic site between 4.9 and 6.8. In alkaline site, the ratios of ergosterol/MBC declined from 0.6 to 1.4% at 5 cm depth to values around 0.2% at 15–25 cm depth, while this ratio varied between 0.4 and 0.1% at all soil depths in acidic site. Further, in both sites, redundancy and variation partition analysis showed that soil microbial properties were driven by soil chemical properties rather than environmental variables. Our results suggest that environmental variables were less disruptive to soil fungal and microbial biomass and inherent soil properties drive changes in soil microbial and fungal biomass stocks and may drive changes in microbial community structure.

Key words: Alpine ecosystems, Elevation gradients, Microbial biomass, Fungal biomass, Carbon sequestration

2.1 Introduction

In Alps, >92% ecosystem carbon (C) is stored in soils (Körner, 2003) thus, alpine ecosystem C storage, and in turn, global C balance is directly influenced by the accumulation and decomposition of soil organic C (SOC). The projected 4 °C increase in temperature by the end of 21st century and consequent upward treeline shift may increase decomposition rate and alter microbial community structure, and C stored in microbial biomass and soil (IPCC, 2014; Djukic et al., 2010b). Changes in vegetation composition, quantity and quality of plant input alter microbial biomass, community structure (Prietzel and Christophel, 2014; Solly et al., 2017; Zhang et al., 2017) and consequently affect SOC storage. Large amount of SOC is stored in the surface layer (0–5 cm depth) of high-elevation sites Alpine soils due to decreased temperature, increased precipitation and altered litter quality compared to low elevation Alpine soils (Djukic et al., 2010b; Prietzel and Christophel, 2014; Siles et al., 2017). Changes in climate induced vegetation modify soil pH, litter quality and soil structure and consequently alter SOC storage potential. In addition, variation in soil parent material plays a significant role in SOC decomposition through regulating soil microbial community structure, microbial biomass and residues (i.e. necromass) (Berger and Berger, 2012; Zhang et al., 2013). However, how changes in climate, litter quality and bedrock affect soil microbial biomass and community structure is still poorly understood, especially in the Alps.

Soil microbial biomass and activity increased (Siles et al., 2017) or decreased with increasing elevation (Ma et al., 2004; Margesin et al., 2009). Also with increasing elevation gradient, microbial community structure shifted towards bacteria (Djukic et al., 2010a), remained constant (Zhang et al., 2013; Lazzaro et al., 2015), or shifted towards fungi (Margesin et al., 2009) as indicated by fungal/bacterial ratio. Overall, the general impact of elevation induced changes in vegetation and climate on microbial biomass and community structure has been rather inconclusive. One reason for these contradictory results might be the unknown interactions between bedrock properties, vegetation, microclimate and soil microorganisms, although methodological constraints cannot be excluded. However, we have limited knowledge regarding changes in microbial biomass and community structure and how these changes regulate SOC storage at different elevation gradients that differ only in bedrock properties and vegetation (i.e. similar climatic conditions). Many studies have investigated changes in microbial community

structure (Margesin et al., 2009; Djukic et al., 2010a), amino sugar derived residues (Zhang et al., 2013), and enzyme activities (Siles et al., 2016) along a variety of altitudinal gradients. As most of previous studies focused on surface soil (0–5 cm), we chose to examine additionally whether elevation induced changes in vegetation influence deeper soil layers (5–15 and 15–25 cm) in two sites that differ in bedrock properties.

The conflicting results make comparison of elevation gradient sites, differing in basic soil characteristics imperative, to explore ecosystem response at a regional level. The elevation gradients are powerful 'natural experiments' in ecological research (Margesin et al., 2009; Djukic et al., 2010a, b). In acidic site (Rauris) three elevation levels were chosen, which are similar in temperature and precipitation regimes, although differing in absolute height from the respective three elevation levels in alkaline site (Hochschwab), intensively studied by Djukic et al. (2010a, b, 2013). The bedrock in acidic site consists of gneiss and schists, whereas the bedrock in alkaline site is dominated by limestone and dolomites. Therefore, simultaneous elevation and soil depth gradient studies offer an understanding of adaptation of microbial communities to local habitat and their patterns through natural gradients of soil conditions with comparison perspective.

Elevation induced changes in microbial biomass, community structure and SOC dynamics in the subsoil depths and their controlling factors, especially in natural ecosystems are largely unexplored. Measurement of microbial biomass C (MBC), microbial biomass N (MBN) and ergosterol stocks using a soil depth approach at both sites provide ideal opportunity to study vertical and spatial variability (Murugan et al., 2013). The quantification of MBC and MBN as sensitive ecological indicators allows understanding of C sequestration potential in soils (Murugan et al., 2014; Khan et al., 2016; Joergensen and Wichern, 2018), but has been rarely applied in alpine elevation gradient studies (Murugan et al., 2019). The importance of microbial biomass as bio-indicators of ongoing climate change in temperate forest soils (Schindlbacher et al., 2011) and also the sensitivity towards changes in tree species and soil type in boreal forests (Bauhus et al., 1998) has been already documented. Further, the fungal cell-membrane is highly specific indicator for most higher fungi in soil (Joergensen and Wichern, 2008; Khan et al., 2016). The aim of this study was to explore soil and microbial biomass C and N dynamics and to access relative changes in their proportion at the two alpine elevation gradients in acidic and alkaline sites. This allowed corroboration of the following

hypotheses: (1) Stocks of MBC, MBN, ergosterol follow those of SOM and increase with increasing elevation in both sites. (2) Lower stocks of MBC and MBN and higher stocks of ergosterol in acidic site than in alkaline site. (3) Soil properties (soil C/N and pH) have stronger effects on microbial parameters (MBC, MBN, and ergosterol) than environmental factors (temperature, moisture, and vegetation), that change similarly at different elevations in acidic and alkaline sites.

2.2 Materials and methods

2.2.1 Site selection and soil sampling

Our study was conducted along three elevation gradients in two Austrian alpine ecosystem sites. Elevation gradients in alkaline (Hochschwab) and acidic (Rauris) sites were selected to exhibit differences only through climate and vegetation while ensuring similar exposition, slope and soil type. In alkaline site, located in the Northern Austrian Limestone Alps (47° 32–36′ N, 15° 02–05′ E), three elevation points selected with south to south-west exposition were at 900 (low elevation) spruce (Picea abies (L.) H. Karst)/beech (Fagus sylvatica L.), 1300 (mid elevation) spruce/beech and 1900 m above sea level (m asl) (high elevation) grassland (Djukic et al., 2013). With increasing elevations, mean annual temperature (MAT) decreased from 6.2 to 2.1 °C, mean annual precipitation (MAP) increased from 1178 to 1725 mm and mean annual snow cover from 123 to 221 days (source: Austrian Federal Ministry of Finance: Table 1). The soils in alkaline site are classified as Leptic Histosols (IUSS WRB, 2015), typical for this area, dominated by Mesozoic limestone bed-rock material (Table 1).

Table 1. General site and soil characteristics information from climosequence study.

Elevation (m asl)	Level	Slope (%)	Aspect (° north)	MAT (°C)	MAP (mm)	Soil type	Bedrock	Vegetation
Alkaline site (Hochschwab)								
900	Low	21	225	6.2	1180	Leptic Histosols	Limestone and Dolomites	Montane spruce-beech forest
1300	Mid	19	225	4.5	1400			Montane spruce-beech forest
1900	High	26	200	2.1	1730			Alpine grassland, with montane pine bushes
Acidic site (Rauris)								
1300	Low	24	190	5.9	1070	Haplic Podzols	Gneisses and Schists	Montane spruce forest
1600	Mid	16	180	3.8	1480			Sub-alpine spruce forest
2100	High	20	180	1.9	1850			Alpine grassland

asl: above sea level; MAT: mean annual temperature; MAP: mean annual precipitation; soil classification according to IUSS WRB 2015; Vegetation as recorded on site; climate data for Hochschwab from Austrian Federal Ministry of Finance; climate data for Rauris by Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Austria.

Our second study site i.e. acidic (Rauris) is in the Central Austrian Alps (47° 14′ N, 13° 0′ E) at Mt. Sonnblick north of Innsbruck/Austria, where spruce is the dominating tree species. The three elevation points along a south-west exposition were at 1300 (low elevation) spruce forest, 1600 (mid elevation) spruce forest, and 2100 m asl (high elevation) grassland. Forty-five years meteorological data recorded at a weather station on Mt. Sonnblick (3106 m asl) show a mean annual temperature (MAT) ranging from 5.9 to 1.9 °C along the increasing elevation gradients, a continuous snow cover from October through May, and the possibility of frost during each summer month. Mean annual precipitation (MAP) ranged from 1067 to 1850 mm, with majority falling during the growing season starting in May through October (Table 1) and the mean annual snow cover vary from 131 to 235 days (source: Austrian Federal Ministry of Finance). The soil type here is Haplic Podzol (IUSS WRB, 2015), a typical soil type of the Central Austrian Alps, dominated by gneisses and schists (Table 1).

During August 2015, soil samples were collected at three soil depths (0–5 (without litter), 5–15 and 15–25 cm) with 12 replicates per elevation per depth, making all together

216 samples. They were kept under cool conditions, transported to the laboratory, sieved (< 2mm mesh), and stored at 4 °C until analysis. For bulk density (BD) analysis, separate 24 samples from 0 to 5 and 15 to 25 cm depths were collected at each site, using soil cores (7 × 5 cm: diameter × height).

2.2.2 Analytical methods

Soil pH was measured in deionised water (1:2.5 or 1:5 w/v wherever required) and BD was calculated on soil dry weight divided by core volume basis. In stone containing soils, difference in total soil volume taken by volume of stones or >2mm fragments was used to calculate volume of soils (cm^{-3}). A fraction of sieved soils was dried (24 h at 105 °C) and finely ground for chemical analyses to determine soil organic C (SOC) and total N after dry combustion followed by gas chromatograph equipped with Vario MAX elemental analyser (Elementar, Hanau, Germany).

Five grams of soil with 40% water holding capacity was used to measure MBC and (Vance et al., 1987) and MBN (Brookes et al., 1985) using chloroform fumigation extraction method with 20 ml 0.5M K_2SO_4 solution. Both fumigated and non-fumigated extracts were analysed using multi N/C 2100S CN analyser (Analytik Jena, Germany). Microbial biomass C was calculated as E_C/k_{EC}, where E_C = difference between organic C extracted from fumigated soils and organic C extracted from non-fumigated soils and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N/k_{EN}, where E_N = difference between total N extracted from fumigated soils and total N extracted from nonfumigated soils and k_{EN} = 0.54 (Joergensen and Mueller, 1996).

The fungal cell-membrane component ergosterol was extracted from 2 g moist soil at 40% water holding capacity with 100 ml ethanol by oscillated shaking at 250 rev. min^{-1} for 30 min (Djajakirana et al., 1996). The extracts were subjected to reverse phase HPLC with 100% methanol mobile phase and final detection at 282 nm wavelength.

2.2.3 Calculations and statistical analysis

All data considering the concentrations were presented on an oven dry basis (105 °C, 24 h) and measured in mg g^{-1} soil, except for ergosterol measured on µg g^{-1} soil basis. Stocks at different elevations (n = 36) were calculated on volume basis and represented in kg m^{-2} for SOC and total N, and those of MBC, MBN and ergosterol in g m^{-2},

considering the bulk density of the respective soil layer. Non-parametric Mann-Whitney U test was performed on pairs of means to determine differences between the stocks at similar elevations (low-to-low, mid-to-mid and high-to-high) of both sites. Correlations between the analysed variables were calculated with non-parametric Spearman's correlation test. All statistical analyses were performed in R 3.4.2 (R Core Team, 2017). To assess the relative importance of each driver (predictor) on microbial indices such as, microbial biomass (MB) – carbon (MBC) and nitrogen (MBN), MB-C/N ratio, ergosterol, MBC as percentage SOC (MBC/SOC (%)), and ergosterol as percentage MBC (ergosterol/MBC (%)), we implemented redundancy analysis (RDA) approach. The following explanatory variables were used as predictors; elevation levels, slope, aspect, mean annual temperature (MAT), mean annual precipitation (MAP), vegetation, soil pH, SOC, total N and soil C/N ratio. In this approach, vectors of higher magnitude forming small angles with an axis indicated strong correlation with that axis. The results from RDA analysis were subsequently used for Variation Partitioning Analysis (VPA) to understand the amount of variability in microbial parameters in response to explanatory variables sub-grouped into environmental variables (elevation levels, slope, aspect, MAT, MAP and vegetation) and soil properties (pH, SOC, total N, soil C/N ratio). Both these analyses were conducted separately for alkaline and acidic site using CANOCO 5.0 software (Šmilauer and Lepš, 2014).

2.3 Results

2.3.1 Soil chemical indices

By default, alkaline site has higher soil pH than acidic site except at low elevation level soils having moderate acidic pH but exceeding those of soils in acidic site (Table 2). Soil pH values usually increased with depth, with the only exception at the low elevation level in alkaline site. The concentrations of SOC and total N (mg g^{-1} soil) were higher at all three elevation levels in alkaline than in acidic site (Table 2). The same was true for soil C/N ratios at low and mid elevation levels. Additionally, the concentrations of SOC and total N always decreased with soil depths and more pronounced in acidic site. The lowest soil C/N ratio was measured at the middle depth position at 4 out of 6 sampling elevations. The stocks of SOC increased with elevation in both sites with median values ranging from 2.4 to 5.7 kg m^{-2} (Fig. 1a) and total N stocks, ranging from 0.15 to 0.40 kg

m^{-2} (Fig. 1b). In alkaline site, stocks of SOC significantly exceeded than those in acidic site only at the mid elevation level (Fig. 1a), whereas such difference in stocks of total N was observed at the high elevation level (Fig. 1b).

Table 2. Basic soil properties along studied elevation gradients in the alkaline (Hochschwab) and acidic (Rauris) sites.

Elevation	Depth	Soil pH	Bulk density	SOC	total N	Soil C/N
(m asl)	(cm)		(g cm^{-3})	(mg g^{-1} soil)		
Alkaline site (Hochschwab)						
900	0-5	5.1 (0.1)	0.10 (0.01)	512 (2)	22.1 (0.4)	23.3 (0.4)
	5-15	4.6 (0.1)		494 (4)	21.0 (0.2)	23.6 (0.4)
	15-25	5.1 (0.2)	0.10 (0.01)	454 (13)	20.3 (0.5)	22.5 (0.9)
1300	0-5	6.0 (0.1)	0.13 (0.03)	495 (9)	21.5 (0.5)	23.1 (0.5)
	5-15	6.5 (0.2)		422 (23)	20.7 (1.2)	20.5 (0.3)
	15-25	7.6 (0.1)	0.60 (0.04)	205 (10)	7.1 (0.7)	30.4 (1.6)
1900	0-5	6.4 (0.1)	0.16 (0.01)	442 (5)	27.3 (0.3)	16.2 (0.3)
	5-15	6.2 (0.1)		403 (6)	27.8 (0.4)	14.5 (0.2)
	15-25	6.9 (0.1)	0.22 (0.01)	270 (9)	19.8 (0.8)	13.6 (0.2)
Acidic site (Rauris)						
1300	0-5	4.1 (0.1)	0.54 (0.03)	243 (25)	11.9 (0.7)	19.9 (0.9)
	5-15	4.3 (0.1)		47 (6)	3.0 (0.4)	15.9 (0.2)
	15-25	4.9 (0.1)	0.66 (0.04)	25 (1)	1.3 (0.1)	19.5 (0.4)
1600	0-5	4.3 (0.1)	0.60 (0.04)	281 (29)	12.4 (1.1)	22.6 (0.8)
	5-15	4.5 (0.1)		46 (5)	2.7 (0.3)	16.7 (0.2)
	15-25	5.3 (0.1)	0.73 (0.03)	24 (1)	1.4 (0.1)	18.0 (0.3)
2100	0-5	4.6 (0.1)	0.46 (0.02)	368 (18)	18.4 (0.7)	20.0 (0.5)
	5-15	4.7 (0.2)		124 (15)	8.1 (0.9)	15.3 (0.2)
	15-25	5.1 (0.1)	0.60 (0.03)	39 (2)	2.5 (0.1)	15.8 (0.3)
CV (± %)			5.0 24	19	19	6.9

CV = mean coefficient of variation between replicate profiles (n = 12); standard error given in brackets.

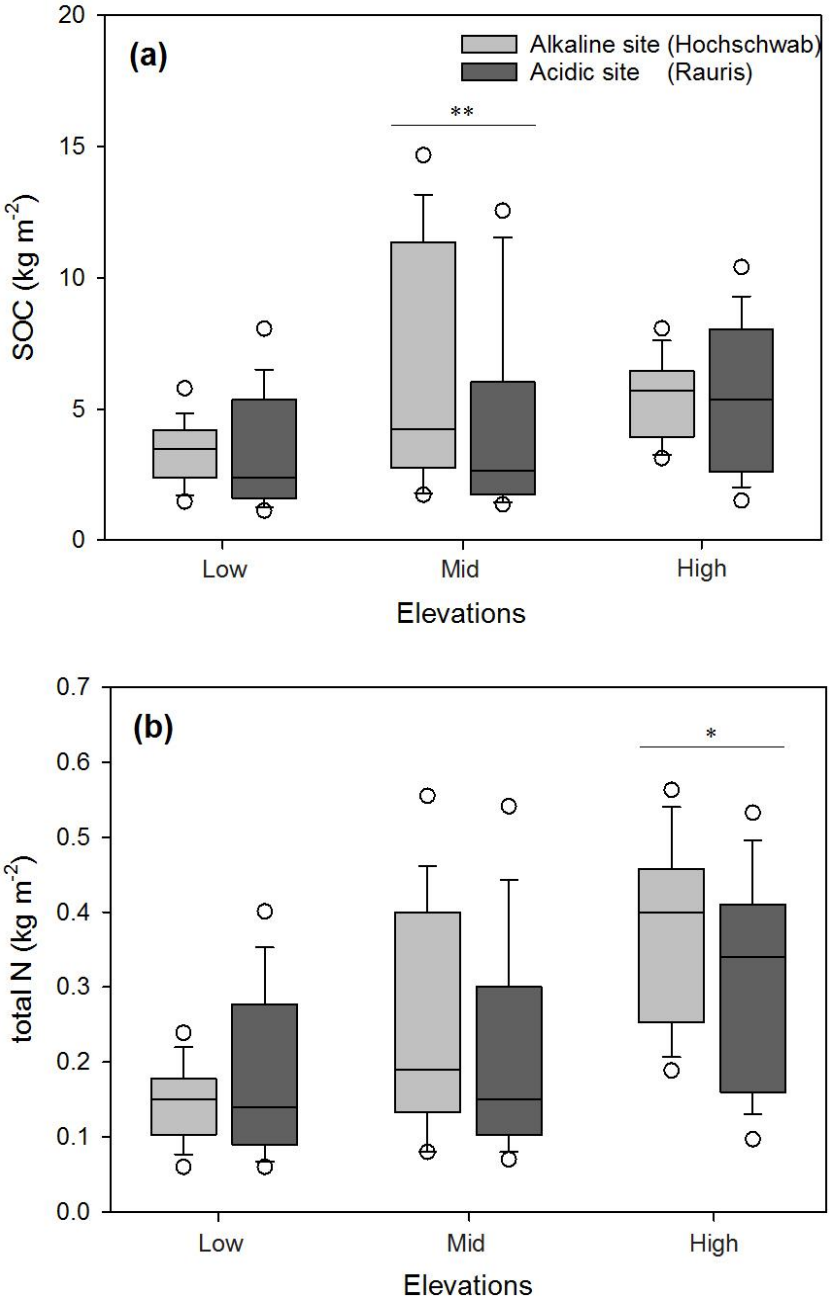

Fig. 1. Boxplots for stocks of (a) SOC and (b) total N along elevation gradients (n = 36 replicates per elevation); * $P < 0.05$ and ** $P < 0.01$ indicate significant elevation-specific differences between the sites (non-parametric Mann-Whitney U-test at); low = 900, mid = 1300, and high = 1900 m asl in alkaline site (Hochschwab) as well as low = 1300, mid = 1600, and high 2100 m asl in acidic site (Rauris).

2.3.2 Soil microbial biomass indices

The median MBC stocks increased with elevation in both sites, ranging from 37.6 to 126.1 gm^{-2} (Fig. 2a), and stocks of MBN from 4.1 to 19.8 gm^{-2} (Fig. 2b). In alkaline site, MBC stocks significantly exceeded than those in acidic site but only at the mid elevation level (Fig. 2a). In contrast, stocks of MBN in acidic site were significantly higher at the low and high elevation levels than in alkaline site (Fig. 2b). The median ergosterol stocks varied around 0.4 gm^{-2} in alkaline site and increased from 0.05 to 0.2 gm^{-2} at acidic site (Fig. 2c). Ergosterol stocks in alkaline site exceeded again than those in acidic site at the low and mid elevation levels.

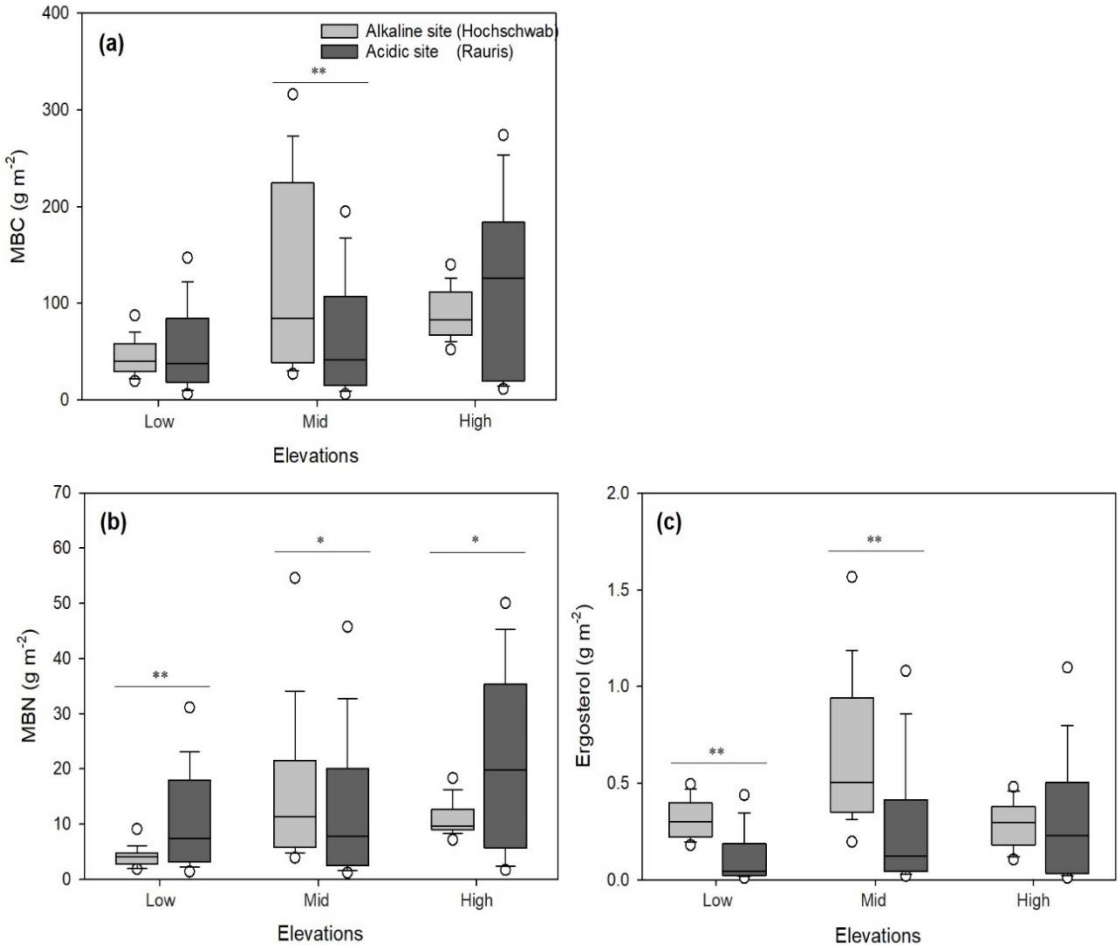

Fig. 2. Boxplots for stocks of (a) MBC), (b) MBN, and (c) Ergosterol along elevations gradients (n = 36, replicates per elevation); * $P < 0.05$ and ** $P < 0.01$ indicate significant elevation-specific differences between the sites (non-parametric Mann-Whitney U-test at); low = 900, mid = 1300, and high = 1900 m asl in alkaline site (Hochschwab) as well as low = 1300, mid = 1600, and high 2100 m asl in acidic site (Rauris).

The concentrations of MBC (Fig. 3a, b), MBN (Fig. 3c, d), and ergosterol (Fig. 3e, f) always declined with depths, except those of MBC and MBN at the low elevation level in alkaline site (Fig. 3a, c). At all elevation levels, the MB-C/N ratio in alkaline site increased with soil depth from 6.0 to 12.3 (Fig. 4a) and remained constant in acidic site between 4.9 and 6.8 (Fig. 4b). The ratio of microbial biomass C to soil organic C (MBC/SOC) generally remained constant with soil depths and averaged around 1.6% (Fig. 4c, d). An exception was the strong decline to 0.8% between 15 and 25 cm soil depths at all elevation levels in acidic site (Fig. 4d). In alkaline site the ratios of ergosterol/MBC declined with values ranging from 0.6 to 1.4% at 5 cm depth to values around 0.2% (Fig. 4e), whereas those in acidic site varied around 0.4 to 0.1% at all soil depths (Fig. 4f).

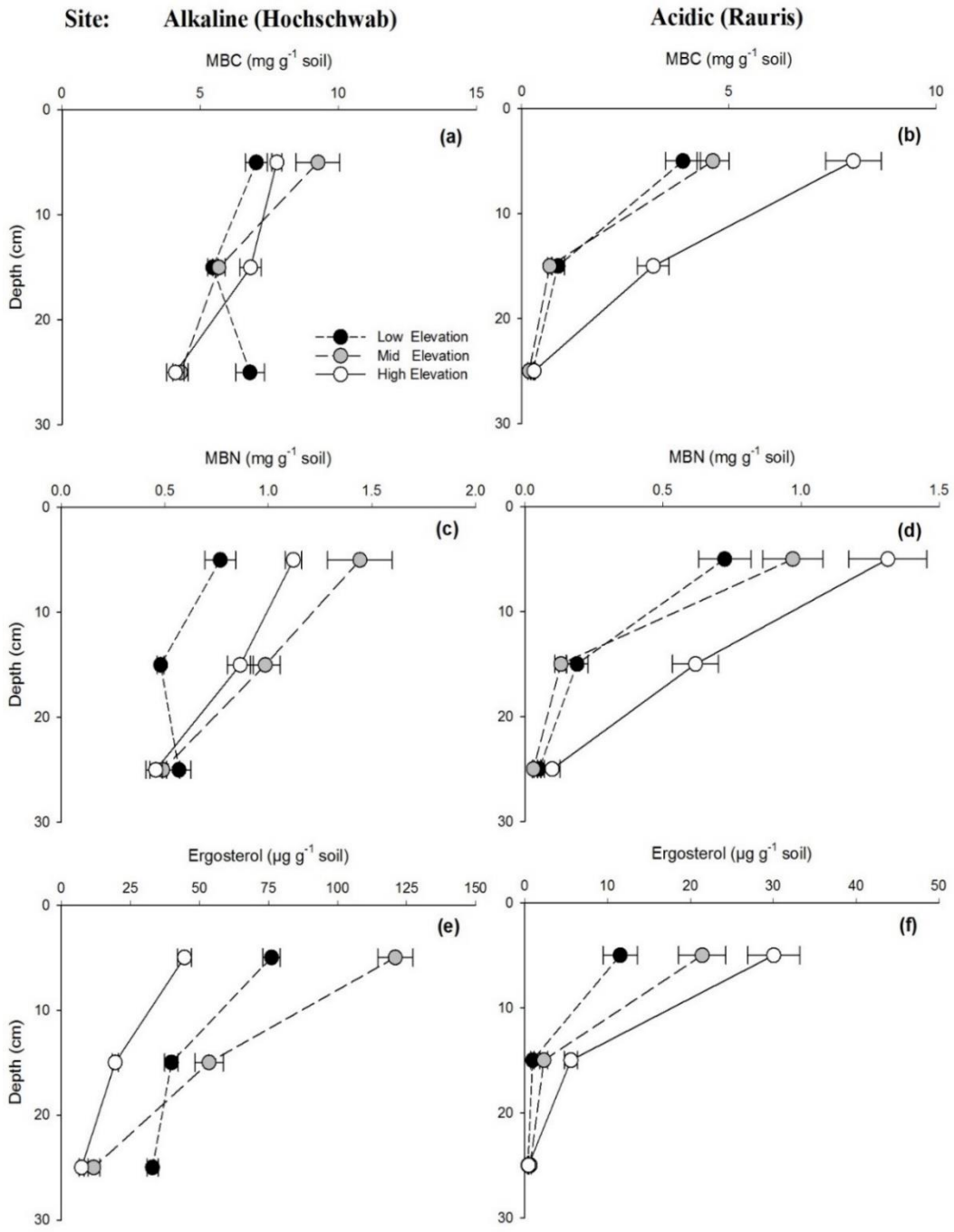

Fig. 3. Depth-specific mean contents of (a) MBC at Hochschwab, (b) MBC at Rauris, (c) MBN at Hochschwab, (d) MBN at Rauris, (e) Ergosterol at Hochschwab, and (f) Ergosterol at Rauris along elevation gradients; error bars indicate standard error of mean (n = 12); low = 900, mid = 1300, and high = 1900 m asl in alkaline site (Hochschwab) as well as low = 1300, mid = 1600, and high 2100 m asl in acidic site (Rauris).

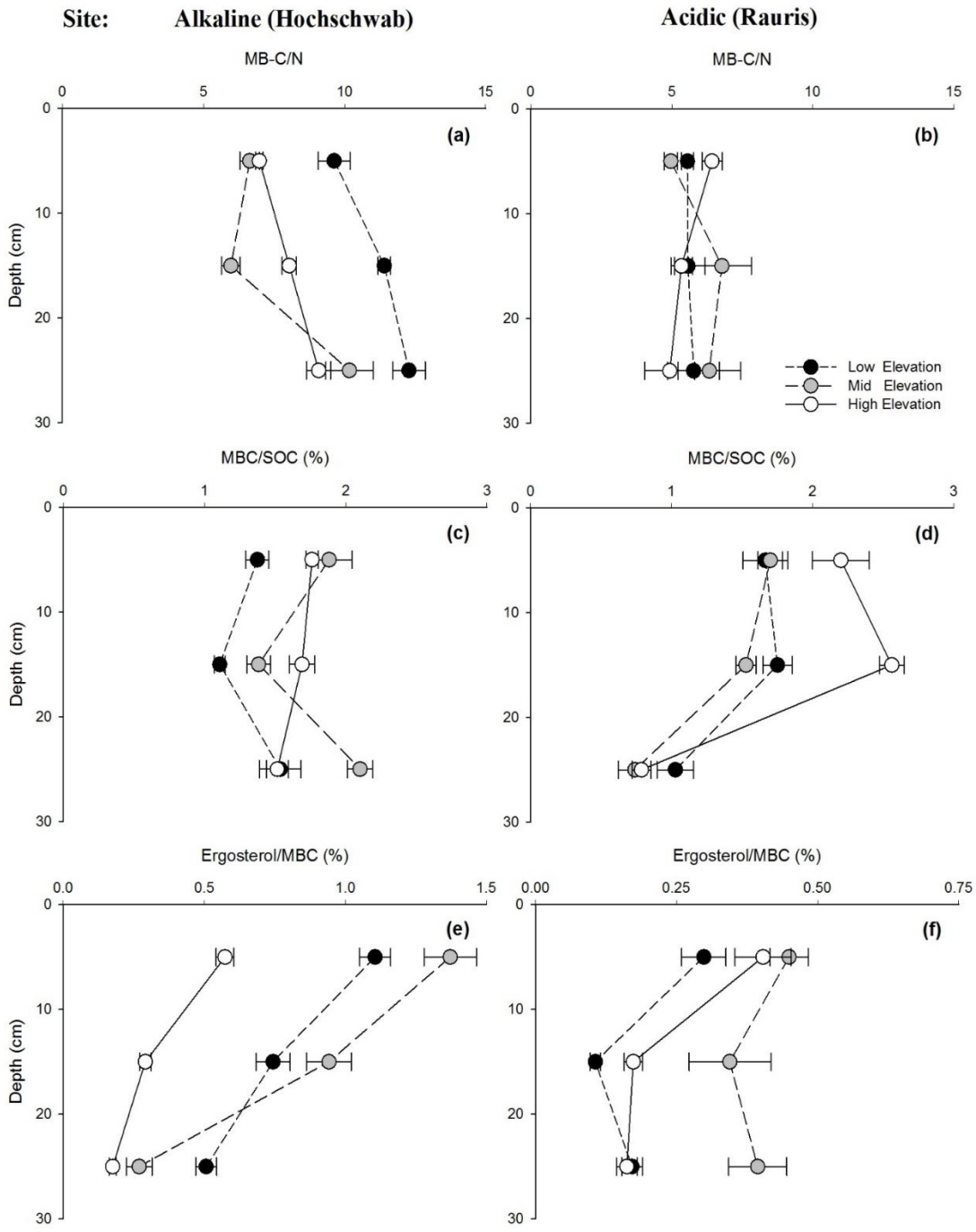

Fig. 4. Depth-specific mean ratios of (a) MB-C/N at Hochschwab, (b) MB-C/N at Rauris, (c) MBC/SOC at Hochschwab, (d) MBC/SOC at Rauris, (e) Ergosterol/MBC at Hochschwab, and (f) Ergosterol/MBC at Rauris; error bars indicate standard error of mean (n = 12); low = 900, mid = 1300, and high = 1900 m asl in alkaline site (Hochschwab) as well as low = 1300, mid = 1600, and high 2100 m asl in acidic site (Rauris).

The concentrations of MBC were always significantly related to the concentrations of SOC (Fig. 5a). An exception was the low elevation level in alkaline site, which added much variation to the overall regression. A significant relationship was also observed between concentrations of MBC and concentrations of ergosterol (Fig. 5b). The regression line was steeper in alkaline than in acidic site, but the relationship was more variable. The ratio of ergosterol/MBC did not exhibit clear relationships to the MB-C/N ratio in both sites (Fig. 5c), although the regression coefficient was significantly negative in alkaline site.

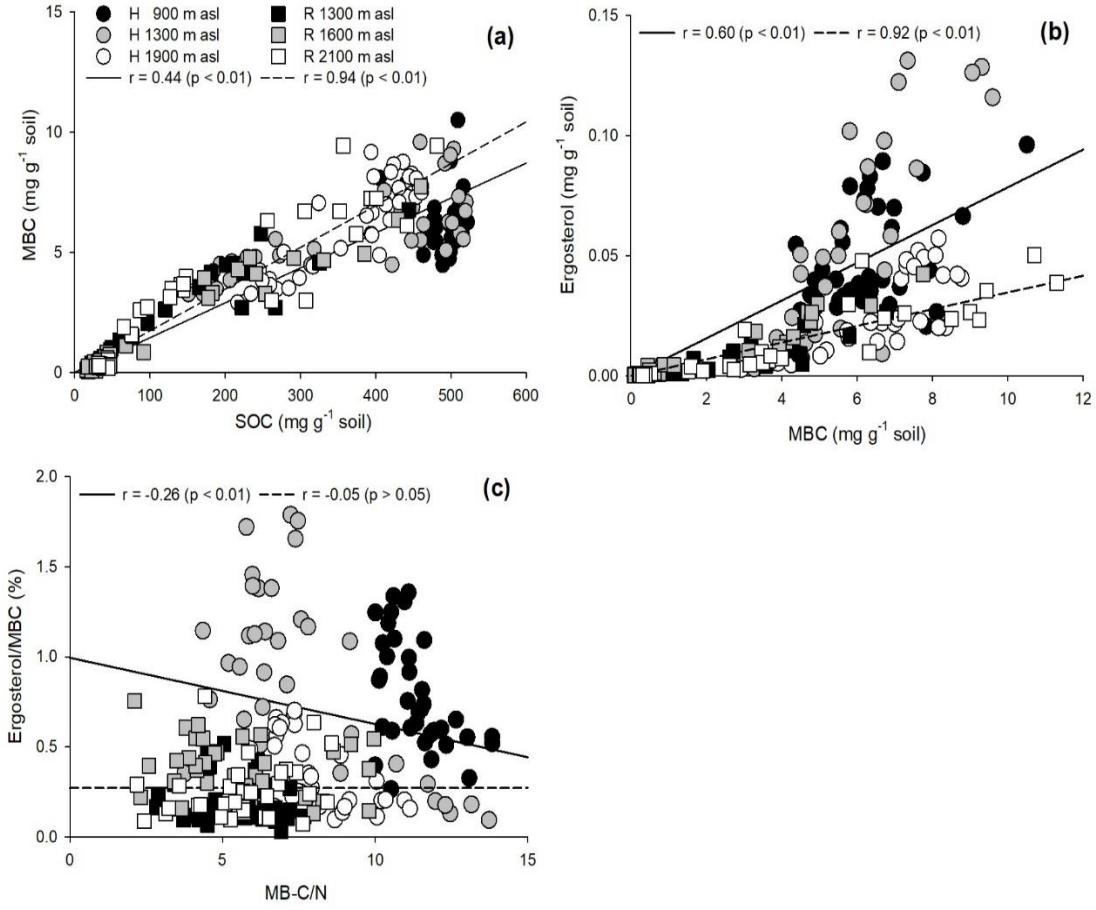

Fig. 5. Linear relationships between (a) SOC and MBC contents (Hochschwab: r = 0.44, $P < 0.01$; Rauris: r = 0.94, $P < 0.01$), (b) MBC and Ergosterol contents (Hochschwab: r = 0.60, $P < 0.01$; Rauris: r = 0.92, $P < 0.01$) as well as (c) MB-C/N and Ergosterol/MBC (Hochschwab: r = -0.26, $P < 0.01$; Rauris: r = -0.05, $P > 0.05$), calculated separately for alkaline site (Hochschwab (H)) and acidic site (Rauris (R)) along the three elevation gradients (n = 108 per site).

In addition to microbial biomass, fine root biomass was significantly higher ($P < .01$) in alkaline site than in acidic site. Fine root biomass at 0–15 cm depth was 180 gm^{-2} at the high, 310 gm^{-2} at the mid, and 278 gm^{-2} at the low elevation level in alkaline site. The respective values in acidic site were 153, 296, and 236 gm^{-2}.

2.3.3 Variations in soil microbial parameters

In alkaline site, the first axis of RDA plot was significantly and positively correlated to SOC and pH (for both $p = .002$) followed by elevation levels, MAT, MAP, vegetation and total N but not with soil C/N ratio ($p = .06$) (Fig. 6a). To the total variation of 66.1% in microbial parameters caused by soil properties, SOC and pH (61.2 and 22.4%) contributed the most. While the contribution of environmental variables ranged from 13.7 to 16.5% to the total of 1.2% variation explained by soil microbial parameters in this site (Fig. 6b). In acidic site, the first axis of RDA plot strongly correlated to total N and SOC ($p = .002$) followed by soil C/N ratio and pH, then elevation levels and vegetation ($p = .006$) (Fig. 6c). The total of 79.2% variation in microbial parameters explained by soil properties, total N (81.7%) and SOC (79.9%) had highest contributions. In addition, environmental variables together explained only 1.5% variation in soil microbial parameters (Fig. 6d) in acidic site. In both sites, approximately 15.5% of variation in soil microbial parameters remained unexplained.

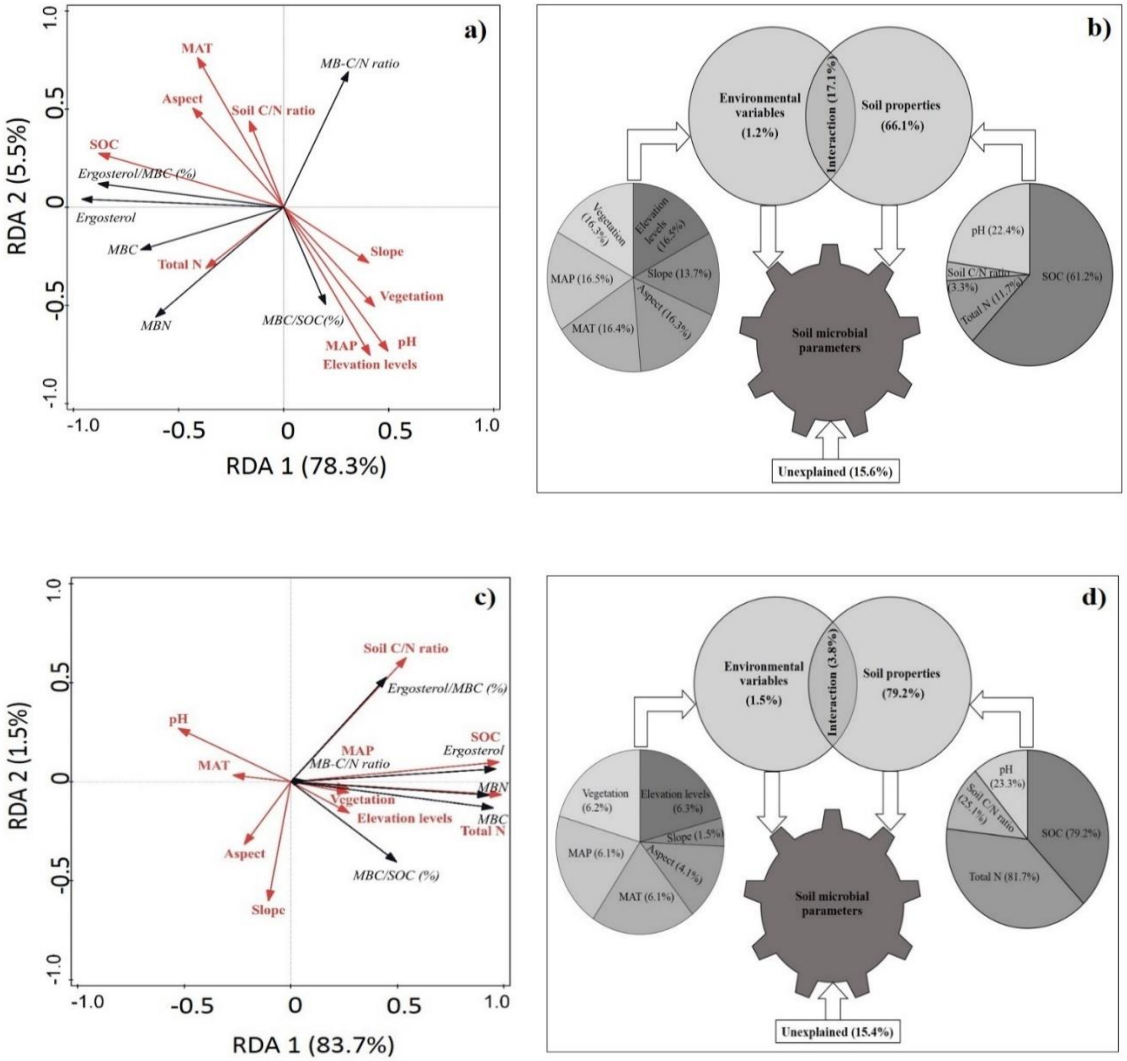

Fig. 6. Redundancy analysis (RDA) ordination biplot of soil microbial parameters (microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MB-C/N ratio, ergosterol, MBC/SOC (%) and ergosterol/MBC (%) ratios indicated by black lines and black arrows, and environmental variables (elevation levels, slope, aspect, mean annual temperature (MAT), mean annual precipitation (MAP) and vegetation) and soil properties (pH, SOC, total N, soil C/N ratio) indicated by red lines and red arrows, followed by Variation Partitioning Analysis (VPA) indicating proportional effects of environmental variables and soil properties on microbial parameters in alkaline site (Hochschwab) (a, b) and acidic site (Rauris) (c, d) separately.

2.4 Discussion

2.4.1 Soil organic matter

High concentration of SOC and high soil C/N ratios in alkaline site (Hochschwab) indicate higher accumulation of less decomposed plant material (Jenkinson et al., 2008; John et al., 2005; Cools et al., 2014) than in acidic site (Rauris). This is surprising as the alkaline limestone and dolomite bedrocks in Hochschwab are more fertile than the acidic gneiss and schists in Rauris. Higher fertility is indicated by the presence of mixed spruce-beech forest stands at the low and mid elevation levels in alkaline site compared to the pure spruce forest stands at the respective elevation levels in acidic site (Berger and Berger, 2012; Oulehle et al., 2016). This is in line with the higher fine root biomass observed in the current alkaline soils, suggesting a higher primary productivity at nutrient rich forest stands (Ponge, 2013; Oulehle et al., 2016; Murugan et al., 2019). In addition, annual litter fall is usually higher in beech than in spruce stands (Augusto et al., 2003; Hansen et al., 2009; Oulehle et al., 2016). Whereas litterbag experiments conducted previously in our alkaline site showed that differences in recalcitrance between beech leaf litter and spruce needle litter did not contribute to the retarded decomposition of the annual C input (Berger and Berger, 2012; Berger et al., 2015). But may be phosphorous (P) limitation retarding microbial activities as seen in contrasting P stocked German forest ecosystems differing in litter and soil parent material (Spohn et al., 2018).

The interactive differences in microclimate, bedrock and soil characteristics as well as in physical structure of the litter form, form the complex cause for the observed retardation of decomposition throughout all elevation levels in alkaline site (Berger et al., 2015). In our study, the bulk density did not exceed 0.16 kg dm^{-3} at 0–15 cm in alkaline site throughout all elevation levels, which is between 60 and 80% lower than that at the respective elevation levels in the acidic site. One reason for such low bulk density is that the coarse rock material across all elevations keep soils less compacted in alkaline site Hochschwab (Djukic et al., 2010b) compared with the weathered fine material of the Podzols (Schulze et al., 2009) in acidic site Rauris. Another possibility is the bulky and voluminous structure of partly decomposed beech leaf litter, remaining less densely packed than spruce needles. This promotes aeration and desiccation (Gavazov, 2010) and obstructs heat transfer (Keith et al., 2010; Ogée and Brunet, 2002; Wilson et al., 2012)

but especially rewetting due to the increase in hydrophobicity and water repellence by drying (Buczko et al., 2005). For this reason, rewetting of a beech forest floor was slower than that of a spruce forest floor (Hömberg and Matzner, 2018), despite the higher hydrophobicity of spruce in comparison with beech litter (Bogner et al., 2008; Butzen et al., 2015).

In both sites, the stocks of SOC and total N generally increased with elevation levels, which support our hypothesis 1. It might be attributed to retarded microbial decomposition due to a decrease in MAT and increase in MAP. This result is in line with other studies investigating elevation levels in Alpine environments (Djukic et al., 2010b; Siles et al., 2016; Murugan et al., 2019), which lacked comparison of study sites formed on different bedrocks. The increase in stocks of SOC and total N in the current study was also in part caused by maximum primary production at the mid elevation level where the climatic conditions are apparently most suitable for mixed beech and spruce forest in alkaline site. The change in vegetation from forest to grassland did not affect the general trend of increasing SOC accumulation with increasing elevation in both sites, despite faster root decomposition rate in grassland (Solly et al., 2014). This suggests that climatic effects override the influence of bedrock and substrate quality on decomposition processes and subsequent changes in SOC storage.

2.4.2 Microbial biomass indices

In general, a positive relationship between MBC and SOC (Fig. 5a) and also between MBN and total N was observed at all elevation levels in both sites. The combination of low temperature and high precipitation might have retarded microbial decomposition but did not affect the relationship between MBC and SOC (Anderson and Domsch, 1993; Anderson and Joergensen, 1997) and between MBN and total N (Joergensen et al., 1995; Zederer et al., 2017) in temperate humid forest soils.

In alkaline (0–25 cm) and acidic (0–15 cm) site soils (Fig. 4c, d), the mean MBC/SOC ratio of 1.6% is in the upper range usually observed in mineral and organic horizons of acidic beech and spruce forests (Anderson and Joergensen, 1997; Khan and Joergensen, 2012; Zederer et al., 2017). These relatively high MBC/SOC ratios indicate the decomposition retardation generally observed in alpine soils (Berger et al., 2015; Djukic et al., 2010a, b; Margesin et al., 2009; Siles and Margesin, 2016; Siles et al., 2016,

2017). However, this reduced decomposition may not be due to a general chemical recalcitrance of the C input by plants, but might be due to the adverse environmental conditions in spruce to spruce-beech strands (Berger and Berger, 2012) and also lack of accessibility (von Lützow et al., 2006).

The low ratio of MBC/SOC at 15–25 cm depth generally observed in the acidic site (Fig. 4d) is more typical for mineral horizons of acidic forests (Anderson and Joergensen, 1997; Khan and Joergensen, 2012). This indicates low C availability to soil microorganisms (Anderson and Domsch, 1989, 2010), presumably caused by the increased presence of Al in the soil solution (Illmer et al., 1995; Mulder et al., 2001; Schwesig et al., 2003). Another reason is the relative enrichment of recalcitrant microbial decomposition residues with increasing profile depth (Marseille et al., 1999; Djukic et al., 2010b). The highest ratios of MBC/SOC observed at high elevation in acidic site might be caused due to changes in vegetation from spruce forest to grassland, which increased the C availability to soil microbial community (Solly et al., 2014; Murugan et al., 2019). The change in MBC/SOC ratio from mixed beech and spruce forest to grassland + pine bushes vegetation in alkaline site was less pronounced because the difference in C availability are smaller.

A striking feature of the current results is that the increase in MB-C/N ratios up to 12.3 with depth in alkaline site, while it remained constant around 6.2 in acidic site. High ratios of MB-C/N up to 17.3 have been observed in acidic beech forests in comparison with alkaline beech forest and explained by shift in the microbial community structure towards fungi (Joergensen et al., 1995). However, the ratios of MB-C/N were not correlated with those of ergosterol/MBC in the current study (Fig. 5c), suggesting the absence of a relationship between the MB-C/N and fungi to bacteria ratio (Khan et al., 2016). High ratios of MB-C/N have been repeatedly observed in tropical soils (Joergensen, 2010), especially in P deficient tropical forest soils (Salamanca et al., 2002). The deficiency of P has been reported to increase the MB-C/N ratio in laboratory experiments with cultured organisms (Anderson and Domsch, 1980) and incubation experiments with litter application (Salamanca et al., 2006). Further, the high ratios of MB-C/N often indicate poor P availability in presence of high C availability (Anderson and Domsch, 1980; Berger and Berger, 2012), but also other nutrient deficiencies may contribute to high MB-C/N ratios (Hartman and Richardson, 2013; Kaiser et al., 2014;

Khan et al., 2016). However, also shifts in the microbial community structure cannot be excluded as reason for increased MB-C/N ratios (Heuck et al., 2015; Murugan et al., 2019).

The ratio of ergosterol/MBC was three to four times higher at low and mid elevation levels in alkaline site compared to that in the acidic site. This indicates higher fungal biomass at the moderately acidic to low alkaline soils than at the strongly acidic soil horizons, contradicting our hypothesis 2 and the general view that the contribution of fungi to the total biomass increases with decreasing pH (Joergensen and Wichern, 2008; Strickland and Rousk, 2010). This result could be explained by smaller fungal hyphae in alkaline site, which increases the ergosterol concentration in fungi (Klamer and Bååth, 2004). While also soil moisture rather than soil pH having stronger effects on fungi in deciduous leaf litter than in needle litter (Swallow and Quideau, 2013). Another reason could be the accumulation of ergosterol in dead fungal tissue (Joergensen and Wichern, 2008) under specific decomposition conditions in alkaline site or differences within the fungal community.

Ergosterol is an important membrane component of Ascomycota and Basidiomycota and many fungal species formerly assigned to Zygomycota (Weete et al., 2010), i.e. in ectomycorrhizal fungi of beech and spruce and in most saprotrophic fungi. The ratio of ergosterol/MBC is similar at both high elevation levels, dominated by grassland vegetation, which usually maintains a considerable community of arbuscular mycorrhizal fungi (Li et al., 2018). However, in contrast to most fungal species, ergosterol does not occur in arbuscular mycorrhizal fungi (Olsson et al., 2003). This is why, the ergosterol/MBC ratio was lower at the high elevation grassland soils than at low and mid elevation forest soils in both sites (Fig. 4e, f). Similar to our study, increased recalcitrance (i.e. soil C/N; Table 2) was attributed to increased accumulation of fungal biomass and residues within aggregate fractions at mid and low elevation forest compared to high elevation grassland soil in acidic Rauris site (Murugan et al., 2019). The current results also indicate that similar vegetation leads to a similar microbial community in organic layers under similar climatic conditions independent of the bedrock properties.

2.4.3 Key influences and variations in soil microbial parameters

Our results provide some insights on important controls on microbial parameters in alpine soils in both alkaline and acidic sites. Although we could not find any relevant literature from other elevation gradient studies to compare these results except that Siles and Margesin (2016) in Italian Alps concluded site specific environmental and soil chemical factors such as soil pH and C/N ratios as important driving factors for abundance and diversity of microbial communities along altitudinal gradients. Our results show that soil properties regulate microbial parameters more than the environmental factors such as elevation and vegetation alone, supporting our hypothesis 3. However, with elevation, changes occur in vegetation and thus the litter input and humus form, therefore intrinsically connecting to the contemporary soil properties. In alkaline site, SOC and pH, and total N, while SOC, soil C/N ratio and pH in acidic site as influencing soil properties led to major variations in soil microbial parameters (Högberg et al., 2007; De Vries et al., 2012). Finally, our results present profound control of abiotic factors on variation in microbial community structure in two different elevation gradient sites in the Alps, differing only in climate induced vegetation, and parent material. Different factors within soil properties cause differential variation in soil microbial parameters and so it is hard to generalise the importance of any specific one factor. This kind of information on edaphic control on soil microbial properties is important for developing modeling frameworks and understanding soil microbial ecology and ecosystem management and to establish better links between them.

2.5 Conclusion

Overall, our results indicate that SOC stocks significantly increased from low to mid elevation and then again decreased at high elevation level only in alkaline site (Hochschwab). A similar pattern in MBC, MBN and ergosterol stocks was also observed. However, the stocks of total N in alkaline site followed that of SOC, total N, MBC, MBN and ergosterol stocks in acidic site (Rauris), showing consistent increase with increasing elevations. All stocks at respective elevations were higher in alkaline site except that of MBC at high, and MBN stock at low and high elevation levels in the acidic site. The concentrations of SOC, total N, MBC, MBN and ergosterol constantly decreased with depth due to variations in soil bulk density and bedrock properties. The highest

MBC/SOC ratio observed at high elevation in acidic site might be caused due to the changes in vegetation from spruce forest to grassland, which increased C availability to soil microbial community. Two times higher MB-C/N ratio in alkaline site might be attributed to P deficiency and high C availability than in acidic site. The ratios, ergosterol/ MBC and MB-C/N were similar at both high elevation levels in both sites, dominated by grassland vegetation, indicating stronger influence of climate induced vegetation change than inherent bedrock properties. In comparison to acidic site, the ratios of soil C/N and ergosterol/MBC were higher at low and mid elevation level in alkaline site, meaning increased accumulation of less easily decomposable organic matter while also the increased accumulation of saprotrophic fungi. Our results suggest profound control of abiotic factors on variation in microbial community structure in two different elevation gradient sites in the Alps that differ only in climate induced vegetation and parent material.

Acknowledgments

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3 Microbial necromass formation and enzyme activities reflect retardation of plant residue decomposition at an alkaline Alpine elevation gradient

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Highlights

- Contribution of microbial necromass C to SOC was higher at the acidic than at the alkaline site.
- Hydrolytic and oxidative enzymes activities were dominant at the alkaline and the acidic sites, respectively.
- Fungal biomass and residues dominated at both sites, but the dominance was stronger at the alkaline site Hochschwab.
- G+ bacteria dominated bacterial PLFAs at both sites, but the dominance was stronger at the acidic site Rauris.

Abstract

This study was carried out with the objective to further investigate the unknown and astonishing phenomenon of retarded plant residue decomposition at the alkaline site Hochschwab in comparison with the acidic site Rauris. For this purpose, samples from three elevation levels were further analysed for amino sugars as microbial necromass indices, extracellular enzymes, and phospholipid fatty acids (PLFAs) as microbial biomass indices. Although the parent material and elevation levels were different in two alpine sites, one acidic (Rauris) and the other alkaline (Hochschwab), the mean annual temperature and mean annual precipitation regimes were similar at the corresponding elevation levels in both sites. Our results on combined analysis of microbial necromass, activity and community composition show that, microbial necromass contributed on average 37% to SOC at the acidic site and only 26% at the alkaline site. Fungi dominated microbial necromass, with 77% at acidic site and 88% at alkaline site. The biomass-specific activities of phenoloxidase and peroxidase enzymes were generally reduced by 96% and 98%, respectively, at the alkaline site, but not those of the hydrolytic enzymes β-glucosidase, exo-glucanase, exo-chitinase, and protease. An exception was the generally 75% lower level of phosphatase activity at alkaline site. The ratio of fungal to bacterial PLFA was unusually high at both sites, but especially high at Hochschwab. G+ bacteria dominated bacterial biomass at both sites, with 70% at Hochschwab and 72% at Rauris. In conclusion, the climatic effects along alpine elevation gradients depended on vegetation induced changes in soil properties, which further interacted with bedrock properties.

Key words: Alpine ecosystems, Amino sugars, Carbon sequestration, Climate change, Elevation gradients, Enzyme activities, PLFA

3.1 Introduction

High altitudinal ecosystems are more susceptible to warming induced changes in vegetation (Körner, 2003), the quality and quantity of plant litter, and subsequently in microbial decomposition and soil organic carbon (SOC) storage (Djukic et al., 2010a/b). Elevation gradients are powerful tools for showing natural effects of temperature decreases and precipitation increases, with increasing altitudes on soil microbial biomass, activities, and communities' (Margesin et al., 2009; Siles et al., 2016, 2017; Murugan et al., 2019). In a previous study (Bhople et al., 2019), this unique situation was used for comparing two sites with strong differences in geological bedrock properties, but with similar mean annual temperature and precipitation at each of the three elevation levels. Stocks of SOC and total N generally increased with increasing elevation levels at both sites (Bhople et al., 2019). At the alkaline site Hochschwab, high SOC/total N ratios indicated the accumulation of non-decomposed plant residues, i.e. a retardation of microbial decomposition (Bhople et al., 2019).

This observation contradicts the higher presence of saprotrophic fungi according to the higher ergosterol to microbial biomass C (MBC) ratio. Usually, the availability of plant residues to soil microorganisms is higher at alkaline sites with deciduous tree stands than at acidic sites with conifer stands (Anderson and Joergensen, 1997; Bauhus et al., 1998; Bååth and Anderson, 2003). This usually results in lower stocks of SOC at alkaline stands (Berger and Berger, 2012; Zederer et al., 2017). Therefore, to further investigate the unknown and astonishing phenomenon of retarded plant residue decomposition, samples from three elevation levels and three depths at the alkaline site Hochschwab and the acidic site Rauris were analysed for amino sugars, extracellular enzymes, and phospholipid fatty acids (PLFA).

Plant residues, such as root and leaf litter, are first incorporated into the biomass of the decomposing microbial community (Six et al. 2006; Khan et al. 2016), followed by the formation of microbial residues (Ding et al., 2017; Joergensen, 2018). This fraction, often summarized as microbial necromass (Liang et al., 2017), comprises non-biomass microbial metabolites such as exo-enzymes, extracellular polymeric substances (EPS) and dead cell remains (Joergensen and Wichern, 2018). The accumulation of microbial necromass can be estimated by measuring cell wall-derived amino sugars (Amelung,

2001; Liang et al., 2011; Joergensen, 2018), integrating environmental effects on soil microorganisms over time (Glaser et al., 2004; Liang et al., 2015; Murugan et al., 2014, 2019). The measurement of bacterial muramic acid (MurN) and fungal glucosamine (GlcN) make it possible to estimate the necromass contribution of these two groups to SOC.

Plant residues are decomposed by a large variety of extracellular enzymes released by soil microorganisms (Allison et al., 2011; Burns et al., 2013), especially fungi (Schneider et al., 2012), which are specifically involved in C and nutrient cycles (Sinsabaugh et al., 2008). Extracellular enzymes depolymerize plant residues through hydrolysis, e.g. by β-glucosidase, or oxidation, e.g. phenoloxidase. Extracellular enzyme activity is usually reduced by low pH, low temperature, and high precipitation (Sinsabaugh, 2010).

Membrane-derived PLFA are important biomarkers for living microorganisms (Frostegård et al., 1993; Zelles, 1997) and give useful information on the large functional groups; Gram-positive (G+) and Gram-negative (G-) bacteria as well as fungi (Joergensen and Wichern, 2008). For this reason, the PLFA linoleic acid (18:2ω6,9) is an independent control for the fungal cell membrane component ergosterol (Weete et al., 2010). In addition, exact information on the ratio of G+/G- bacteria is important for calculating bacterial necromass C from the soil MurN content as G+ bacteria, i.e. firmicutes, and actinobacteria contain considerably more MurN than G- bacteria (Appuhn and Jorgensen, 2006). G+ bacteria show a higher presence on more recalcitrant soil organic matter, while the presence of G- bacteria increased with availability of more labile C sources (Whitaker et al., 2014; Fanin et al., 2019).

The objective of this work was to investigate the retarded plant residue decomposition at the alkaline site Hochschwab in comparison with the acidic site Rauris, based on the following hypotheses: (1) Microbial necromass formation is generally reduced at the alkaline site Hochschwab. (2) The biomass-specific activities of extracellular hydrolytic and oxidative enzymes are generally reduced at the alkaline site Hochschwab. (3) Fungi generally dominate microbial necromass and biomass. (4) G+ bacteria dominate bacterial biomass at the alkaline site Hochschwab. (5) The vegetation-induced differences in microbial necromass, activity, and community structure between

the two sites are reduced in the highest elevation level, due to the increased contribution of grassland species to vegetation.

3.2 Materials and methods

3.2.1 Site characteristics and soil sampling

Soils from an alkaline site (Hochschwab) located in the Northern Limestone Alps (47° 32-36′ N, 15° 02-05′ E) and from an acidic site (Rauris) in the Central Austrian Alps (47° 14′ N, 13° 0′ E) were sampled in August 2015, at three elevation levels - low, mid and high. These levels corresponded to 900, 1300 and 1900 m above sea level (m asl) at the alkaline, and 1300, 1600 and 2100 m asl at the acidic site, respectively. Deciduous spruce/beech trees (*Fagus sylvatica*) dominated the low and mid elevations at the alkaline and Norway spruce (*Picea abies*) at the acidic site. The high elevations in both sites represented typical alpine grasslands. With increase in elevations, mean annual temperature (MAT) decreased from 6.2 to 2.1 °C and 5.9 to 1.9 °C and mean annual precipitation (MAP) increased from 1178 to 1725 mm and 1067 to 1850 mm at the alkaline (Austrian Federal Ministry of Finance) and acidic sites (Zentralanstalt für Meteorologie und Geodynamik - ZAMG Austria), respectively. Further, mean annual snow cover increased from 123 to 221 days at the alkaline and continued from October to May at the acidic site. Soils were classified as Leptic Histosols formed on calcareous limestone and dolomites parent material at the alkaline site and Haplic Pozols on gneiss and schists at the acidic site (IUSS WRB, 2015).

At each elevation, 12 soil replicates, each from 0-5 (without litter), 5-15 and 15-25 cm depths were collected in separate polyethylene bags and transported to the laboratory in cool conditions. After sieving (< 2 mm mesh), 2 sites × 3 elevations × 3 depths × 4 replicates resulted in 72 samples which were stored at -80 °C until analysis.

3.2.2 Soil analytical parameters

Soil pH was measured in deionized water (1:2.5 w/v). SOC and total N were determined from finely ground samples dried at 105 °C for 24 h, using gas chromatography equipped with a Vario MAX elemental analyzer (Elementar, Hanau, Germany). Microbial biomass C (MBC) and N were estimated from 20 ml 0.5 M K_2SO_4 extracted soil samples, either fumigated with ethanol-free chloroform for 24 h or non-

fumigated (Brookes et al., 1985; Vance et al., 1987). Calculation of MBC was based on E_C/k_{EC}, where E_C = difference between organic C extracted from fumigated and non-fumigated soils and k_{EC} = 0.45 (Wu et al., 1990). Calculations of MBN were based on E_N/k_{EN}, where E_N = difference between total N extracted from fumigated soils and non-fumigated soils and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996). The fungal cell membrane component ergosterol was determined from 2 g moist soil according to Djajakirana et al. (1996), followed by measurement with reverse-phase HPLC, using 100% methanol as mobile phase and detection at 282 nm.

3.2.3 Phospholipid fatty acid (PLFA) analysis

Lipids were extracted by modified Bligh and Dyer method (Hackl et al., 2005) from 1 g lyophilised soil and subjected to gas-chromatography using HP-6890 Series GC-System equipped with 7683 series injector, auto sampler, HP-5 capillary column and flame ionization detector. An external standard (BAME mix from SUPELCO, St. Louis Missouri, USA) helped identification of fatty acid methyl esters. Methyl nonadecanoate fatty acid (19:0) was used as an internal standard for peak quantification. Since *Firmicutes* and *Actinobacteria* are Gram-positive (G+) (Barka et al., 2016), the sum of iso- and anteiso-branched saturated (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), and actinobacteria specific ester linked 10-methyl branched saturated fatty acids (10Me 16:0, 10Me 17:0 and 10Me 18:0) (Zelles, 1997) represented G+ bacteria (Frostegård and Bååth, 1996; Zelles, 1997). The sum of cyclopropyl (cy17:0, cy19:0) and monounsaturated fatty acids (16:1ω9, 17:1ω8 and 18:1ω7) represented Gram-negative (G-) bacteria (Frostegård et al., 1993; Zelles, 1997). Lipids 16:1ω5 represented arbuscular mycorrhizal fungi (AMF), and 18:1ω9 and 18:2ω6 represented saprotrophic and ectomycorrhizal fungi (Olsson, 1999; Joergensen and Wichern, 2008). As PLFAs 14:0 and 17:0, are present in all bacteria, they were used to account for total bacterial PLFA together with G+ and G- indicator PLFAs (Kaiser et al., 2010). PLFAs 15:0 and 16:0 common to both bacteria and fungi (Kaiser et al., 2010) and 20:4ω6 to protozoa (Fierer et al., 2003), were used only to sum the total microbial PLFA contents.

3.2.4 Enzyme assay

Extracellular enzyme activities were assayed using a microplate method (Kaiser et al., 2010). Fluorimetric analysis was based on MUF (4-methylumbelliferyl) labelled

substrates: β-D-glucopyranoside for β-glucosidase (BGL), β-D-cellobioside for exoglucanase (EGL), N-acetyl-ß-D-glucosaminide for exochitinase (ECH), and phosphate for phosphatase (PHO). For protease (PRO), L-leucine-7-amido-4-methyl-coumarin (AMC) was used as substrate. Phenoloxidase (POX) and peroxidase (POD) were measured photometrically using L-3,4-dihydroxyphenylalanine (DOPA) substrate. Opaque 96-microwell plates with assay reactions for BGL, EGL, ECH, PHO and PRO were sealed and incubated at 20 °C for 120 min using sodium acetate buffer (100 mM, acetic acid adjusted pH 5.5) prior to fluorimetric measurements (excitation 365 nm and emission 450 nm with instrument setting at 30 flashes). For photometric (absorbance at 450 nm) POX and POD enzyme activity measurements, transparent 96-well plates were used. Difference in the colour intensity at the start and end of the incubation time (20 h at 20 °C) represented net activities of POX and POD enzymes.

3.2.5 Amino sugar analysis

Amino sugars, muramic acid (MurN), glucosamine (GlcN) and galactosamine (GalN) were determined by HPLC according to Indorf et al. (2011). Briefly, 0.5 g moist soil samples were subjected to acid hydrolysis for 6 h at 105 °C in 10 ml 6 M HCl. After pre-column derivatization with *ortho*-phthaldialdehyde, amino sugar residues were separated using a Hyperclone C18 HPLC column (125 mm length × 4 mm diameter) at 35 °C. The HPLC system was equipped with a Dionex (Germering, Germany) P580 gradient pump, an analytical auto sampler (Dionex Ultimate WPS – 3000TSL) with in-line split-loop injection and thermostat as well as a fluorescence detector (Dionex RF 2000) set at 445 nm emission and 330 nm excitation wavelengths. Bacterial GlcN was subtracted from total GlcN to calculate fungal GlcN, assuming that MurN and GlcN occur at a 1:2 molar ratio in bacteria (Engelking et al., 2007). Thus, µg fungal GlcN g^{-1} soil = (µmol GlcN g^{-1} soil – 2 × µmol MurN g^{-1} soil) × 179.17 (i.e. molecular weight of GlcN). Fungal C was calculated by multiplying fungal GlcN by 9, while bacterial C = µg MurN g^{-1} soil × 45 (Appuhn and Joergensen, 2006). Total microbial residue was estimated as the sum of all amino sugars.

3.2.6 Statistical analysis

Difference between the sites were tested using the t-test for the total site-specific means and the elevation-specific means, i.e. low, mid, and high, respectively. Shapiro-Wilk and Levene tests (R-package "car"; Fox et al., 2012) were used to confirm normality and heteroscedasticity in data and, if violated, data were log/Box-Cox transformed (R-package "MASS"; Ripley et al., 2011).

Differences in enzyme activity measurement methods were normalized by log transforming, standardizing, and calculating the proportion of each enzyme to their sum. Euclidean distance matrices from log transformed-standardized enzymes, relative abundance of individual PLFAs and amino sugar biomarkers, were used for nonmetric multidimensional scaling (NMDS) plots. Differences between elevations and depths causing distribution of PLFAs, amino sugar residues and enzymes, were evaluated by perANOVA, using distance matrices generated *via* ADONIS function in R-package "vegan" (Oksanen, 2015). Linear relationships calculated at each site were based on Spearman's non-parametric test. Principal component analysis (PCA) was carried out using R-package "vegan". Two-way ANOVA was used for testing if differences between depth and elevations were stronger for PLFA groups, amino sugar residues and all enzyme classes, by log-transforming the data when required. Mantel test based on Spearman correlations (at significance level $p < 0.05$) was used to study relationships between microbial community composition and residues with soil variables and with calculated enzyme matrices. All statistics were performed using R version 3.4.2 (R Core Team, 2017).

3.3 Results

Soil pH at mid and high elevation was significantly higher than at low elevation in alkaline site (Table 3). SOC and soil C/N ratio, MBC and MB-C/N ratio and ergosterol contents had significantly higher overall mean values at the alkaline in comparison to the acidic site.

Table 3. Mean soil analytical parameters indicated by soil pH, soil organic carbon (SOC), soil organic carbon-to-nitrogen (soil C/N) ratio, microbial biomass carbon (MBC), microbial biomass carbon-to-nitrogen (MB-C/N) ratio, as well as contents of ergosterol over all soil depths at the three elevation levels in the two study sites calculated on per gram dry soil basis.

Elevation (m asl - level)	Soil pH (H_2O)	SOC (mg g^{-1} soil)	Soil C/N	MBC (mg g^{-1} soil)	MB-C/N	Ergosterol (µg g^{-1})
Alkaline site (Hochschwab)						
Overall	6.0 A	410 A	20.8 A	6.35 A	8.7 A	45.2 A
900 - Low	4.9 a	487 a	23.5 a	6.43 a	11.0 a	49.6 a
1300 - Mid	6.7 a	374 a	24.2 a	6.41 a	7.2 a	62.0 a
1900 - High	6.5 a	372 a	14.8 ns	6.24 ns	8.0 a	23.8 ns
Acidic site (Rauris)						
Overall	4.6 B	133 B	18.2 B	2.45 B	5.2 B	8.2 B
1300 - Low	4.4 b	105 b	18.5 b	1.68 b	5.1 b	4.3 b
1600 - Mid	4.7 b	117 b	19.2 b	1.82 b	5.4 b	8.1 b
2100 – High	4.8 b	177 b	17.1 ns	3.83 ns	5.1 b	12.0 ns
CV (± %)	3	12	3	19	19	27

Values presented are sample means per site (n = 36) and per elevation (n = 12); mean values followed by capital letters (for Overall i.e. site specific means) and lower-case letters (for elevation levels i.e. elevation specific means) are significantly different according to t-test ($p < 0.05$); CV = mean coefficient of variation between elevation- and depth-specific replicate samples (n = 4); ns = not significant.

Total PLFA and contribution of fungal PLFA to total PLFA (mol%) were significantly higher at the alkaline than at the acidic site (Table 4). The contribution of G+ bacteria was significantly higher at the acidic site, especially at the mid and high elevation levels. Fungal necromass C was significantly higher at the alkaline site at all elevation levels than at the acidic site, whereas bacterial necromass C differed only for the overall mean.

Table 4. Mean contents of the total PLFA and contribution of fungal PLFA, Gram positive (G+) and Gram negative (G-) bacterial PLFA to total PLFA as its mole percentage (mol%) as well as the contents of amino sugars; Muramic acid (MurN) and, fungal glucosamine (GlcN) over all soil depths at the three elevation levels in the two study sites. Calculations are based on per gram dry soil basis for total PLFA and amino sugars, respectively.

Elevation	Total PLFA	Fungal PLFA	G+ PLFA	G- PLFA	MurN	Fungal GlcN
levels	(nmol g^{-1} soil)	(mol%)			(mg g^{-1} soil)	
Alkaline site (Hochschwab)						
Overall	80 A	47.7 A	21.9 B	9.6 ns	0.28 A	10.0 A
Low	58 a	44.1 ns	24.0 ns	9.6 b	0.25 ns	10.4 a
Mid	96 a	48.0 ns	21.7 b	9.4 b	0.25 ns	10.5 a
High	87 a	51.1 a	19.8 b	10.0 ns	0.34 ns	8.9 a
Acidic site (Rauris)						
Overall	22 B	39.5 B	26.7 A	10.5 ns	0.20 B	4.4 B
Low	23 b	40.0 ns	25.6 ns	11.6 a	0.20 ns	4.5 b
Mid	18 b	42.1 ns	24.7 a	11.3 a	0.17 ns	4.6 b
High	23 b	36.3 b	29.7 a	8.6 ns	0.22 ns	4.1 b
CV (± %)	14	4	4	5	14	12

Values presented are sample means per site (n = 36) and per elevation (n = 12); mean values followed by capital letters (for Overall i.e. site specific means) and lower-case letters (for elevation levels i.e. elevation specific means) are significantly different according to t-test ($p < 0.05$); CV = mean coefficient of variation between elevation- and depth-specific replicate samples (n = 4); ns = not significant.

Elevations had higher R^2 values, considering the distribution of microbial PLFAs (ADONIS: elevations $R^2 = 0.53$, depths $R^2 = 0.17$; Fig. 7a) and residues (ADONIS: elevations $R^2 = 0.63$, depths $R^2 = 0.05$; Fig. 7b). MBC was higher at the alkaline site, down all depth gradients, including increasing contribution of fungal PLFA to total PLFA (Fig. 8a). At the acidic site, it decreased with depth following low total PLFA (Fig. 8b). Likewise, microbial residue contents were also higher at the alkaline site, especially the contribution of fungal GlcN to total amino sugars, than at the acidic site (Fig. 8cd).

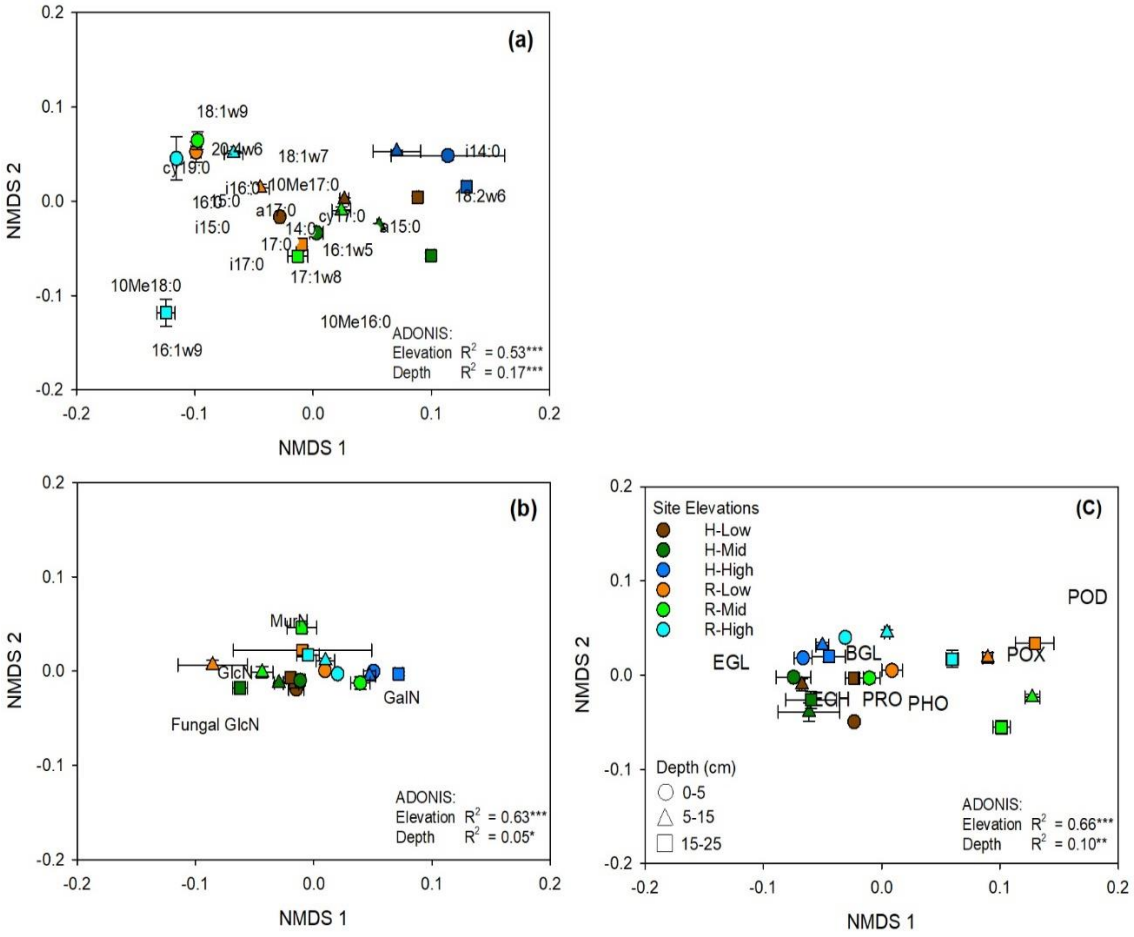

Fig. 7. (a) NMDS plot of microbial community composition based on distance matrix of relative abundance of individual PLFAs; (b) NMDS plot of Amino sugar residues based on distance matrix of relative abundance of individual amino sugars; (c) NMDS plot of enzyme patterns based on distance matrix of standardized enzyme activities at three depths and three elevations levels; low = 900, mid = 1300, and high = 1900 m asl in alkaline site (Hochschwab – H) as well as low = 1300, mid = 1600, and high = 2100 m asl in acidic site (Rauris – R). Symbols represent means of individual replicates and error bars confidence intervals.

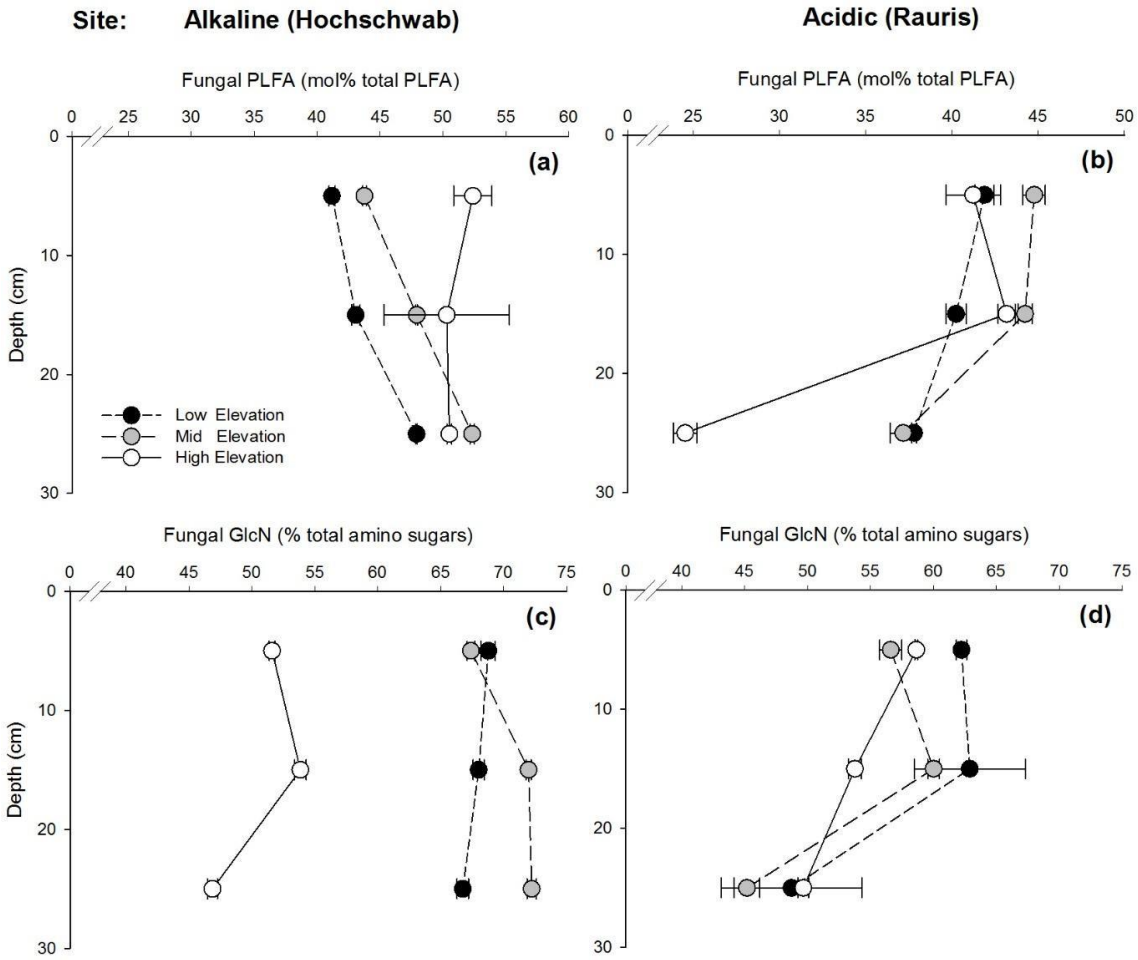

Fig. 8. Depth-specific mean contribution of fungal PLFA to total PLFA contents as its mole percentage (mol%) and mean contribution of fungal glucosamine (fungal GlcN) to total amino sugar contents as its percentage (%) in alkaline site (Hochschwab) (left panels – a, c) and acidic site (Rauris) (right panels – b, d) respectively along elevation gradients; error bars indicate standard error of means (n = 4); low = 900, mid = 1300, and high = 1900 m asl in alkaline site, as well as low = 1300, mid = 1600, and high = 2100 m asl in acidic site.

Mean MBC-specific exo-glucanase activity was significantly higher at the alkaline than at the acidic site, whereas the reverse was true for protease (Table 5). The activities of β-glucosidase and exo-chitinase did not generally differ between the sites. MBC-specific phosphatase was on average 75% lower at the alkaline than at the acidic site. Phenoloxidase and peroxidase enzyme activities were on average 96 and 98% lower at the alkaline than at the acidic site. Elevations more strongly differentiated enzyme patterns than depths (ADONIS: elevations $R^2 = 0.66$, depths $R^2 = 0.10$; Fig. 7c). Notable were, high phosphatase activities at all depths in both sites, especially at the surface soil

layer (Fig. 9ab). Oxidative POX and POD enzyme activities were higher up to 5-15 cm soil depth at the acidic than at the alkaline site, at all elevation levels (Fig. 9c-f).

Table 5. Mean activity rates for the hydrolytic enzymes BGL (ß-glucosidase), EGL (exo-glucanase), ECH (exo-chitinase), PRO (protease) and PHO (phosphatase) as well as the oxidative enzymes POX (phenoloxidase) and POD (peroxidase) over all soil depths at the three elevation levels in the two study sites; calculations based on per mg microbial biomass carbon (MBC).

Elevation levels	BGL	EGL	ECH	PRO	PHO	POX	POD
	(nmol h^{-1} mg^{-1} MBC)					(µmol h^{-1} mg^{-1} MBC)	
Akaline site (Hochschwab)							
Overall	74 ns	15.8 A	77 ns	4.6 B	220 B	0.4 B	0.2 B
Low	58 ns	10.3 ns	85 a	4.0 ns	240 b	0.4 b	0.1 b
Mid	57 ns	8.4 a	74 ns	2.8 b	140 b	0.4 b	0.1 b
High	108 ns	28.7 ns	73 ns	7.0 ns	270 b	0.4 b	0.5 b
Acidic site (Rauris)							
Overall	102 ns	8.4 B	88 ns	14.5 A	860 A	9.1 A	10.0 A
Low	68 ns	4.4 ns	55 b	5.2 ns	910 a	10.9 a	12.9 a
Mid	105 ns	2.0 b	85 ns	25.9 a	890 a	8.8 a	7.6 a
High	134 ns	19.0 ns	124 ns	12.5 ns	780 a	7.4 a	9.5 a
CV (%)	24	32	22	41	27	26	39

Values presented are sample means per site (n = 36) and per elevation (n = 12); mean values followed by capital letters (for Overall i.e. site specific means) and lower-case letters (for elevation levels i.e. elevation specific means) are significantly different according to t-test ($p < 0.05$); CV = mean coefficient of variation between elevation- and depth-specific replicate samples (n = 4); ns = not significant.

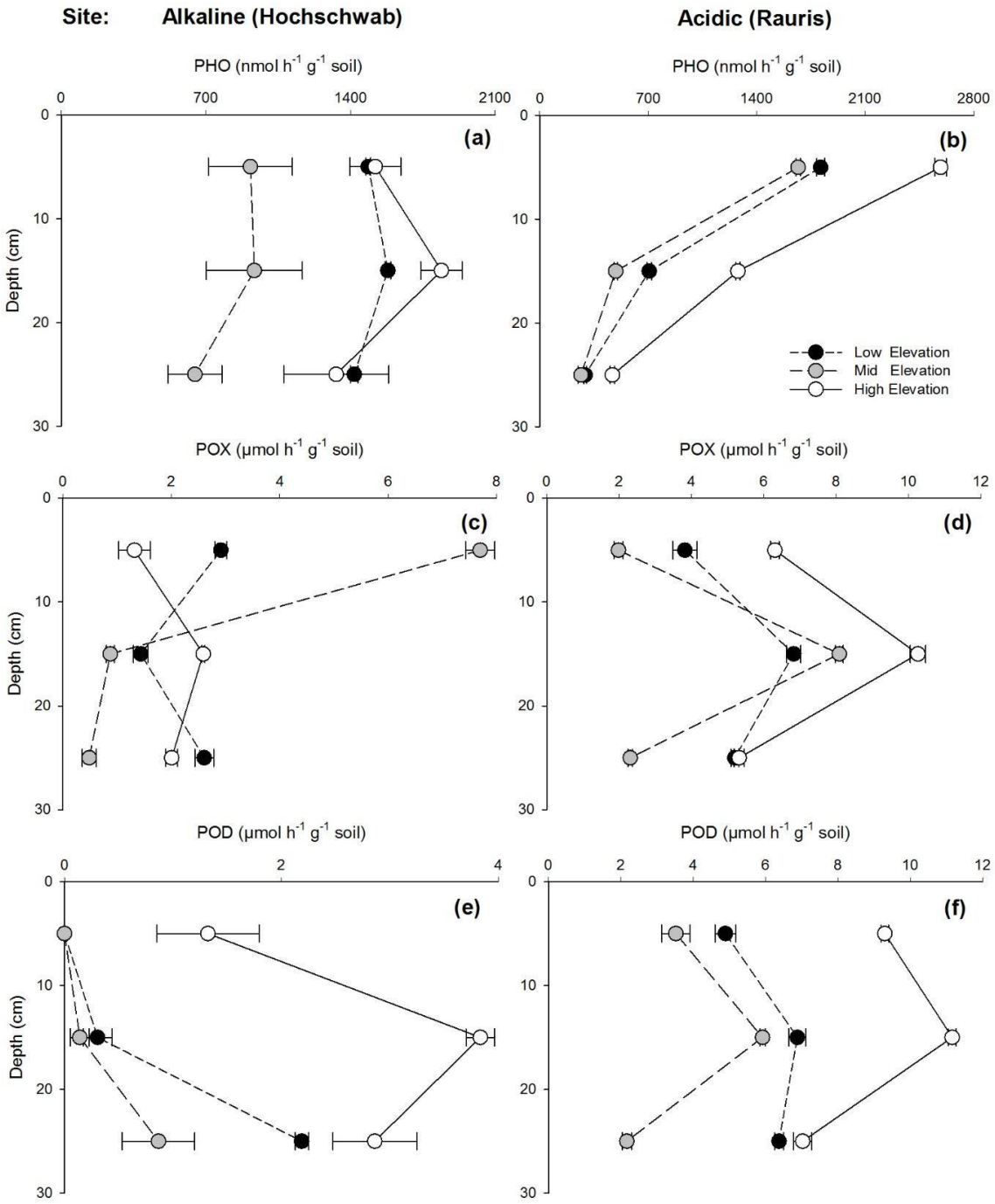

Fig. 9. Depth-specific mean potential enzyme activities on dry soil basis for hydrolytic enzyme phosphatase (PHO) and oxidative enzymes Phenoloxidase (POX) and Peroxidase (POD) in alkaline site (Hochschwab) (left panels – a, c, e) and acidic site (Rauris) (right panels – b, d, f) respectively along elevation gradients; error bars indicate standard error of means (n = 4); low = 900, mid = 1300, and high = 1900 m asl in alkaline site, as well as low = 1300, mid = 1600, and high = 2100 m asl in acidic site.

The ratios of ergosterol/fungal GlcN and bacterial PLFA/MurN were significantly higher at the alkaline than at the acidic site (Table 6). The fungal/bacterial PLFA and fungal/bacterial necromass C ratios were significantly higher at the alkaline site than at the acidic site. In contrast, the contribution of microbial necromass C to SOC was higher at the acidic site than at the alkaline site.

Table 6. Mean ratios of ergosterol/fungal glucosamine (fungal GlcN), bacterial PLFA/Muramic acid (MurN), fungal/bacterial PLFA, fungal carbon (C)/bacterial carbon (C) as well as contribution of microbial C to soil organic carbon (SOC) as its percentage over all soil depths at the three elevation levels in the two study sites.

Elevation level	Ergosterol/ fungal GlcN ($\mu g\ mg^{-1}$)	Bacterial PLFA/ MurN ($nmol\ mg^{-1}$)	Fungal/ bacterial PLFA	Fungal C/ bacterial C	Microbial C/ SOC (%)
Alkaline site (Hochschwab)					
Overall	4.3 A	107 A	1.4 A	7.5 A	26 B
Low	4.7 a	85 ns	1.2 ns	8.8 a	22 b
Mid	5.5 a	132 a	1.4 ns	8.4 a	29 b
High	2.9 ns	83 a	1.6 a	5.2 ns	26 ns
Acidic site (Rauris)					
Overall	1.5 B	50 B	1.0 B	3.4 B	37 A
Low	1.0 b	66 ns	1.0 ns	3.1 b	38 a
Mid	1.5 b	48 b	1.1 ns	4.1 b	44 a
High	2.1 ns	36 b	1.0 b	3.1 ns	28 ns
CV (± %)	31	22	7	7	17

Values presented are sample means per site (n = 36) and per elevation (n = 12); mean values followed by capital letters (for Overall i.e. site specific means) and lower-case letters (for elevation levels i.e. elevation specific means) are significantly different according to t-test ($p < 0.05$); CV = mean coefficient of variation between elevation- and depth-specific replicate samples (n = 4); ns = not significant.

At the acidic site, the correlations between MBC and total PLFA as well as MurN and bacterial PLFA contents were significant but not at the alkaline site. (Fig. 10ab). The biomarkers; ergosterol and PLFA 18:2ω6 and fungal GlcN and fungal PLFA contents were positively correlated at the acidic site in contrast to negative relationships at the

alkaline site (Fig. 10cd). The PCA plots consisting of ergosterol, enzymes, group specific PLFAs (G+, G- and fungal) and three amino sugar residues (MurN, GalN and fungal GlcN) did not show high correlations between different parameters. However, at the alkaline site, the samples were grouped by elevation and high elevation level samples were clearly separated from those of low and mid elevation levels (Fig. 11a). At the acidic site, soil samples were grouped more clearly by depth and correlation occurred between bacterial PLFAs and oxidative enzymes (POX and POD). Other parameters (fungal GlcN, GalN, and MurN) correlated with hydrolytic enzymes, thus forming two distinct clusters (Fig. 11b).

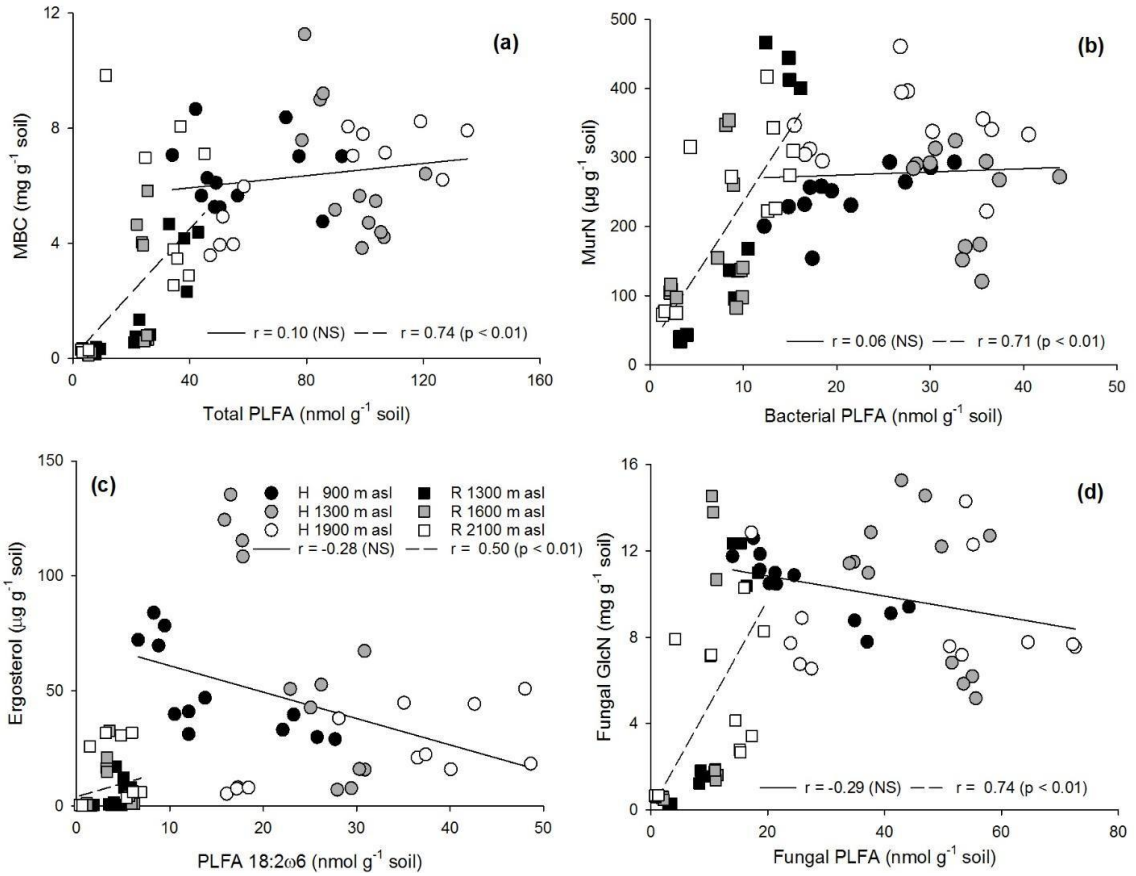

Fig. 10. Linear relationships between (a) Microbial biomass C (MBC) and Total PLFA contents; (b) Muramic acid (MurN) and Bacterial PLFA contents; (c) Ergosterol and PLFA 18:2ω6 contents; and (d) Fungal glucosamine (Fungal GlcN) and Fungal PLFA contents. Relationships calculated using Spearman's non-parametric test for each site (n = 36 per site) separately; NS = not significant. H indicates the alkaline site Hochschwab and R indicates the acidic site Rauris.

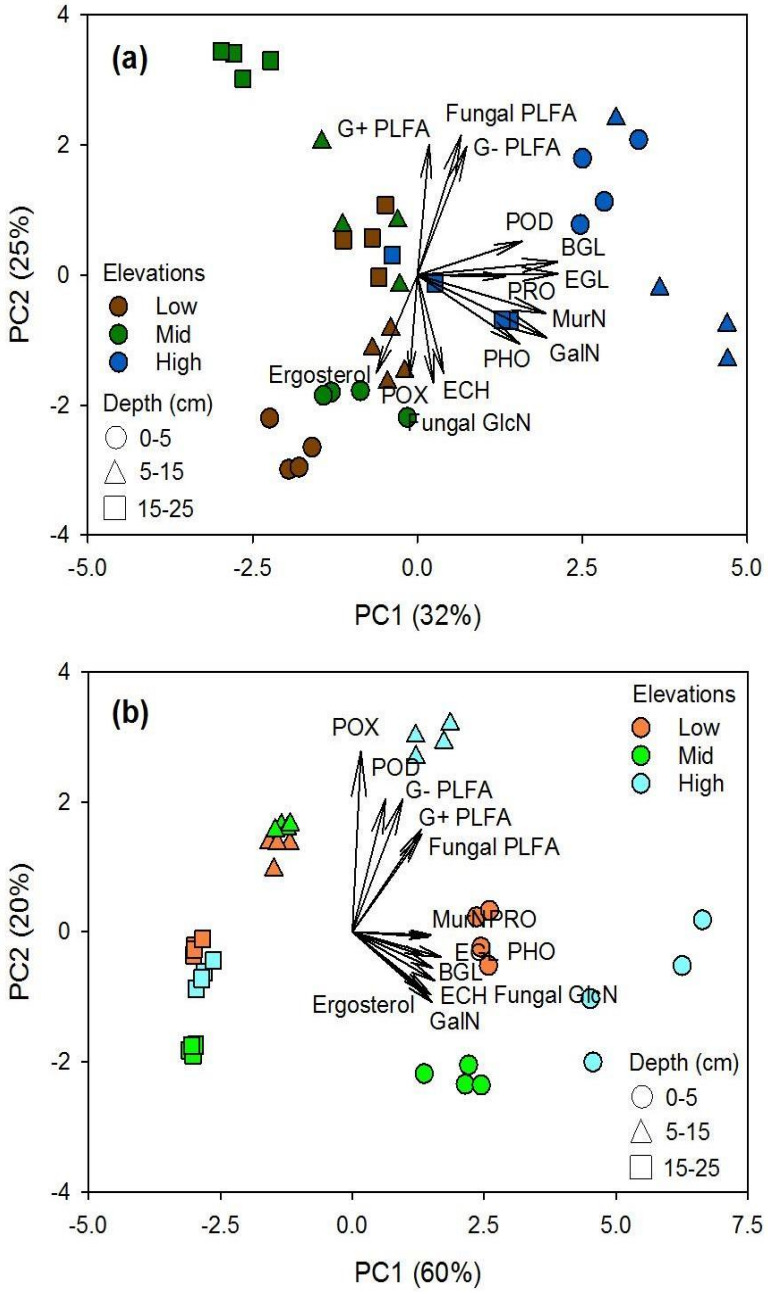

Fig. 11. PCA plot of enzymes measured on dry soil basis, group specific PLFA's (Fungal PLFA, Gram positive (G+) and Gram negative (G-) bacterial PLFA), amino sugars (muramic acid (MurN), galactosamine (GalN) and fungal glucosamine (Fungal GlcN)) and ergosterol contents over all elevation and soil depth gradients in (a) Alkaline and (b) Acidic site.

3.4 Discussion

3.4.1 Contribution of microbial necromass

The generally lower contribution of microbial necromass C to SOC at the alkaline site Hochschwab, suggest retardation of plant residue decomposition. This view is in accordance with the higher SOC contents and the higher soil C/N ratios at this site. High soil C/N ratios usually indicate the accumulation of less decomposed plant residues (John et al., 2005; Jenkinson et al., 2008). The lower accumulation of microbial necromass C also led to higher ratios of ergosterol to fungal GlcN and bacterial PLFA to MurN. This means that changes in microbial biomass are not necessarily always reflected by the contribution of microbial residue to SOC (Potthoff et al., 2008). Furthermore, ordination analysis showed that the distribution of amino sugars was different to that of PLFA (Fig. 11ab). This further suggest that microbial necromass distribution varies from that of living microorganisms, as their biomass is more prone to the actual turnover conditions (Liang et al., 2015; Siles et al., 2016).

The low formation of microbial necromass C at the alkaline site Hochschwab is apparently caused by a reduction in microbial biomass turnover. The possibility of an increased microbial necromass turnover, i.e. amino sugar turnover, which has been observed in non-paddy rice fields (Roth et al., 2011), is unlikely, as it should be accompanied by low soil C/N ratios. Acidity is often the main reason for a low contribution of 25% microbial necromass C to SOC in forest soils (Khan et al., 2016), a percentage similarly observed at the current alkaline site Hochschwab. Microbial growth and activity are both known to decrease under water limited conditions, thereby reducing organic matter decomposition and its conversion into necromass (Crowther et al., 2015). Water and P limitation most likely cause this retardation in plant residue decomposition (Gavazov, 2010; Gavazov et al., 2017; Bhople et al., 2019). As the general precipitation is similar at both sites, low moisture might be induced by the physical structure, i.e. the low bulk density, leading to high aeration, especially during dry summer periods. However, this view needs further experimental evidence, which cannot be provided by the current study, focussed on additional microbial necromass, biomass, and activity markers.

3.4.2 Enzyme activities as indicators

Lower biomass-specific phosphatase activity at the alkaline site indicates that not only water, but also P limitation is another important reason for the retarded decomposition of plant residues at alkaline site Hochschwab. Microorganisms seem to form less phosphatase per biomass at lower concentrations of available organic phosphate esters to be cleaved (Nannipieri et al., 2011; Margalef et al., 2017) and in relatively young organic matter, especially in the absence of inorganic material (Turner et al., 2014). In contrast, biomass specific -glucosidase activity did not differ between the sites at all three elevation levels. This points to a close relationship between ß-glucosidase, a key enzyme providing available C to soil microorganisms (Leitner et al., 2012), enabling microbial biomass growth (Kaiser et al., 2010). The hydrolytic enzymes ß-glucosidase, exo-glucanase, exo-chitinase, and protease differed only at one elevation level between the sites, making general explanations for the different enzyme formation impossible. Reasons might be site and elevation level-specific hot-spots in the presence of respective substrates (Kuzyakov and Blagodatskaya, 2015).

A striking feature of the current results is the 96 and 98% lower activity of phenoloxidases and peroxidases at the alkaline site Hochschwab. The low activity levels of these oxidative core enzymes in lignin degradation (Sinsabaugh 2010; Leitner et al., 2012; Miyamoto et al., 2017) might be another reason for the retarded decomposition of plant residues at the alkaline site Hochschwab. Such a relationship between a reduction in enzyme activity and a retardation of plant residue decomposition has been repeatedly described in incubation experiments (He et al., 2007; Leitner et al., 2012), but rarely in the field (Chigineva et at., 2012), especially not for the alkaline sites (Sinsabaugh, 2010). Water limitation might result in lower oxidative enzyme production (Burns et al., 2013), but this effect has not been observed for the hydrolytic enzymes exo-glucanase, exo chitinase and protease. A more important reason might be the absence of microbially available P, Cu, and Mn for oxidases (Bárta et al., 2010; Sterkenburg et al., 2018; Kaur et al., 2019). It is unlikely that phenoloxidase and peroxidase producing species are missing in a microbial community strongly dominated by fungi. However, more information on the fungal community composition might elucidate this question.

3.4.3 Fungal contribution to microbial residues and biomass

Fungi generally dominate microbial residues at both sites, but the dominance is stronger at the alkaline site, where fungi contributed 88% and bacteria 12% to microbial residues. At the acidic site, fungi contributed 77% and bacteria 23% to this fraction. The current results contradict the general view that acidification promotes fungi (Strickland and Rousk, 2010; Khan et al., 2016). Other factors override the pH effects in the current ecosystems. The percentages at Rauris are nearly identical to the fungal 79% and bacterial 21% reported by Zhang et al. (2013) for Chinese subalpine and alpine soils, which can be calculated from the GlcN and MurN data according to Engelking et al. (2007). These values at the Rauris site and in China are similar to the mean contribution of these two main functional microbial groups observed in forest soils (Joergensen and Wichern, 2008). Fungi contributed 58% to the microbial PLFA at the alkaline site and 50% at the acidic site. These are much higher percentages than those usually obtained by PLFA analysis in Alpine forest soils: Siles et al. (2016, 2017), Zhang et al. (2013), and Margesin et al. (2009) observed mean fungal contributions to the microbial community of 25%, 20%, 13%, and 7%, respectively. Whitaker et al. (2014) calculated a mean fungal contribution to the microbial biomass of 87% by converting fungal PLFA to fungal biomass. However, the contribution of fungi to total PLFA depends on the number of PLFA used as indices for fungi and bacteria, which is highly variable (Joergensen and Wichern, 2008). In most cases, only linoleic acid (18:2ω6,9) is used as a fungal biomarker (Margesin et al., 2009; Zhang et al., 2013; Siles et al., 2016, 2017). In some cases, even general microbial biomarkers, such as the straight saturated PLFA (14:0 to 18:0), are assigned as bacterial biomarkers (Malik et al., 2016), lowering the ratio of fungal to bacterial PLFA.

The fungal indicator PLFA 18:2ω6,9 is mainly present in Basidiomycota and Ascomycota, like ergosterol (Klamer and Bååth, 2004; Joergensen and Wichern, 2008). It occurs only in minor percentages in the former fungal phylum Zygomycota (Klamer and Bååth, 2004) and not in AMF (Olsson et al., 1995). The PLFA 18:1ω9 is more specific for Zygomycota (Klamer and Bååth, 2004; Joergensen and Wichern, 2008) and 16:1ω5 for AMF (Olsson et al., 1995). However, none of the three fungal indicators are fully specific as 16:1ω5 occurs in trace percentages in G- bacteria (Nichols et al., 1986;

Zelles, 1997), 18:1ω9 in bacteria (Zelles, 1997), and 18:2ω6,9 occurs in plants (Zelles, 1997). This might be the reason for the negative relationship between ergosterol and the PLFA 18:2ω6 as well as between fungal GlcN and fungal PLFA at the alkaline site. The specificity of PLFAs might be additionally hampered by the ability of microorganisms to recycle foreign PLFAs after uptake (Dippold and Kuzyakov, 2016). More work with cultured organisms seems to be necessary to re-assess the specificity of indicator PLFAs.

3.4.4 Contribution of Gram-positive bacterial to PLFA

G+ bacteria generally dominated bacterial PLFA at both sites, but the dominance is slightly stronger at the acidic site Rauris, where G+ bacteria contributed 72 mol% and G- bacteria 28 mol% to the total bacterial PLFA, an indicator for bacterial biomass. At the alkaline site Hochschwab, G+ bacteria contributed 70 mol% and G- bacteria 30 mol% to total bacterial PLFA. This difference is small but significant, looking at the mol% of the bacterial indicator PLFA (Table 4). Margesin et al. (2009) observed a mean G+/G- PLFA ratio of 84 to 16% in sup-Alpine and Alpine soils, whereas Zhang et al. (2013) and Whitaker et al. (2014) found mean G+/G- PLFA ratios of roughly 50 to 50% at high elevation levels in the Changbai Mountains and the Andes, respectively. A G+/G- PLFA ratio of 60 to 40% (Joergensen and Potthoff, 2005) has been used as a basis for the conversion of MurN to bacterial necromass C (Appuhn and Joergensen, 2006). As the MurN concentration of G+ bacteria is on average 2.7 times higher than that of G- bacteria (Appuhn and Joergensen, 2006), a strong shift towards G+ bacteria leads to an overestimation of bacterial necromass C.

However, it should also be considered that G- bacteria have an additional outer membrane containing PLFA and lipopolysaccharides. This markedly increases the PLFA concentration in the biomass of G- bacteria and lowers their true contribution to total bacterial biomass. The dominance of G+ bacteria obtained by PLFA, contrasts studies using DNA extraction, enrichment and sequencing, where G+ soil bacteria contributed only between 3 and 15% to total gene copies in Alpine soils (Siles et al., 2017). One reason might be the insufficient extraction of DNA from G+ bacteria, due to their nearly three times thicker murein layer. However, this explanation needs experimental evidence by comparing PLFA and DNA based approaches. In most cases, only firmicutes are assigned as G+ (Margesin et al., 2009; Zhang et al., 2013; Whitaker et al., 2014; Fanin et

al., 2019), although actinobacteria are also an important G+ bacterial phylum. Actinobacteria are slow growing bacteria, capable of decomposing complex polymers, especially the chitin (Siles et al., 2016, 2017). On the other hand, lactobacilli are the most rapidly growing bacterial genus. Consequently, the ecological meaning of the G+/G- ratio should not be overemphasized, contrasting the views of Whitaker et al. (2014) and Fanin et al. (2019).

3.4.5 Depth and elevation gradients

The differences between the low and mid elevation level were in most cases small for most of the microbial biomass and necromass markers within each site. In contrast, the differences between the two sites were often significant at the low and mid elevation levels. This indicates interacting effects of bedrock and vegetation. At the high elevation level, where trees are unable to grow anymore, the vegetation converges, due to the higher presence of grassland species and higher contribution of AMF to the fungal community, indicating an increasing influence of climate induced vegetation change rather than inherent bedrock properties. Consequently, microbial biomass (MBC, ergosterol, G-PLFA), activity (ß-glucosidase, exo-glucanase, exo-chitinase, and protease), and necromass (MurN, ergosterol/fungal GlcN, fungal/bacterial necromass C, and microbial necromass C/SOC) indices differ less often significantly. A warming induced shift in vegetation will have strong effects on these indices in the future.

Bedrock and vegetation also have interacting effects on soil development. The physical breakdown of gneiss and schists leads to the formation of finer gravel and sand. This allows growing spruce trees to create a strong but shallow rooting system at the acidic Rauris site. This means that spruce trees supply more root residues as C input to the topsoil horizon and that the fungal community must consist to a higher percentage of ecto-mycorrhizal fungi. In contrast, the physical breakdown of limestone and dolomites leads to larger lumps of stone fragments, where huge cracks enable the growth of deep rooting deciduous trees. The extremely slow chemical breakdown of limestone by the dissolution of carbonates leads to the observed low contribution of inorganic material to the topsoil horizons at the alkaline site Hochschwab. Deciduous trees supply more leaf litter as C input as their roots reach deeper layers, i.e. the fungal community should contain more of saprotrophic and less of mycorrhizal fungi (Purahong et al., 2016;

Sterkenburg et al., 2018). However, this possible shift contradicts the markedly lower presence of oxidases at the alkaline site Hochschwab. This points to the great need for more information about which fungal species produce oxidases under which environmental conditions.

3.5 Conclusion

Microbial necromass formation is reduced at all elevation levels of the alkaline site Hochschwab in comparison with those of the acidic site Rauris, which is another strong indication of retarded microbial plant residue decomposition. The biomass-specific activities of phenoloxidase and peroxidase enzymes are generally reduced at the alkaline site Hochschwab, but not those of the hydrolytic enzymes. An exception was the general level of phosphatase activity at Hochschwab. It should be assessed whether Cu, Mn, and P limitation contribute to the observed reduced enzyme activities. Fungi generally dominate microbial necromass, with 85% at Hochschwab and 72% at Rauris. The ratio of fungal to bacterial PLFA was unusually high at both sites, but especially high at Hochschwab. G+ bacteria dominate bacterial biomass at both sites, but the contribution was slightly higher at the acidic site Rauris. The specificity of indicator PLFAs should be re-assessed with cultured microorganisms in the near future. Climatic effects along alpine elevation gradients depend on vegetation induced changes in soil properties, which interact with bedrock properties.

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4 Variations in fungal community structure along elevation gradients in contrasting Austrian Alpine ecosystems

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Highlights

- The number of fungal sequences were higher at alkaline site Hochschwab than at acidic site Rauris.
- Fungal diversity indices showed mid-domain maxima at both sites.
- In both sites the fungal diversity was in the order of phylum Ascomycota > Basidiomycota > Zygomycota.
- Saprotrophic and symbiotrophic trophic modes dominated both alkaline and acidic sites.
- Environmental factors (elevations, MAT and MAP) affected fungal community structure more, followed by soil pH and SOC contents in both sites, in addition to soil C/N ratio only in alkaline site.

Abstract

The variability in fungal community along elevation gradients is less known especially with comparison perspective. Such insights provide comprehensive understanding on the effects of changing climatic conditions on fungi as they are integral part of decomposition and nutrient cycles. In our preceding work at the two Austrian alpine elevation gradient sites, differing in vegetation and parent material, the stocks of fungal biomass were significantly higher at the alkaline site Hochschwab in comparison to the acidic site Rauris. Therefore, in this work, we highlight soil fungal community distribution at soil depths over six elevation gradients across 900 to 2100 m above sea level (m asl). The fungal communities were characterized using high-throughput Illumina sequencing of internal transcribed spacer (ITS2) region. Fungal diversity properties were significantly higher at Hochschwab that at Rauris. However, both sites displayed diversity maxima at mid elevation levels. The differences in fungal community structure and main fungal classes were mainly related to environmental factors (elevations, mean annual temperature (MAT) and mean annual precipitation (MAP)) followed by edaphic factors, soil pH and organic carbon (SOC) contents in both sites. Additionally, only at alkaline site Hochschwab, soil C/N ratio significantly affected fungal community structure. In both sites the fungal diversity was in the order of phylum Ascomycota > Basidiomycota > Zygomycota. In terms of functional guilds, saprotrophism (Ascomycota) and symbiotrophism (Basidiomycota) dominated in these sites. To our knowledge this is a promising metagenomic and climosequence study, leading to better insights on variations in fungal community structure as affected by environmental and soil chemical properties along elevations and soil depth gradients in contrasting alpine sites.

Key words: Alpine ecosystems, Elevation gradients, Illumina sequencing, Internal transcribed spacer region, Fungal community structure, Fungal functional guilds.

4.1 Introduction

Climate warming in Alps is likely to continue with greater magnitudes (IPCC, 2014). In response to this, tree line is predicted to shift upward and significantly affect microbial community composition and functioning (Djukic et al., 2010a). Fungi are important soil microbial groups and their role in soil carbon sequestration is implicit due to close relationships with plants, and the ability to internally cyclize soil organic carbon (SOC) through aggregation (Murugan et al., 2019). They contribute significantly to microbial tissue in soils and are also requisites in decomposition (Joergensen and Wichern, 2008; Setala and McLean, 2004). Hence it is fundamental to decipher fungal diversity and factors that drive variations in their community structure especially over the alpine elevation gradients. In our previous work (Bhople et al., 2019), elevation gradients having similar mean annual temperature and precipitation at corresponding three levels served as natural laboratories to compare two alpine sites with disparate bedrock material. The study showed, exceeded ergosterol stocks at alkaline site Hochschwab than at acidic site Rauris especially at low and mid elevation levels where spruce/beech dominated forest vegetation at Hochschwab unlike spruce strands at Rauris. .

At alkaline site Hochschwab, three to four times higher ergosterol to microbial biomass C (MBC) ratio indicated higher presence of saprotrophic fungi (Bhople et al., 2019). However, in our subsequent work, reduced enzyme activities of phosphatase and oxidases, despite the higher fungal contribution to microbial biomass and necromass formation were seen at Hochschwab in comparison to those at Rauris (Chapter 3 of the dissertation). The interaction of bedrock and vegetation at low and mid elevation forests in both sites change soil properties which may further drive changes in microbial community structure (Bhople et al., 2019). Differences in forest tree species inevitably lead to differences in fungal community composition (Asplund et al., 2019; Awad et al., 2019; Bödeker et al., 2016). Hence for further investigating fungal diversity, soil samples from three elevation levels at alkaline site Hochschwab and acidic site Rauris, were subjected to metagenomic sequencing of fungal biomarker (ITS2) gene.

The disparity in vegetation with increasing elevations in Alps lead to alterations in quantity and quality of C resources (Djukic et al., 2010b). The passage of plant C resource to fungi differs among fungal groups, for example ectomycorrhizal (EMF) and

endomycorrhizal fungi acquire C from host plant roots while saprotrophic fungi (SAP) derive C by decomposing plant material (Ishida et al., 2007, Lindahl et al., 2010). The subsequent changes in temperature and precipitation indirectly affect C allotment to mycorrhiza by modifying host plant roots regulatory mechanisms (Treseder and Allen, 2002, Öpik et al., 2006). On the other hand, SAP are more affected by soil properties such as pH, moisture and oxygen levels (Setala and McLean, 2004). Both these scenarios are decisive in functional attributes of fungal community composition. At high elevations in Alps, vegetation converges into grasslands where microbial residues are differently activated (Murugan et al., 2019) and bacteria and arbuscular mycorrhizal fungi (AMF) contribute more to SOC sequestration at the cost of SAP (Murugan et al., 2013). Hence, cataloguing fungal diversity along elevation gradients and their response to biotic and abiotic factors will help to understand which factors determine fungal life in alpine ecosystems, especially under changing climatic conditions.

Despite of the considerable interest in general fungal distribution (Tedersoo et al., 2012, 2014), many elevation-based studies investigated only specific fungal groups i.e. either EMF and/or AMF (Gómez-Hernández et al., 2012, Shi et al., 2014, Jarvis et al., 2015, Geml et al., 2017). Thus, the general fungal diversity and its elevational patterns in alpine ecosystems remain elusive (Siles and Margesin, 2017; Ogwu et al., 2019). Further paucity exists on generality of results as most of the studies were conducted across single elevation (i.e. same bedrock) or short elevational range or along elevations with same vegetation with special focus only on top-soils. Hence it is of further interest to understand fungal community structure at deeper soils in alpine sites differing in geology and vegetation (i.e. similar climate), with comparison perspective.

Till to date, only Siles and Margesin (2016, 2017) have investigated shifts in soil fungal communities in high altitude European Alps, using comprehensive state-of-the-art tools like high-throughput sequencing, albeit only along single elevation gradient (i.e. same bedrock material). While in Japanese high mountain Alps, fungal elevational patterns are studied but effects of vegetation along altitudes were not considered (Ogwu et al., 2019). Therefore, our aim in this study was to investigate fungal community structure along elevation gradients in contrasting alpine ecosystem sites differing in bedrock and vegetation but exhibiting similar climate in-terms of mean annual temperature and precipitation regimes at corresponding elevation levels. It was of interest

to address three main questions; 1) How fungal community structure vary along the elevation and depth gradients in two contrasting alpine ecosystem sites? 2) What is the status of fungal community composition at different elevations within the two sites in terms of their functional perspective? And 3) Which factors primarily affect fungal community structure in these sites? For these questions, our hypothesis is that significantly different fungal communities characterizing contrasting Alpine sites would differentially respond in taxonomic and functional terms in response to the observed changes in soil and microbial properties along the elevation gradients.

4.2 Materials and methods

4.2.1 Study sites and soil sampling

The study sites are located at Hochschwab (H) in the Northern and Rauris (R) in the Central Austrian Alps. At both sites three elevations starting from highest to lowest at each site (H1, H2, H3 in Hochschwab and R1, R2, R3 in Rauris) spanning across 900 to 2100 m above sea level (m asl) were sampled in August-September 2015. Soils in Hochschwab were characterized as Leptic Histosols formed on limestone and dolomites and in Rauris as Haplic Podzols formed from gneiss and schists, parent material (IUSS WRB, 2015 (update). The Mean annual temperature (MAT) decreased from 6.2 to 2.1 °C and 5.9 to 1.9 °C, and mean annual precipitation (MAP) increased from 1178 to 1725 mm and 1067 to 1850 mm at Hochschwab (Austrian Federal Ministry of Finance) and at Rauris (Zentralansalt für Meteorologie und Geodynamik (ZAMG)) respectively. In Hochschwab low and mid elevation forests were dominated by mixed spruce-beech (*Fagus sylvatica*) trees while at Rauris by spruce (*Picea abies*) trees. At high elevations in both sites the vegetation was of alpine grassland type.

At each elevation in both sites, 12 soil replicates were sampled from three soil depths 0-5 (without litter), 5-15 and 15-25 cm respectively. All soil samples were transported in cooling boxes to laboratory and sieved immediately (< 2 mm mesh). After sieving 72 samples were generated (4 replicates × 3 depths × 3 elevations × 2 sites) and stored at -80 °C until molecular analysis. The details of analysis of soil analytical and microbial parameters from these samples are given in Bhople et al. (2019 submitted/chapter 3 of the dissertation).

4.2.2 Metagenomic DNA extraction

DNA was extracted from 0.5 g soil sample using commercially available Fast DNA spin kit (MP Biomedicals) according to the manufacturer's recommendations. For samples with high humus content the homogenization step was extended from 40 to 60 seconds and speed setting of FastPrep® instrument at 6, with two repetitions. The immediate step of centrifugation was extended to 15 min at 14,000 × g. All extracted DNA pellets were washed twice with 70% ethanol, dissolved in 50 µl TE buffer after air drying and stored at -20 °C for subsequent use. The size of extracted metagenomic DNA was checked by electrophoresis on 1% agarose gel.

4.2.3 Internal Transcribed Spacer (ITS2) region amplification, paired-end library preparation and Illumina MiSeq sequencing

Fungal internal transcribed spacer (ITS2) gene was amplified using combination of ITS3-Mix as forward and ITS4-Mix as reverse primer set (Tedersoo et al., 2014; 2015). PCR amplification of the target region was achieved using following thermal profile of 30 cycles after initial 3 min denaturation step at 95 °C followed by denaturation at 98 °C for 30 s; annealing at 55 °C for 30 s; and extension at 72 °C for 30 s; and final extension for 5 min at 72 °C in BIOMETRA thermal cycler. This amplification was performed in a 25 µl reaction volume each containing; 5 µl 5x KAPA HiFi fidelity buffer, 0.75 µl 10 mM dNTP mix, 0.75 µl each of 10 µM forward (ITS3-Mix) and reverse (ITS4-Mix) primers, 1 µl of 20 ng template DNA, 0.5 µl of 1 U/µl KAPPA HotStart DNA polymerase and 16.25 µl PCR grade water. Excess primers and dimers were removed using AMPure XP beads (Beckman Coulter, Brea, CA, USA) to get pure amplicon mix with target DNA fragment.

Purified and cleaned PCR product from amplicon PCR was used as template for Index PCR. In index PCR, cleaned templates were amplified using Index 1 (N701-N709) and Index 2 (S517-S508) primers, from Nextera XT Index Kit (Illumina, San Diego, CA, USA). For indexing these primers, an 8 cycle PCR was performed in a 50 µl reaction volume each containing; 5 µl (10 ng) cleaned amplicon DNA template, 5 µl (10 µM) each forward (N701-N709) and reverse (S517-S508) index primers, 25 µl 2x KAPA HiFi HotStart Ready Mix and 10 µl PCR grade water. PCR program for indexing comprised of 1 cycle of initial denaturation for 3 min at 95 °C, followed by 8 cycles of denaturation

at 95 °C for 30 s; annealing at 55 °C for 30 s and extension at 72 °C for 30 s; and 1 cycle of final extension at 72 °C for 5 min. Different combination of indices [9 different Index 1 adapters (N701-N709) and 8 different Index 2 adapters (S517-S508)] for different templates were used for parallel MiSeq sequencing. Indexed PCR products were cleaned using AMPure XP beads (Beckman Coulter, Brea, CA, USA) according to manufacturer's say.

qPCR was performed using KAPA Library Quantification kit recommended for Illumina sequencing platforms and library insert size of 500-550 bp. Library concentration was validated on Agilent 2100 Bioanalyzer. 5 µl aliquots were pooled from each library with unique indices and mixed with 10 mM pH 8.5 Tris buffer to achieve final concentration of 4 nM of the cleaned library. The pooled library was again validated using Agilent 2100 Bioanalyzer. Using pre-chilled hybridization buffer (supplied along with Illumina MiSeq Reagent Kit) and freshly prepared 0.2 N NaOH, pooled library of 4 nM was further diluted to 20 pM concentration. The library was denatured at 96 °C for 2 min on thermomixer R (Eppendorf). Just prior to denaturation PhiX DNA of 4 pM concentration was spiked with the library to enhance the data quality of low diversity samples. After denaturation, the library was immediately placed on ice-water bath to keep the single stranded DNA unbound. A final concentration of 4 pM denatured DNA was subjected to MiSeq sequencing (Illumina, San Deigo, CA, USA) using MiSeq Reagent v3 Kit with paired end, 2 × 300 bp cycle run and applying Generate FASTQ reporter workflow.

4.2.4 Bioinformatics and reads processing

After completing the sequencing procedure, the quality of raw data from MiSeq was quarantined in FastQC software (Andrews, 2010) and further screening of reads for PhiX contamination using Bowtie 2.2.6 (Langmead and Salzberg, 2012). Before merging paired-end reads by PEAR 0.9.6 ($p < 0.001$) (Zhang et al., 2013), error correction was achieved by applying Bayesian clustering on the reads (Nikolenko et al., 2013; Schirmer et al., 2015). From these merged reads, in the next step, forward and reverse primers were stripped, employing Cutadapt 1.8.3 tool (Martin and Wang, 2011) and quality filtered *via* USEARCH v8.0.1517 (maximum expected error = 0.5) (Edgar, 2013; Edgar and Flyvbjerg, 2015). ITSx was used to target the extraction and to verify ITS2 region of the

sequences (Bengtsson-Palme et al., 2013). Targeted reads were then labelled according to original sample name and combined in QIIME (Caporaso et al., 2010). At 97% of similarity, the sequences were dereplicated, sorted and clustered using VSEARCH 1.1.1 (Rognes et al., 2016). As a routine of the above-mentioned tool, chimeras were checked adopting both a *de-novo* and a reference based approach. The UNITE reference dataset (v7 2016-01-01) was used for the reference-based chimera detection in UCHIME. Afterwards in VSEARCH, an optimal global alignment was applied to generate a BIOM table containing OTUs. The naïve Bayesian RDP classifier v.2.10.2 (Wang et al., 2007) with a minimum confidence of 0.6 against the UNITE database (v7 2016-01-31) (Kõljalg et al., 2013) was used for taxonomy assignment of the reads.

4.2.5 Statistical analyses

One-way analysis of variance (ANOVA) determined whether elevations had significant ($p < 0.05$) effects across sites on soil physiochemical parameters. Later, Tukey HSD post-hoc test allowed multiple comparison of means at 95% confidence interval, using R-package "agricolae" (Mendiburu, 2015). The diversity of fungal community was characterized by calculating richness (number of OTUs), evenness, Shannon index, Simpson's index, Chao1 and ACE (abundance-based coverage estimation) using package "RAM" (Chen et al., 2018). One-way analysis of variance (ANOVA) was applied for each site separately to determine if there were significant effects of elevations and depths on different diversity indices (package "agricolae" Mendiburu, 2015). If significant treatment effects were observed ($p < 0.05$) means were separated with Tukey HSD test. The normal and homogeneous distribution of residuals was examined using Shapiro-Wilks' W-Test and Levene tests (R-package "car"; Fox et al., 2012). If violated, data were log10/Box-Cox transformed (R-package "MASS"; Ripley et al., 2011) before the analysis. Data on relative abundances of the top 10 fungal classes had mainly unequal variances and transformations could not restore homoscedasticity for many groups. Thus, the Kruskal-Wallis test (Conover, 1999) was used to identify whether there were significant effects of different elevations and depths within the sites. If significant treatment effects were observed ($p < 0.05$), mean ranking values were separated with the Kruskal multiple comparison test (Conover, 1999). Benjamini and Hochberg (1995)

stepwise adjustment of *p* values allowed control of false discovery rate (FDR) to reduce type I errors in post-hoc test.

Mantel test based on Spearman's method was used to study the relationship between fungal community structure from OTU data and environmental and soil physiochemical properties, as well as the fungal indices. Bray-Curtis similarities between fungal community structure of different sites were related based on Bray-Curtis matrix with 9,999 permutations including all the environmental, and soil properties and the fungal indices. The significance of factors; sites, elevations and depths on fungal community structure was tested using analysis of similarity (ANOSIM) tests with Bray-Curtis similarities at 9,999 permutations. Non-metric multidimentional scaling (NMDS) analysis, based on Bray-Curtis similarities at OTU level was used to assess differences in the fungal communities at the two sites, at their elevation and soil depth gradients. All statistical analyses were done in R software version 3.4.2 (R Core Team, 2017).

Fungal OTUs were taxonomically parsed by ecological guilds based on trophic modes by using FUNGuild tool (https://github.com/UMNFuN/FUNGuild/blob/master/README.md) (Nguyen et al., 2016). Although FUNGuild data base is presently evolving yet it presents a nearly accurate functional annotation of fungal OTUs using previously established guilds. The communal and taxa roles at each elevation were interpreted using the relative abundance of FUNGuild results (trophic mode).

4.2.6 DNA sequence data accessibility

Sequence data are made available at NCBI SRA database under the SRA accession: PRJNA624806.

4.3 Results

4.3.1 Soil physiochemical properties

The mean values of soil properties; soil pH, SOC, TN and soil C/N ratio were significantly higher at all elevation levels in alkaline site Hochschwab than at acidic site Rauris (Table 7). The same is true for ergosterol contents, fungal PLFA and Fungal GlcN residues which were significantly higher in alkaline site in comparison to acidic site, especially at the mid elevation level (Table 8).

Table 7. Environmental and soil characteristics at elevation gradients in the two alpine ecosystem sites.

Elevation (m asl)	Level	MAT (°C)	MAP (mm)	Soil pH* (in H_2O)	SOC* (mg g^{-1} soil)	TN*	C/N*	Soil type	Bedrock	Vegetation
Alkaline site (Hochschwab)										
1900	High (H1)	2.1	1730	6.50 a	372 a	25 a	14.8 c	Leptic Histosol	Limestone and Dolomites	Alpine grassland, with montane pine bushes
1300	Mid (H2)	4.5	1400	6.70 a	374 a	16 b	24.2 a			Montane spruce-beech forest
900	Low (H3)	6.2	1180	4.94 b	487 a	21 ab	23.5 a			Montane spruce-beech forest
Acidic site (Rauris)										
2100	High (R1)	1.9	1850	4.79 bc	177 b	10 c	17.1 bc	Haplic Podsol	Gneiss and Schists	Alpine grassland
1600	Mid (R2)	3.8	1480	4.69 bc	117 b	5 c	19.2 b			Sub-alpine spruce forest
1300	Low (R3)	5.9	1070	4.42 c	105 b	5 c	18.5 b			Montane spruce forest
CV (± %)				3	12	12	3			

asl: meters above sea level; mm, millimetres; MAT: mean annual temperature; MAP: mean annual precipitation; SOC, Soil organic carbon; TN, total nitrogen; Soil C/N, SOC to TN ratio; Values represent sample means at each elevation level (n = 12). Means within a column are followed by different letters of significance according to Tukey's HSD test ($p < 0.05$) conducted across elevations in the two sites; Soil classification according to IUSS-WRB 2015 (update); Vegetation as recorded on site; climate data for alkaline site (Hochschwab) from Djukic et al. (2010a); and for acidic site (Rauris) from Zentralanstalt für Meteorologie und Geodynamik (ZAMG), Austria. *Data taken from Chapter 3 of the dissertation.

4.3.2 Taxonomic characteristics of fungal communities

Across 72 soil samples analysed, the Illumina amplicon sequencing had total counts of 25,09,988 high-quality sequence reads belonging to 4,188 distinct OTUs. The number of fungal sequences exceeded in alkaline site than in acidic site with maximum number of average sequences (39,222) at high elevation level in alkaline site (Table 8). Except evenness, other fungal diversity indices (richness, Shannon index, Chao1 and ACE) were significantly higher in alkaline site than in acidic site. In both sites, mid elevation levels showed significant diversity maxima in comparison to the other two elevation levels. The fungal diversity comprised of 5 different phyla with predominance of Ascomycota (with number of classified sequences in this phylum ranging from 37.62-62.73% across soil samples at different elevations) > Basidiomycota (18.79-50.17%) > Zygomycota (6.17-13.32%) (Fig. 12). At depth gradients, the dominance of Ascomycota was highest at all depths only at high elevation in acidic site in contrast to the dominance of Basidiomycota (49.10-55.40%) at 05-15 cm soil depths at all elevation levels the in alkaline site. The predominant classes comprised of Agaricomycetes and Tremellomycetes among Basidiomycota; Leotiomycetes, Eurotiomycetes, Sordariomycetes, Pezizomycetes, Saccharomycetes, Archaeorhizomyctes, Dothideomycetes and Incertae sedis among Ascomycota; and Incertae sedis (including Mortierellomycotina and Mucoromycotina) among Zygomycota (Fig. 13) in all samples. The dominance of Agaricomycetes at all elevation levels ranged between 13.23-42.79%, except at high elevation level in acidic site, where Leotiomycetes were in higher abundance (17.91%). A total of 80 fungal OTUs were most abundant (i.e OTUs with a relative abundance of ≥ 0.25%), of which 59 were classified at genus level.

Table 8. Fungal community diversity properties and indices at elevation gradients in the two alpine ecosystem sites.

Diversity properties & fungal attributes	Elevations in alkaline site			Elevations in acidic site		
	H1	H2	H3	R1	R2	R3
Number of sequences	39222 ns	35657 ns	35096 ns	34191 ns	32608 ns	32392 ns
Richness	504 b	677 a	378 c	381 c	400 bc	342 c
Evenness	0.13 ns	0.15 ns	0.11 ns	0.15 ns	0.13 ns	0.11 ns
Shannon index	4.10 ab	4.46 a	3.75 b	3.94 b	3.90 b	3.62 b
Chao1	595 b	860 a	508 b	487 b	543 b	486 b
ACE	595 b	856 a	502 b	480 b	524 b	476 b
Ergosterol*	23.8 ab	62.0 a	49.6 a	12.0 bc	8.10 c	4.30 c
Fungal PLFA*	51.1 a	48.0 ab	44.1 bc	36.3 c	42.1 c	40.0 c
Fungal GlcN*	50.8 c	70.5 a	67.9 a	54.0 bc	53.9 bc	58.0 b

Ergosterol represented as μg g^{-1} soil, fungal PLFA as mol% total PLFA and fungal GlcN as %total amino sugars. Values represent sample means at each elevation level (n = 12). Means within a row are followed by different letters of significance according to Tukey's HSD test ($p < 0.05$) conducted across all elevations in the two sites; ns, not significant.
* Data taken from Chapter 3 of the dissertation.

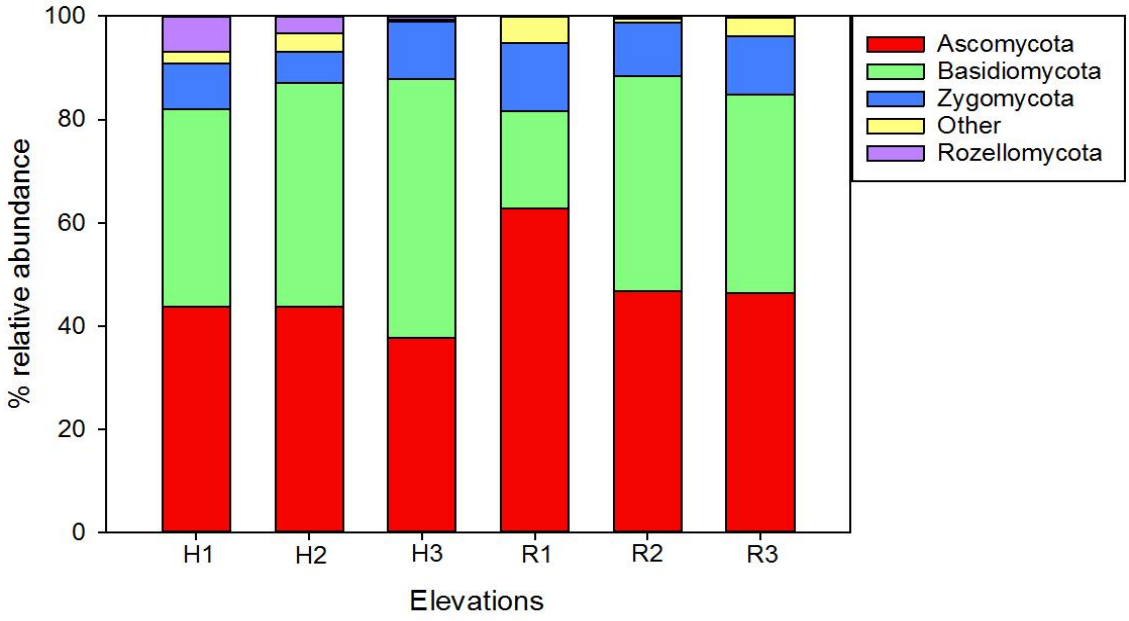

Fig. 12. Relative abundance of top 5 fungal phyla at elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl).

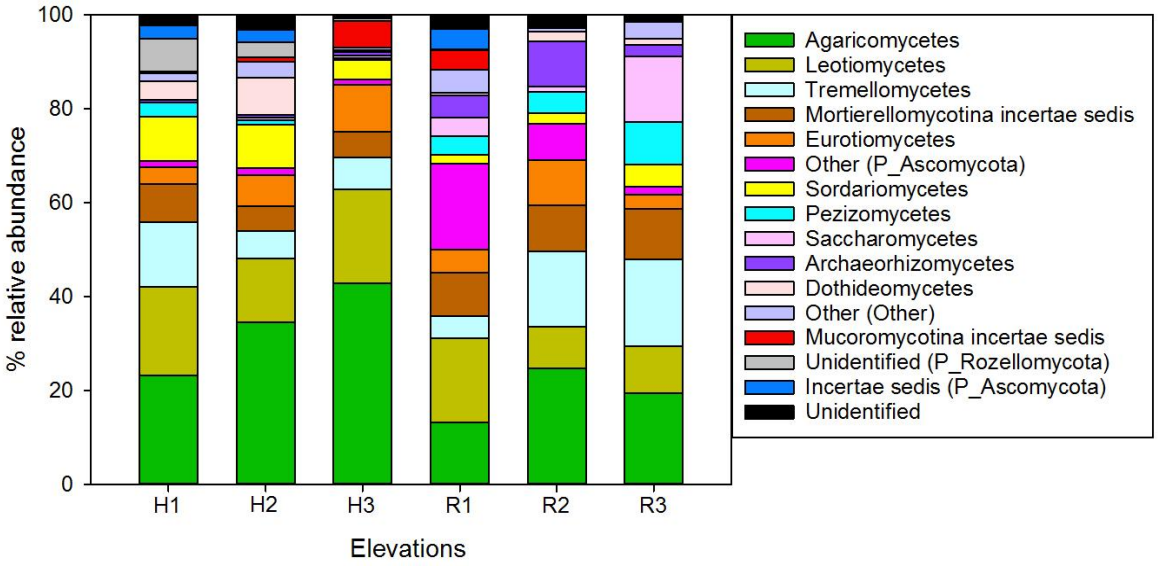

Fig. 13. Relative abundance of top 15 fungal classes at elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl).

4.3.3 Functional categories based on trophic mode of fungal communities

In both the alpine sites, seven recognized fungal trophic levels were found which included; pathotroph, pathotroph-saprotroph, pathotroph-saprotroph-symbiotroph, pathotroph-symbiotroph, saprotroph, saprotroph-symbiotroph and symbiotroph, considering the OTUs ranked as probable and highly probable at the confidence level recommended in the FUNGuild user manual V1.0. OTUs with possible and unassigned confidence ranking were excluded from the analysis. Saprotrophic mode dominated in alkaline site at high and mid elevation levels, while in acidic site similar dominance occurred only at the high elevation level. At low elevation level in both sites, symbiotrophic mode was most abundant (Fig. 14). Dominant trophic mode amongst Ascomycota was saprotrophic while amongst Basidiomycota was symbiotrophic at both sites.

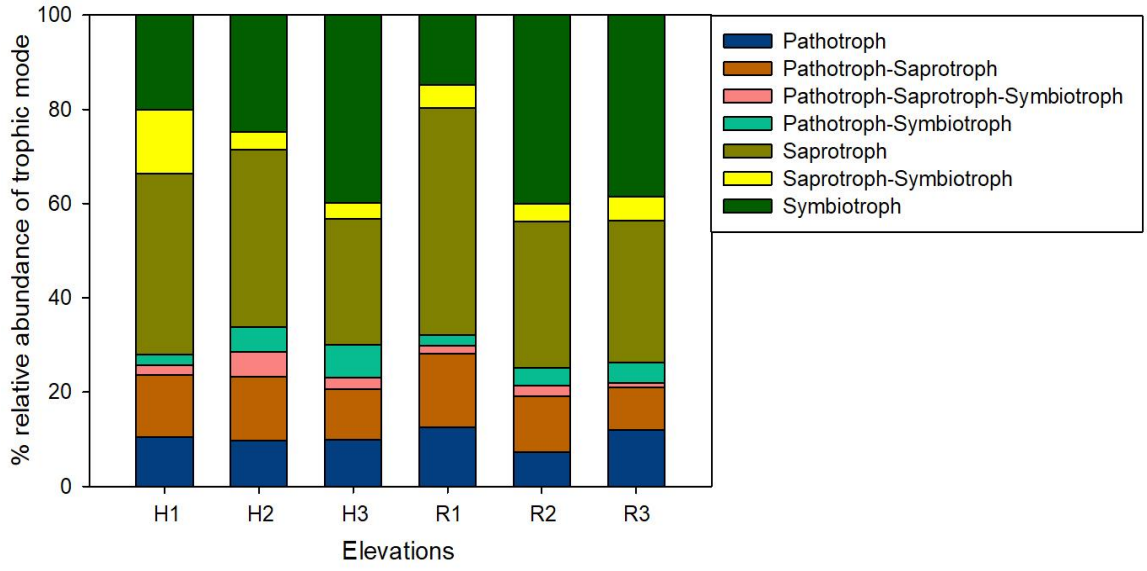

Fig. 14. Relative abundance of fungal trophic mode along elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl), excluding OTUs unassigned at trophic level.

4.3.4 Effects of elevation and depth gradients on diversity of fungal community structure

ANOSIM analysis demonstrated that OTU based fungal community structure significantly varied by elevations (R = 0.98, p = 0.001) but not by depths (R = 0.04, p = 0.07). In concordance with these results, visualisation of the Bray-Curtis distances on NMDS scaling plot indicated significant variability across elevations further grouping samples in five clusters by elevation levels across the two sites (Fig. 15). The single grouped cluster comprised of samples from mid and low elevation levels of acidic site. While soils from similar climatic biomes harboured similar fungal communities as samples of different depths within single elevation grouped together as single cluster at the alkaline site.

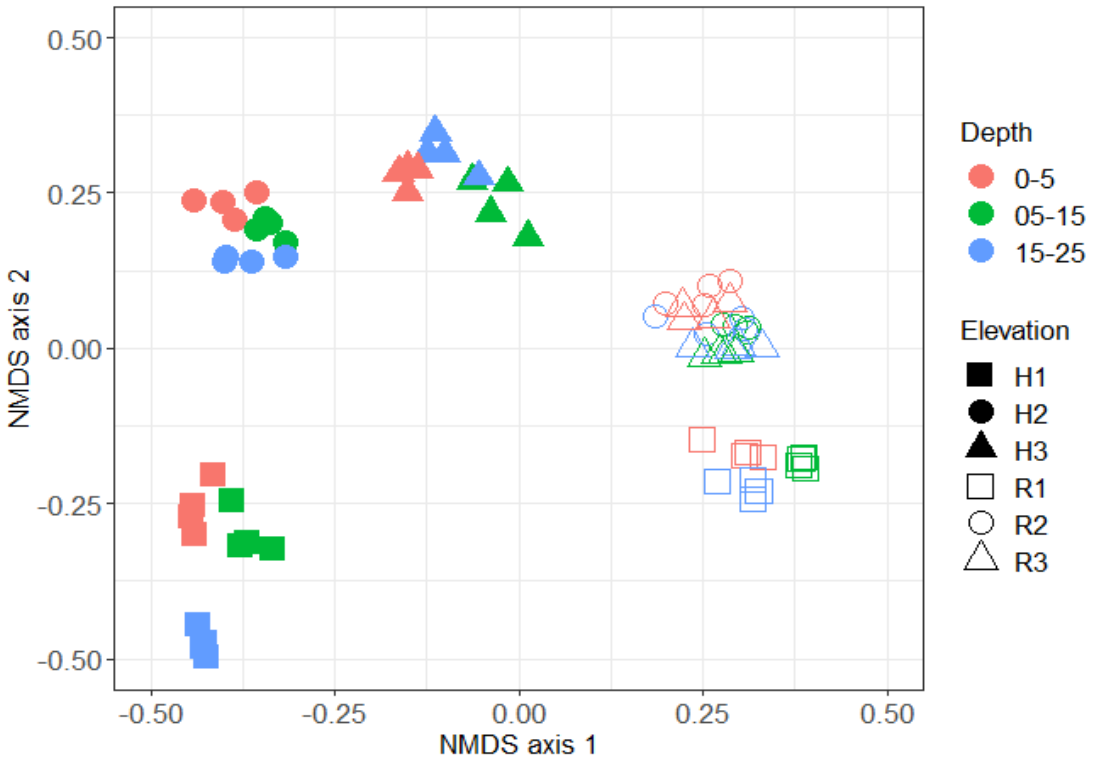

Fig. 15. Nonmetric multi-dimensional scaling (NMDS) ordination of fungal community structure based on Bray-Curtis similarities at elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl).

4.3.5 Factors affecting fungal community structure and correlations with fungal indices

Our correlation analysis included elevations as environmental parameter as it is related to range of variables that affect ecosystem properties like temperature, precipitation, and vegetation cover. Significant high correlations occurred between environmental factors (elevation, MAT and MAP) and fungal OTU based Bray-Curtis distances in Mantel test results (Table 9), especially at the alkaline site. In alkaline site, among all soil properties, soil C/N ratio correlated the most with fungal community structure but not at the acidic site. In the multiple correlation analysis of top 10 most abundant fungal classes and the environmental, soil properties and fungal indices; elevation, MAP, pH and Fungal PLFA positively correlated with 4 fungal classes *(*Agaricomycetes, Eurotiomycetes, Saccharomycetes and Archaeorhizomycetes) in alkaline site. Such correlation occurred only for 2 fungal classes (Tremellomycetes and Pezizomycetes) in the acidic site.

Table 9. Mantel test results considering fungal community structure, environmental and soil variables in the two alpine ecosystem sites.

Factors	Fungal community structure	
	Alkaline site	Acidic site
Elevation	0.86	0.70
Depth	0.11	0.40
MAT	0.86	0.44
MAP	0.86	0.44
Soil pH	0.44	0.33
SOC	0.24	0.25
TN	Ns	0.27
C/N	0.63	ns
Overall	0.14	ns

Values represent statistical significance at $p < 0.05$; Overall, sum of all factors; ns, not significant.

4.4 Discussion

4.4.1 Diversity and composition of fungal communities at elevation and soil depth gradients

The increase in mean fungal 'proxy species' (OTUs/number of sequences) along the elevation gradients in alkaline site Hochschwab as well as acidic site Rauris, is a noteworthy pattern in our study. This is in line with the fungal Rapoport elevational (RE) pattern in a Japanese high mountain (Ogwu et al., 2019), and fungal abundance and diversity patterns in the Northern Austrian (Djukic et al., 2010b) and the Italian Alps (Siles and Margesin, 2017). Such an increase in fungal sequences with increasing elevations could probably be attributed to: i) better adaptability of fungi to cold environments (Djukic et al., 2010b) and, ii) changing C resources through changes in vegetation coupled with changes in temperature, precipitation directly or indirectly affecting soil fungi (Fierer et al., 2011; Geml et al., 2017; Okada et al., 2011). While overall higher fungal sequences along elevations in the two sites compared to other European Alps (Italian Alps - Siles and Margesin, 2017) could be due to environmental filtering and interspecific competition in fungal communities (Veter et al., 2013) and also the variations and distribution of microbial properties in these sites (Bhople et al., 2019, Murugan et al., 2019). At depth gradients, the differences in fungal community structure could be in response to changes in C and N availability with soil depths (Vořiškova et al., 2014).

In our study, the significantly higher fungal beta-diversity (richness, evenness) and alpha-diversity indices (Shannon index, Chao-1 and ACE) at mid elevation level in both sites is in agreement with studies linking RE pattern and mid-domain maxima (Ogwu et al., 2019; Sundqvist et al., 2013). However, the higher diversity and OTU richness at mid elevation levels further align with the study of Gómez-Hernández et al. (2012) showing significant pattern in macromycete (Ascomycota and Basidiomycota) fungal community assemblage on account of alterations in temperature, soil water content, vegetation structure and tree species richness at mid altitudes. In between the two sites, the differences in diversity patterns especially at low and mid elevation level forests are attributed to the differences in tree species composition (Ishida et al., 2007; Yang et al., 2017). While also site-specific environmental characteristics may have determined fungal

community composition and distribution (Geml et al., 2017). Spruce and beech locally determine forest fungal communities with more abundance of saprotrophs (SAP) and ectomycorrhiza (EMF) in beech than in spruce stands (Asplund et al., 2019). This further complement our results of higher ergosterol, which is an important membrane component of fungal species like SAP and EMF within Ascomycota and Basidiomycota phyla and in some species formerly placed in Zygomycota phylum (Weete et al., 2010). This is true for low and mid elevation levels in alkaline and acidic sites, where symbiotrophic mode increased and saprotrophic mode decreased. This is in line with the study showing accumulation of EMF at the expense of SAP in presence of spruce trees (Awad et al., 2019).

In alkaline site at low elevation level plant diversity was more (Djukic et al., 2010b), which may have reduced the selective pressure on specific fungal communities and hence may have decreased their attributes (diversity, ergosterol and PLFA based abundance). Whereas in acidic site, low fungal abundance (based on PLFA measurements) and symbiotic nature of fungal communities may have compounded their diversity pattern in soils. This is factual at depth gradients as soils in alkaline site were more homogeneous than in the acidic site. In acidic site, soil physiochemical properties drastically changed along depths especially at 15-25 cm. Such chemical changes create differential habitats for microorganisms and change the vertical distribution of fungal taxa in podzol soil profile (Rosling et al. 2003). At high elevation in both sites, vegetation converged into grassland with less complex litter and less stable and poorly developed soils. Such heterogenous environmental conditions may have allowed fungal growth and caused increased diversity at this elevation. The nearly similar dominance of mycorrhiza and saprotrophic fungi as usually seen in temperate cold soils (Voříškova et al., 2014), might be the reason for no clear pattern among top 10 fungal classes most of which belonged to Ascomycota and Basidiomycota. These two larger fungal groups compete for same substrates but differentially affect decomposition (Bödeker et al., 2016). This justifies their indistinct detection in organic rich alkaline site soils and as well as in less organic acidic site soils. The clustering of soil samples from low and mid elevation levels in acidic site might be attributed due to the overlapping of fundamental niches and interference competition between the fungal communities (Bödeker et al., 2016; Lindahl et al., 2010), against the elevation and substrate-dependent depth partitioning.

4.4.2 Functional attributes of fungal communities along elevation gradients

The dominance of saprotroph and symbiotroph trophic modes mostly sharing soil or plant-wood saprotrophic and, ectomycorrhizal or endophytic guilds, is in line with the dominance and distribution of ectomycorrhiza in cold environments (Ishida et al., 2007, Shi et al., 2014, Siles and Margesin, 2017), while also along short elevational range (Jarvis et al., 2015). The great biodiversity and distribution ability of ectomycorrhizal fungi (Tedersoo et al., 2012) support their increased detection in our study. The high symbiotrophic mode at low and mid elevation level forests in contrast to increased saprotrophic mode at high elevation grasslands in both sites, suggest a possible competition within these groups for C resources which were significantly higher at high elevation level in both sites. The possibility of such competition is evident from a study showing removal of EMF dominance from fungal community which increased relative abundance of free-living fungal groups, whose abundance was earlier compromised due to the presence EMF (Lindahl et al., 2010). Such guild-specificity and higher EMF in sites with higher productivity (in our case low and mid elevation forests) and predominant effects of host plant growth in natural grasslands of Tibetan Plateau were recently demonstrated (Yang et al., 2017).

The variations in distribution of trophic modes among two most abundant phyla Ascomycota and Basidiomycota, is difficult to explain as it may require deeper understanding of the interactions within different fungal groups and associated variations with factors that are changing along the elevation gradients (Phillips et al., 2013). However, the dominance of symbiotroph especially such as EMF belonging to Basidiomycota at low elevation in both sites, especially in the acidic site is relative to the prevalence of EMF trees such as spruce that out-compete SAP (Asplund et al., 2019, Awad et al., 2019).

4.4.3 Factors affecting fungal community structure along elevation gradients and correlations

In agreement to our hypothesis, we found significantly different fungal community structure in the two contrasting Alpine ecosystem sites. These fungal communities differed in taxonomy and functional guilds at different elevation gradients between the two sites. This agrees with our work on understanding fungal abundance (PLFA based)

and residue distribution (amino sugar based), showing higher dominance of fungi in alkaline site than in the acidic site (Chapter 3 of the dissertation). Recent study (Murugan et al., 2019) have demonstrated differential strategies of soil microbial abundance and residue activation in alpine site soils. While former investigations have specifically confirmed significant differences in fungal community composition at different elevations in high mountain environments (Wang et al., 2015, Jarvis et al., 2015, Siles and Margesin, 2017), indicating elevations as key factors in driving variation in fungal community composition. In this regard, despite that elevation is not a direct environmental variable by itself, but elevation induced changes in vegetation, regulate soil microbial communities' structure (Sundqvist et al., 2013, Siles and Margesin, 2017). This comply our results showing significant effects of elevations on fungal community structure in both sites. The significant correlation results of MAT and MAP are in line with previous studies considering strong effects of these climatic factors on composition of soil fungi and their functional guild (Okada et al., 2011; Timling et al., 2014, Yang et al., 2017).

Of multiple soil variables examined, soil pH strongly correlated with fungal community structure in alkaline as well as acidic site. This is line with the altitudinal gradient study in Italian Alps (Siles and Margesin, 2016) and in Tibetan forest systems, where pH determined only alpha diversity and not the beta diversity of soil fungal communities (Wang et al., 2015). This may be due to the ambiguous role of pH in determining fungal diversity patterns. Rousk et al. (2010) demonstrated that pH had weak effects on fungal diversity while Bååth and Anderson (2003) observed strong correlations in bacterial and fungal diversity with increasing pH gradient. The fungal community composition was seen to be closely related with soil organic matter (SOM) characteristics such as carbon and nitrogen and its quality (Siles and Margesin, 2016). Our results in NMDS plot showing clustering of samples according to the vegetation zones confirm correlation of fungal community structure with SOC in both sites. However, Agaricomycetes and Archaeorhizomycetes in both sites and Mortierellomycotina incertae sedis in alkaline site and Leotiomycetes in acidic site (all of which are fast growing saprotrophic fungal forms), negatively correlated with SOC. This may possibly be due to the limitations in these fungal members to grow despite high SOC contents, especially in the alkaline site. While in acidic site this may be because of the poor quality of spruce

litter (Djukic et al., 2010b). Like the study of Jarvis et al. (2015), soil C/N ratio strongly correlated with fungal community structure only in the alkaline site. The absence of no correlation in acidic site indicate higher recalcitrance of needle litter resulting in greater C sequestration and low nutrient immobilization rates (Siles and Margesin, 2016). The variation in main fungal classes and their driving factors in two contrasting alpine sites demonstrate that their shifts are driven by complex microbial interaction with elevation related environmental factors.

4.5 Conclusion

The study highlights that while comparing two alpine ecosystem sites differing in parent material and forest vegetation cover at low and mid altitude levels (spruce/beech forests at alkaline site *vs*. spruce forests at acidic site), elevations and soil physiochemical properties had significant effects on fungal diversity. The depth effect mattered strongly only in the acidic site presumably due to clear separate soil horizons. The investigation on variations in fungal community structure revealed interesting pattern of increase in elevation range in fungal OTUs/sequences but diversity maxima only in the mid elevation level in both alkaline and acidic sites. The existence of differential abundance of fungal phylum and classes in corresponding climatic zones within two sites is intriguing which further differed in trophic modes. This indicates ectomycorrhiza (EMF) and saprotrophs (SAP) as major players in the studied alpine ecosystems. This may lead to improved understanding in fungal ecology and their ability to respond functionally to discreet habitat changes. The correlation analysis showed that environmental factors had significant effects on overall fungal diversity while edaphic factors (soil pH, SOC) displayed less stronger effects. The variation in main fungal classes and their driving factors in two contrasting alpine sites demonstrate that their shifts are driven by complex microbial interaction with elevation related environmental factors. To deepen the understanding on transition in fungal communities along the elevation and depth gradients in contrasting alpine ecosystems, the current data should be complimented with more accurate functional analysis of metatranscriptome or metaproteome and reveal elevational range changes in physiology of individual taxa including the mycorrhizal and saprotrophic extent.

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Authors contributions

R.M. and R.G.J. conceived the original research idea. R.M. designed the experiment and was involved in selection of the sampling locations and sample collection along with I.D., F.Z. and P.B. P.B. and Ab.S. carried out the laboratory experimental work. L.A. conducted bioinformatics analysis of the MiSeq data. An.S. provided research facilities. K.K. and S.Z-B gave insights on the initial results and interpretations to P.B. Joint support to the original research idea of R.M. was provided by R.G.J. and S.Z-B. Ad.S. performed ANOVA and calculations of the fungal diversity indices on OTU data and aided in the interpretation of the results. P.B. performed other numerical calculations, designed figures, drafted the tables and wrote the manuscript in consultation with R.G.J. and R.M. R.G.J. and R.M. were in-charge of the overall direction of the project and R.G.J. supported in receiving financial assistance to P.B. at the University of Kassel.

5 General discussion

This PhD thesis characterizes soil and microbial properties along elevation and depth gradients in contrasting Austrian Alpine ecosystem sites, with comparison viewpoint. Therefore, two sites were considered in the present study (Table 1), one formed on alkaline parent material in the Northern Limestone Alps (Hochschwab) and the other on acidic parent material in the Central Alps (Rauris), in Austria. Both sites differed in bedrock material and vegetation (i.e similar climatic conditions), which have interacting effects on soil development and soil microbial properties. Chapter 2 of the dissertation explored soil and microbial biomass C and N dynamics, changes in their relative proportion and important factors influencing microbial parameters in alpine soils in the alkaline (Hochschwab) and acidic (Rauris) sites. The subsequent chapters leveraged the use of natural gradients represented by elevation levels, soil depths, contrasting forest types and parent material to study environmental controls on soil microbial community composition, activity, and residual contribution to soil C storage potential and, fungal community structure in alpine soils of contrasting sites. Thus, the main objective of the work was to investigate how climate change induced shifts in community structure, biomass and residues of soil fungi could explain organic matter turnover as well as carbon (C) and nitrogen (N) dynamics along alpine elevation and depth gradients. The major findings of this dissertation are detailed below.

5.1 Soil organic matter, microbial biomass indices and influencing factors

In chapter 2, the aim of the study was to explore soil and microbial biomass C and N dynamics and to access relative changes in their proportion at the two alpine elevation gradients in acidic and alkaline sites. The results show that SOC stocks significantly increased from low to mid elevation and then again decreased at high elevation level only in alkaline site Hochschwab. A similar pattern in MBC, MBN and ergosterol stocks was also observed. However, the stocks of total N in alkaline site followed that of SOC, total N, MBC, MBN and ergosterol stocks in acidic site Rauris, showing consistent increase with increasing elevations. All stocks at respective elevations were higher in alkaline site except that of MBC at high, and MBN stocks at low and high elevation levels at acidic site Rauris. The concentrations of SOC, total N, MBC, MBN and ergosterol constantly decreased with depths in both sites. The ratio of MBC/SOC was highest at high elevation

in acidic site. Although, MB-C/N ratio was strikingly two times higher in alkaline site especially at low and mid elevation level, it was similar at high elevation in both sites. In comparison to acidic site, the ratio of ergosterol/MBC was three to four times higher in alkaline site Hochschwab. Major variations (66.1-79.2%) in soil microbial parameters were caused by soil properties compared to variations by environmental factors (1.2-1.5%) in both alkaline site Hochschwab and acidic site Rauris.

The higher SOC concentration and high soil C/N ratio in alkaline site Hochschwab compared to acidic site Rauris suggest higher accumulation of less decomposed plant material (Jenkinson et al., 2008; John et al., 2005; Cools et al., 2014). This accumulation was due to higher primary productivity especially at low and mid elevation levels at Hochschwab than that at Rauris. While also in these elevations, less densely packed beech leaf litter at Hochschwab than spruce needle litter at Rauris, promoted aeration and desiccation (Gavazov, 2010) which may have further retarded the microbial decomposition of plant residues. Along with retarded decomposition and maximum primary productivity, suitable climatic conditions for mixed spruce/beech forests at mid elevation level in Hochschwab (Djukic et al., 2010b) generated increased SOC and total N stocks than at Rauris. Positive relationships between MBC and SOC and total N and MBN (Anderson and Domsch, 1993; Anderson and Joergensen, 1997; Joergensen et al., 1995; Zederer et al., 2017) demonstrated follow-up pattern in stocks of MBC and MBN in both sites. Change in vegetation from spruce forest to grassland increased C availability to soil microorganisms (Solly et al., 2014; Murugan et al., 2019) especially with greater magnitude in acidic site, resulting in highest ratios of MBC/SOC at high elevation in acidic site Rauris. The high MB-C/N ratio in alkaline site Hochschwab is attributed the shift in microbial community structure towards fungi (Heuck et al., 2015; Murugan et al., 2019). This further supports the observed high ratio of ergosterol/MBC in alkaline site Hochschwab. Such high ratio was usually observed in presence of higher fungi like saprotrophs (SAP) having smaller fungal hyphae and consequently the increased concentration of ergosterol (Klamer and Bååth, 2004). While also other reasons could exist like accumulation of ergosterol from dead fungal tissue (Joergensen and Wichern, 2008) under specific decomposition conditions in alkaline site or differences within the fungal community. Our results with higher regulation of microbial parameters by soil properties (SOC, total N, soil C/N and pH) suggest less disruptive nature of environmental

factors as seen in earlier studies (Högberg et al., 2007; de Vries et al., 2012). However, with elevation, vegetation changes and consequent litter input and humus form, thus intrinsically connecting to contemporary soil properties. This warranted further investigation of accumulated SOM and retarded microbial decomposition especially in the alkaline site Hochschwab.

5.2 Soil microbial communities, activities and microbial residues

In the preceding Chapter (3), accumulation of non-decomposed plant residue i.e. retarded microbial decomposition in alkaline site Hochschwab contradicts the higher presence of saprotrophic fungi indicated by higher ergosterol/MBC ratio. Generally, in alkaline sites with deciduous trees, availability of plant residues to soil microorganisms is more compared to acidic conifer strands (Anderson and Joergensen, 1997; Bauhus et al., 1998). This should lower the stocks of SOC (Berger and Berger, 2012; Zederer et al., 2017). The results of Chapter 3 of the dissertation investigating the unknown and astonishing phenomenon of retarded plant residue decomposition, showed that microbial residue formation was reduced at all elevation levels in the alkaline site Hochschwab compared to those in acidic site Rauris. The biomass-specific activities of phenoloxidase and peroxidase enzymes were generally reduced in the alkaline site Hochschwab, but not those of the hydrolytic enzymes. An exception was the generally lower level of phosphatase activity in alkaline site. Total PLFA and contribution of fungal PLFA to total PLFA (mol%) was significantly higher in the alkaline site than in the acidic site. Also, in both sites, microbial necromass was dominated by fungi. The fungal/bacterial PLFA and fungal/bacterial necromass C ratios were slightly higher in the alkaline site than in the acidic site. In contrast, Gram positive (G+) bacteria dominated bacterial biomass in both sites but the contribution was slightly higher in the acidic site Rauris.

The low formation of microbial necromass C in alkaline site Hochschwab is apparently caused by a reduction in microbial biomass turnover. Apart from acidity in forest soils (Khan et al., 2016), water scarcity and P limitation (Anderson and Domsch, 1980; Berger and Berger, 2012) especially at Hochschwab might be potential reasons for low microbial necromass formation. Further, water limitation may also have reduced phenoloxidase and peroxidase enzyme activities (Burns et al., 2013). But more important reason could be the absence of microbially available P, Cu, and Mn for oxidases (Bárta

et al., 2010; Sterkenburg et al., 2018; Kaur et al., 2019). Microorganisms seem to form less phosphatase per biomass at lower concentrations of available phosphate-esters to be cleaved (Nannipieri et al., 2011; Margalef et al., 2017) this may have resulted in lower phosphatase activity as seen in the alkaline site. The contribution of fungi to total PLFA depends on the number of PLFA used as indices for fungi and bacteria, which is highly variable (Joergensen and Wichern, 2008). In most cases only linoleic acid (18:2ω6,9) is used as fungal biomarker (Margesin et al., 2009; Zhang et al., 2013; Siles et al., 2016, 2017). In some cases, even general microbial biomarkers, such as the straight saturated PLFAs (14:0 to 18:0) are assigned as bacterial biomarkers (Malik et al., 2016) thus lowering the ratio of fungal to bacterial PLFA like that in acidic site Rauris. In most cases, only firmicutes were assigned as G+ (Margesin et al., 2009; Zhang et al., 2013; Fanin et al., 2018), although actinobacteria are also important G+ bacterial phylum and grouped together with G+ bacteria in the work done in Chapter 3. This may have represented higher contribution of G+ bacteria to soil bacterial biomass and have also conflicted earlier estimations based on G+ bacterial DNA extraction and sequencing approach in alpine soils (Siles et al., 2017). Work done in this Chapter (3) shows interactive effects of bedrock and vegetation on soil development, which leads to inevitable differences in fungal community structure (Purahong et al., 2016; Sterkenburg et al., 2018). This prompted the need to investigate fungal community structure at genomic level in alkaline site Hochschwab and acidic site Rauris.

5.3 Fungal diversity

Fungi generally dominated microbial necromass and biomass in alkaline and acidic sites Hochschwab and Rauris, respectively (Chapter 3). Whereas the disparity in vegetation with increasing elevations in the Alps lead to alterations in quantity and quality of C resources (Djukic et al., 2010b). The passage of plant C resource to fungi differs among fungal groups, for example ectomycorrhizal (EMF) and endomycorrhizal fungi acquire C from host plant roots while saprotrohic fungi (SAP) derive C by decomposing plant material (Ishida et al., 2007, Lindahl et al., 2010). Whereas bedrock and vegetation have interacting effects on soil development and may lead to differences in fungal community structure at two alpine sites. The characterization of fungal community structure using metagenomic sequencing of fungal biomarker (ITS2) gene in soil samples

of 6 elevation levels in Chapter 4 of this dissertation, showed an increase in fungal sequences with increasing elevations, plus a common trend in diversity maxima at mid elevation levels in both sites. The differences in fungal diversity in both sites were more strongly related to environmental factors (elevations, MAT and MAP) than edaphic factors like soil pH and SOC contents. However only in alkaline site Hochschwab, soil C/N ratio also had significant effects on fungal community structure. Overall, the diversity of soil fungi showed phylum Ascomycota > Basidiomycota > Zygomycota. In terms of functional guilds saprotrophism (Ascomycota) and symbiotrophism (Basidiomycota) dominated both sites.

The increase in fungal sequences along elevations could be attributed to better adaptability of fungi to cold environments (Djukic et al., 2010b) and changes in temperature, precipitation, vegetation cover. Such alteration lead to changes in important food reserves, directly or indirectly affecting fungal community (Fierer et al., 2011). At depth gradients, changes in C and N availability (Voříškova et al., 2014) decreased fungal sequences but not at high elevation level in acidic site Rauris. The diversity maxima at mid elevation levels in both sites can be related to the observed changes in temperature, soil water content and tree species richness (Djukic et al., 2010b; Murugan et al., 2019). The dominance of Ascomycota and Basidiomycota phyla corresponds with the significant patterns in these macromycete fungal communities observed along elevation gradients (Gómez-Hernández et al., 2012). Further, spruce and beech locally determine forest fungal communities and in presence of spruce, ectomycorrhizal fungi (EMF) accumulate at the cost of saprotrophic fungi (SAP) (Asplund et al., 2019; Awad et al., 2019). Therefore, the dominance of symbiotrophic EMF belonging to Basidiomycota at low elevation in alkaline site Hochschwab and at mid elevation level in acidic site Rauris was higher. Fungal diversity indices increased at high elevation in both sites, mainly due to the heterogenous environmental conditions in grassland soils (Djukic et al., 2010a). With increasing elevations, vegetation, MAT and MAP change, and hence although elevation is not a direct environmental variable by itself, it significantly governs soil microbial communities' structure (Sundqvist et al., 2013, Siles and Margesin, 2017). Altogether, this work gives better insights on variations in fungal community structure as affected by environmental and soil chemical properties at natural elevations and vertical soil profiles in alpine ecosystems.

6 General conclusions

Jointly my PhD research highlights soil and microbial biomass C and N dynamics as well as factors influencing microbial parameters in high mountain alpine sites soils. In particular, the work points out the contribution of fungi to microbial biomass and necromass formation and, activities by using seven enzymes for two contrasting alpine ecosystem sites along elevation as well as soil depth gradients. The study sites differed only in bedrock and vegetation (i.e. similar climatic conditions). Also, the fungal community structure was characterized and factors affecting the fungal ecology. The findings suggest that triggering fungal community composition may alter patterns of C dynamics pertaining to the higher contribution of fungi to microbial biomass and necromass formation in these alpine site soils. The results of my research presented in preceding chapters have furnished some novel insights and contributed to our understanding of climatic impacts on soil microbial communities especially on fungi and soil driven ecosystem process. The main conclusions spawned according to the specific objectives (Chapter 1.7) are:

(i) In alkaline site Hochschwab, high concentrations of SOC and soil C/N ratio indicate retarded decomposition of plant material than in the acidic site Rauris. This phenomenon is attributed to the interactive differences in microclimate, bedrock, and soil characteristics as well as in physical structure of the litter form. While SOC accumulation at high elevation in both sites, despite the faster root decomposition in grasslands, indicate climatic effects override influence of bedrock and substrate quality on decomposition processes.

(ii) Three to four times higher ratios of ergosterol/MBC at low and mid elevations in alkaline site Hochschwab than acidic site Rauris contradict general assumption of higher contribution of fungi to total microbial biomass with decreasing pH. In-turn constancy in this ratio at high elevation levels in both sites mean that similar vegetation leads to similar microbial community in organic layers under similar climatic conditions independent of bedrock properties. However, regulation of soil microbial parameters more by soil properties (66.1–79.2%) than by environmental factors (1.2–1.5%), present profound control of abiotic factors on variations in microbial community structure in the two alpine elevation gradient sites.

(iii) Microbial biomass turnover was reduced in alkaline site Hochschwab, leading to low formation of microbial necromass C. The strikingly low biomass-specific phosphatase, and exceptionally, phenoloxidase and peroxidase enzyme activities in the alkaline site further support the astonishing retarded microbial decomposition of plant residue. While the higher contribution of fungi to total PLFA and of G+ bacteria to total bacterial PLFA in alkaline and acid sites is a consequence of differential but more appropriate consideration of PLFA biomarkers to specific microbial groups.

(iv) Although the differences in microbial biomass and necromass markers within low and mid elevations were small for individual sites, they were more when sites were compared, indicating interacting effects of bedrock and vegetation at these elevations. While at high grassland elevation, higher contribution of arbuscular mycorrhizal fungi (AMF) to the fungal community, points to the increasing influence of climate induced vegetation change than inherent bedrock properties.

(v) The existence of differential abundance of fungal phylum Ascomycota and Basidiomycota and the corresponding fungal classes belonging to these phyla at similar climatic zones/elevations between the two sites is intriguing. The differences in fungal community structure were further observed in trophic modes, illustrating the key importance of functional role of ectomycorrhiza (EMF) and saprotrophs (SAP) in the studied elevation gradient soils.

(vi) Finally, the variation in main fungal classes and their driving factors in the two contrasting alpine sites Hochschwab and Rauris, suggest that the shifts in fungal communities are strongly driven by complex microbial interaction with elevation related environmental factors.

7 Outlook

The astonishing feature of first study is the retarded microbial decomposition of plant residue in the alkaline site Hochschwab compared to the acidic site Rauris, in presence of high SOC contents, soil C/N and MBC/SOC ratios. Although a combination of low temperature and high precipitation may have retarded microbial decomposition, for a better understating on this phenomenon, limitation of phosphorous (P) and water availability arising from less densely packed litter especially at low and mid elevation spruce/beech dominated forests in alkaline site, needs further experimentation. Likewise, in acidic site Rauris, low C availability to soil microorganisms indicated by low MBC/SOC ratio generally at 15-25 cm depth is presumed to be due to increased presence of aluminium (Al) in soil solution. This is often the case in Podzol soils of acidic forests. Examining the effects of such mineral toxicity to microbial biomass will extend our knowledge about interactive effects of bedrock and vegetation on decomposition process in acidic alpine soils. In further steps, research should also be extended to more elevation levels and deeper soil depths and at more sites spanning similar climate but different bedrock material and vegetation. This would allow to clarify the combined effects of different litter and root C input and soil mineral characteristics under climate change scenario.

From the second study we understand that although moisture limitation may have reduced phosphatase, phenoloxidase and peroxidase enzymes in the alkaline site Hochschwab, compared to those in the acidic site Rauris. However, such an effect was not observed for other biomass-specific hydrolytic enzymes such as the exo-glucanase, exo-chitinase and protease in our second study. This warrants further assessment of whether Cu, Mn, and P limitation contribute for reduced enzyme activities, as it is unlikely that phenoloxidase and peroxidase producing species would be missing in microbial community strongly dominated by fungi. Furthermore, given the differences in bedrock and vegetation composition in alkaline and acidic sites especially at low and mid elevation levels, it will be highly effective to get more information on which fungal species produce oxidases under which environmental conditions. Further, the ambiguity persisting on the usage of PLFA biomarkers for specific microbial groups require critical work with cultured organisms to re-assess the specificity of indicator PLFAs especially those of fungi. Such work might provide a more specific quantitative data on their

biomass estimates that marks significant importance for the stability of soil organic matter (SOM) and turnover rates.

Furthermore, as this research unfolds fungal diversity in elevation and depth gradient soils in Alps future research must substantiate information on functional diversity of soil fungi. If possible, by isolating key fungal groups and understanding their interaction with SOM and numerous enzymes they would produce specially under changing climatic conditions. Also, understanding on transition in fungal communities can be strengthened by metatranscriptomic or metaproteomic techniques with a focus on quantification of the decomposer gene.

The work in my thesis presents important role of fungal communities in essential ecosystem processes such as SOM turnover and C sequestration in high mountain alpine sites under changing climatic conditions. However, it is far from the end of the story. The next research ventures could consider reciprocal soil translocations within and in-between the sites to apprehend crucial shifts in fungal communities and factors driving such shifts. It would help to address home field advantage strategy of soil fungi within and across the alpine sites. This future work can bank on the results presented in this thesis and provide a larger view on response of soil fungal communities to environmental change and their interaction with other ecosystem components and, overall consequences on ecosystem functionality. Nevertheless, the kind of data generated in the present thesis supply substantial information for developing modeling frameworks and understanding soil microbial ecology and ecosystem management and to establish better links between them. This may help to predict more accurately the effects of rising temperatures on soil and microbial dynamics and ecosystem processes as climate change continues. Finally, in line with earlier studies, the work in my thesis furthers the understanding that effects of climate warming in the Alps are impossible to overlook.

8 Supplementary materials

Supplementary Table 1. Mean contents of total PLFA, contribution of fungal and bacterial PLFA as mole percent (mol%) total PLFA, fungal/bacterial PLFA ratio, amino sugars muramic acid (MurN) and fungal glucosamine (Fungal GlcN), over all soil depths at each elevation level in two study sites. Calculations for total PLFA and amino sugar contents based on per gram dry soil.

Elevation	Depth	Total PLFA	Fungal PLFA	Bacterial PLFA	Fungal/ bacterial PLFA	MurN	Fungal GlcN
levels	(cm)	(nmol g^{-1})	(mol% total PLFA)			(mg g^{-1})	
Alkaline site (Hochschwab)							
	0-5	42.7 (6.5)	41.2 (0.5)	35.7 (0.3)	1.15 (0.02)	0.20 (0.04)	11.7 (0.9)
Low	5-15	49.8 (5.1)	43.1 (0.7)	38.4 (0.5)	1.12 (0.03)	0.25 (0.01)	10.9 (0.3)
	15-25	81.9 (8.5)	47.9 (0.1)	35.3 (0.1)	1.36 (0.01)	0.30 (0.01)	8.8 (0.7)
	0-5	81.9 (3.7)	43.8 (0.3)	35.8 (0.3)	1.22 (0.02)	0.30 (0.01)	11.7 (0.8)
Mid	5-15	103.1 (13.1)	47.9 (0.1)	36.4 (0.2)	1.32 (0.01)	0.30 (0.03)	13.7 (1.5)
	15-25	103.1 (3.5)	52.3 (0.4)	33.5 (0.4)	1.56 (0.03)	0.15 (0.02)	6.0 (0.7)
	0-5	115.1 (15.6)	52.4 (3.1)	31.2 (2.0)	1.70 (0.20)	0.35 (0.01)	7.5 (0.3)
High	5-15	93.7 (27.8)	50.3 (14.0)	33.1 (9.4)	1.67 (0.70)	0.40 (0.10)	11.8 (2.9)
	15-25	50.9 (3.3)	50.5 (0.4)	33.2 (0.3)	1.52 (0.03)	0.30 (0.02)	7.5 (1.1)
Acidic site (Rauris)							
	0-5	38.2 (4.1)	41.9 (1.1)	38.1 (0.7)	1.10 (0.05)	0.45 (0.03)	11.5 (1.0)
Low	5-15	22.9 (2.5)	40.2 (1.2)	41.1 (1.2)	0.98 (0.06)	0.15 (0.03)	1.6 (0.3)
	15-25	8.2 (0.8)	37.8 (0.3)	41.8 (0.3)	0.90 (0.01)	0.05 (0.01)	0.3 (0.1)
	0-5	23.7 (1.5)	44.8 (1.3)	34.5 (1.1)	1.30 (0.08)	0.30 (0.10)	11.5 (3.4)
Mid	5-15	25.0 (0.5)	44.3 (0.8)	38.8 (0.8)	1.14 (0.05)	0.10 (0.03)	1.7 (0.2)
	15-25	5.5 (0.5)	37.2 (1.5)	43.6 (1.9)	0.86 (0.07)	0.10 (0.01)	0.6 (0.1)
	0-5	29.4 (14.7)	41.2 (3.2)	35.5 (2.0)	1.17 (0.15)	0.35 (0.06)	8.4 (1.3)
High	5-15	36.1 (2.5)	43.2 (1.0)	37.6 (0.7)	1.15 (0.05)	0.30 (0.06)	3.2 (0.7)
	15-25	3.6 (1.2)	24.5 (1.4)	50.5 (1.9)	0.50 (0.04)	0.10 (0.01)	0.7 (0.1)

Values presented are sample means per depth (n = 4); standard deviations given in brackets.

Supplementary Table 2. Two-way ANOVA R^2 for enzyme classes, PLFA and amino sugars, calculated on dry soil basis in the two study sites.

	Alkaline site (Hochschwab)			Acidic site (Rauris)		
	Elevation	Depth	Interaction	Elevation	Depth	Interaction
PLFA						
Total PLFA	0.25	ns	***	ns	0.91	***
Fungal PLFA	0.28	ns	***	ns	0.91	***
G+ PLFA	ns	ns	***	ns	0.90	**
G- PLFA	0.35	ns	***	ns	0.82	***
Fungal/bacterial PLFA	0.24	ns	ns	ns	0.80	***
Amino sugars						
MurN	0.34	ns	ns	ns	0.77	*
GalN	0.49	ns	*	ns	0.88	**
Fungal GlcN	ns	0.58	*	ns	0.96	***
Microbial C	ns	0.56	ns	ns	0.94	***
Enzymes						
ß-Glucosidase (BGL)	0.53	ns	***	ns	0.70	*
Exoglucanase (EGL)	0.50	ns	*	0.40	0.46	***
Exochitinase (ECH)	ns	0.78	**	ns	0.72	*
Protease (PRO)	ns	ns	ns	0.31	0.52	*
Phosphatase (PHO)	ns	ns	ns	ns	0.86	ns
Phenoloxidase (POX)	ns	0.24	*	ns	0.64	*
Peroxidase (POD)	0.40	0.24	**	ns	ns	ns

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant

Supplementary Table 3. Mantel test results based on Spearman correlations of microbial community composition (distance matrix of relative abundance of PLFAs), microbial residues (distance matrix of relative abundance of fungal and bacterial C residues), and enzyme patterns (distance matrix of standardized enzyme activities) with abiotic and biotic variables in the two study sites.

	Alkaline site (Hochschwab)			Acidic site (Rauris)		
	Low	Mid	High	Low	Mid	High
Microbial community composition						
pH	ns	0.60	ns	0.83	0.64	0.49
SOC	0.54	0.65	0.26	0.69	0.76	0.50
Total N	0.27	0.45	ns	0.73	0.76	0.54
Soil C/N	0.25	0.42	ns	ns	0.78	ns
MBC	ns	0.72	ns	0.69	0.79	0.54
MB-C/N	0.31	ns	0.24	ns	ns	0.45
Ergosterol	0.72	0.92	ns	0.59	0.64	0.52
Fungal C/bacterial C	0.77	ns	ns	0.82	0.90	0.53
Microbial residues	0.75	ns	ns	0.82	0.70	0.51
Enzyme patterns	0.62	0.62	ns	0.83	0.76	0.80
Microbial residues						
pH	ns	ns	ns	0.75	0.90	0.44
SOC	0.71	ns	ns	0.69	0.44	0.88
Total N	0.48	ns	ns	0.75	0.47	0.91
Soil C/N	ns	0.27	ns	ns	0.35	0.69
MBC	ns	ns	ns	0.71	0.52	0.88
MB-C/N	0.22	ns	ns	ns	ns	0.27
Ergosterol	0.54	ns	ns	0.60	0.32	0.89
Fungal/bacterial PLFA	0.67	ns	0.38	0.60	0.91	0.48
Microbial community composition	0.75	ns	ns	0.82	0.70	0.51
Enzyme patterns						
pH	ns	0.29	ns	0.73	0.42	0.62
SOC	ns	0.30	ns	0.76	0.78	0.68
Total N	ns	ns	ns	0.81	0.78	0.73
Soil C/N	ns	ns	0.24	ns	0.80	0.30
MBC	ns	0.37	ns	0.74	0.81	0.69
MB-C/N	ns	ns	ns	ns	ns	0.56
Fungal/bacterial PLFA	ns	0.40	ns	0.66	0.36	0.77
Microbial community composition	0.62	0.62	ns	0.83	0.76	0.80

R-values indicate significant relations ($p < 0.05$); ns = not significant.

Supplementary Table 4. Mean activity rates for the hydrolytic enzymes; ß-glucosidase (BGL), Exo-glucanase (EGL), Exo-chitinase (ECH), Protease (PRO) and Phosphatase (PHO), as well as for the oxidative enzymes Phenoloxidase (POX) and Peroxidase (POD) over all soil depths at each elevation levels in the two study sites separately. Calculations based on per gram dry soil.

Elevation	Depth	BGL	EGL	ECH	PRO	PHO	POX	POD
levels	(cm)	(nmol $h^{-1}g^{-1}$)					(µmol $h^{-1}g^{-1}$)	
Alkaline site (Hochschwab)								
Low	0-5	195 (22)	20 (2)	630 (20)	45 (1)	1485 (23)	2.9 (0.2)	0
	5-15	540 (34)	130 (20)	495 (32)	15 (2)	1580 (29)	1.5 (0.3)	0.3 (0.3)
	15-25	300 (23)	40 (12)	460 (26)	20 (3)	1420 (35)	2.6 (0.4)	2.2 (0.1)
Mid	0-5	455 (94)	100 (16)	600 (93)	10 (4)	915 (406)	7.7 (0.6)	0
	5-15	360 (25)	35 (5)	520 (33)	25 (24)	935 (465)	0.9 (0.2)	0.2 (0.2)
	15-25	250 (11)	35 (4)	270 (10)	10 (5)	650 (260)	0.5 (0.3)	0.9 (0.7)
High	0-5	770 (30)	195 (7)	570 (2)	70 (35)	1520 (250)	1.3 (0.6)	1.3 (1.0)
	5-15	760 (19)	240 (15)	510 (26)	45 (26)	1840 (200)	2.6 (0.1)	3.8 (0.5)
	15-25	460 (27)	105 (13)	290 (17)	20 (10)	1330 (510)	2.0 (0.2)	2.9 (0.8)
Acidic site (Rauris)								
Low	0-5	240 (34)	20 (6)	220 (23)	12 (3)	1810 (50)	3.8 (0.7)	4.9 (0.6)
	5-15	40 (02)	03 (1)	35 (10)	05 (1)	710 (30)	6.8 (0.4)	6.9 (0.5)
	15-25	20 (06)	01 (1)	15 (4)	02 (1)	295 (20)	5.2 (0.2)	6.4 (0.3)
Mid	0-5	320 (32)	25 (10)	350 (50)	30 (4)	1670 (30)	2.0 (0.3)	3.5 (0.8)
	5-15	35 (14)	00	30 (5)	12 (1)	490 (22)	8.1 (0.2)	5.9 (0.2)
	15-25	30 (06)	00	25 (4)	10 (1)	265 (40)	2.3 (0.1)	2.2 (0.3)
High	0-5	995 (62)	230 (15)	745 (26)	62 (20)	2590 (75)	6.3 (0.3)	9.3 (0.2)
	5-15	260 (27)	45 (3)	200 (10)	40 (10)	1280 (25)	10.3 (0.4)	11.2 (0.2)
	15-25	60 (07)	04 (1)	65 (2)	5 (3)	470 (30)	5.3 (0.3)	7.0 (0.5)

Values presented are sample means per depth (n = 4); standard deviations given in brackets.

Supplementary Table 5. Mean activity rates for the extracellular enzymes over all soil depths at the three elevation levels in the two study sites. Hydrolytic enzymes include ß-glucosidase (BGL), Exo-glucanase (EGL), Exo-chitinase (ECH), Protease (PRO) and Phosphatase (PHO). Oxidative enzymes include Phenoloxidase (POX) and Peroxidase (POD). Values are calculated on a per gram dry soil basis.

Elevation	BGL	EGL	ECH	PRO	PHO	POX	POD
levels	(nmol h^{-1} g^{-1})					(µmol h^{-1} g^{-1})	
Alkaline site (Hochschwab)							
Overall	450 A	100 A	485 A	30 ns	1295 ns	2.4 B	1.3 B
Low	340 a	60 a	530 a	26 ab	1490 ab	2.3 c	0.8 d
Mid	350 a	56 a	460 a	16 bc	830 bc	3.0 c	0.3 d
High	660 a	180 a	460 a	45 a	1560 a	2.0 c	2.7 c
Acidic site (Rauris)							
Overall	225 B	35 B	190 B	20 ns	1070 ns	5.6 A	6.4 A
Low	100 b	8 b	90 c	6 c	940 abc	5.3 ab	6.1 b
Mid	130 b	8 b	130 bc	18 ab	810 c	4.1 bc	3.9 c
High	440 b	92 b	340 ab	35 ab	1450 ab	7.3 a	9.2 a
CV (± %)	12	27	9	31	14	13	26

Values presented are sample means per site (n = 36) and per elevation (n = 12); mean values followed by capital letters (for Overall) and lower-case letters (for elevation levels) are significantly different according to Tukey's HSD test ($p < 0.05$); CV = mean coefficient of variation between elevation- and depth-specific replicate samples (n = 4); ns = not significant.

Supplementary Table 6. Mean contents of individual bacterial PLFAs in nmol g^{-1} soil basis over all soil depths at each elevation level in two study sites.

Elevation levels	Depth (cm)	i14:0	i15:0	a15:0	i16:0	a17:0	i17:0	cy17:0	cy19:0	16:1ω9	17:1ω8	18:1ω7
		Gram+ bacteria						Gram- bacteria				
Alkaline site (Hochschwab)												
	0-5	0.06 (0.01)	2.84 (0.54)	1.50 (0.27)	1.52 (0.29)	0.07 (0.01)	0.70 (0.10)	0.72 (0.10)	0.22 (0.03)	0.15 (0.02)	1.99 (0.28)	1.11 (0.14)
Low	5-15	0.05 (0.01)	3.46 (0.36)	2.08 (0.23)	2.22 (0.19)	0.10 (0.01)	1.05 (0.11)	1.00 (0.12)	0.33 (0.04)	0.17 (0.02)	1.82 (0.21)	1.46 (0.17)
	15-25	0.06 (0.01)	5.38 (0.64)	3.67 (0.44)	2.96 (0.26)	0.12 (0.02)	1.71 (0.18)	1.78 (0.21)	0.35 (0.05)	0.29 (0.10)	2.99 (0.33)	2.23 (0.26)
	0-5	0.18 (0.03)	5.31 (0.32)	3.35 (0.15)	2.84 (0.14)	0.12 (0.01)	1.29 (0.11)	1.52 (0.05)	0.31 (0.01)	0.38 (0.03)	3.91 (0.17)	1.54 (0.05)
Mid	5-15	0.10 (0.02)	7.74 (1.08)	4.36 (0.60)	3.85 (0.58)	0.12 (0.02)	1.75 (0.25)	2.51 (0.37)	0.46 (0.08)	0.41 (0.08)	4.44 (0.58)	2.37 (0.34)
	15-25	0.08 (0.01)	5.90 (0.19)	4.66 (0.11)	2.91 (0.07)	0.08 (0.02)	1.72 (0.06)	2.77 (0.10)	0.27 (0.04)	0.40 (0.01)	3.38 (0.11)	2.33 (0.09)
	0-5	0.20 (0.06)	4.72 (0.75)	4.14 (0.63)	3.95 (0.52)	0.09 (0.06)	2.11 (0.28)	2.35 (0.32)	0.42 (0.06)	0.33 (0.23)	4.77 (0.67)	2.80 (0.38)
High	5-15	0.10 (0.02)	3.68 (0.60)	3.22 (0.52)	3.10 (0.46)	0.10 (0.02)	1.76 (0.27)	1.81 (0.27)	0.43 (0.05)	0.36 (0.06)	3.78 (0.56)	2.84 (0.41)
	15-25	0.07 (0.00)	1.95 (0.22)	2.23 (0.25)	1.49 (0.10)	0.07 (0.00)	0.98 (0.06)	0.97 (0.07)	0.23 (0.01)	0.20 (0.10)	2.34 (0.15)	1.54 (0.08)
Acidic site (Rauris)												
	0-5	0.01 (0.00)	3.05 (0.40)	1.00 (0.12)	1.47 (0.21)	0.10 (0.01)	0.72 (0.08)	0.77 (0.08)	0.35 (0.04)	0.08 (0.01)	1.37 (0.14)	0.98 (0.11)
Low	5-15	0.00 (0.00)	1.66 (0.20)	0.61 (0.06)	0.91 (0.10)	0.06 (0.01)	0.63 (0.04)	0.66 (0.04)	0.14 (0.01)	0.07 (0.01)	0.71 (0.08)	1.14 (0.05)
	15-25	0.00 (0.00)	0.49 (0.08)	0.22 (0.03)	0.26 (0.04)	0.02 (0.00)	0.29 (0.03)	0.25 (0.03)	0.05 (0.01)	0.04 (0.00)	0.51 (0.05)	0.26 (0.03)
	0-5	0.01 (0.00)	1.67 (0.22)	0.56 (0.07)	0.88 (0.14)	0.03 (0.00)	0.35 (0.02)	0.34 (0.03)	0.20 (0.02)	0.08 (0.02)	0.86 (0.04)	0.47 (0.07)
Mid	5-15	0.01 (0.01)	1.85 (0.08)	0.62 (0.03)	0.70 (0.05)	0.04 (0.01)	0.51 (0.02)	0.53 (0.02)	0.25 (0.03)	0.08 (0.02)	1.45 (0.04)	0.83 (0.06)
	15-25	0.00 (0.00)	0.38 (0.10)	0.18 (0.06)	0.17 (0.02)	0.01 (0.01)	0.15 (0.01)	0.12 (0.02)	0.04 (0.02)	0.04 (0.01)	0.41 (0.02)	0.10 (0.01)
	0-5	0.02 (0.01)	2.24 (1.12)	0.60 (0.30)	1.33 (0.58)	0.02 (0.01)	0.44 (0.20)	0.41 (0.21)	0.17 (0.07)	0.14 (0.05)	0.74 (0.35)	0.76 (0.42)
High	5-15	0.01 (0.01)	2.55 (0.20)	0.80 (0.05)	1.85 (0.13)	0.02 (0.00)	0.75 (0.05)	0.61 (0.04)	0.16 (0.00)	0.20 (0.03)	1.00 (0.08)	1.43 (0.19)
	15-25	0.00 (0.00)	0.30 (0.11)	0.12 (0.04)	0.16 (0.07)	0.01 (0.01)	0.14 (0.05)	0.06 (0.01)	0.01 (0.01)	0.07 (0.04)	0.13 (0.05)	0.05 (0.02)

Values presented are sample means per depth (n = 4); standard deviations given in brackets.

Supplementary Table 7. Mean contents of individual PLFAs in nmol g^{-1} soil basis over all soil depths at each elevation level in two study sites.

Elevation levels	Depth (cm)	10Me 16:0	10Me 17:0	10Me 18:0	14:0	17:0	16:1ω5	18:1ω9	18:2ω6	20:4ω6	15:0	16:0
		Gram+ Actinobacteria			All bacteria		AMF	SAP		Protozoa	Fungi + bacteria	
Alkaline site (Hochschwab)												
	0-5	0.08 (0.01)	1.00 (0.12)	2.02 (0.23)	0.50 (0.11)	0.78 (0.12)	3.54 (0.61)	5.79 (0.86)	8.28 (1.21)	0.31 (0.03)	0.50 (0.08)	9.06 (1.43)
Low	5-15	0.09 (0.01)	1.42 (0.15)	2.43 (0.19)	0.46 (0.05)	0.98 (0.11)	2.98 (0.37)	6.42 (0.70)	12.07 (1.33)	0.32 (0.03)	0.55 (0.05)	8.33 (0.83)
	15-25	0.08 (0.02)	2.06 (0.20)	3.02 (0.27)	0.70 (0.07)	1.51 (0.13)	5.44 (0.68)	9.15 (0.96)	24.70 (2.52)	0.36 (0.06)	0.81 (0.08)	12.61 (1.22)
	0-5	0.19 (0.02)	1.35 (0.05)	3.11 (0.13)	1.73 (0.10)	2.21 (0.10)	9.35 (0.62)	9.55 (0.27)	16.98 (0.97)	0.73 (0.06)	0.85 (0.04)	15.19 (0.69)
Mid	5-15	0.16 (0.03)	2.65 (0.40)	2.49 (0.63)	1.64 (0.22)	2.42 (0.33)	10.85(1.42)	12.30 (1.63)	26.25 (3.35)	0.65 (0.10)	0.79 (0.11)	14.73 (1.82)
	15-25	0.13 (0.03)	3.38 (0.11)	2.75 (0.09)	1.51 (0.05)	2.22 (0.18)	14.37(0.26)	9.93 (0.30)	29.63 (1.28)	0.52 (0.06)	0.62 (0.03)	13.48 (0.66)
	0-5	0.26 (0.04)	3.02 (0.41)	3.51 (0.65)	0.91 (0.18)	2.19 (0.29)	4.53 (0.50)	17.43 (2.26)	38.44 (8.71)	1.05 (0.15)	0.74 (0.10)	17.11 (2.23)
High	5-15	0.21 (0.03)	2.46 (0.39)	3.16 (0.51)	0.70 (0.13)	1.65 (0.25)	4.26 (0.74)	14.72 (2.37)	30.64 (21.12)	0.82 (0.14)	0.59 (0.09)	13.39 (2.09)
	15-25	0.16 (0.01)	1.20 (0.03)	2.06 (0.09)	0.46 (0.09)	0.97 (0.06)	2.97 (0.21)	5.48 (0.32)	17.24 (0.95)	0.51 (0.04)	0.40 (0.06)	7.36 (0.48)
Acidic site (Rauris)												
	0-5	0.02 (0.01)	1.42 (0.19)	2.06 (0.19)	0.34 (0.05)	0.84 (0.08)	2.60 (0.26)	8.36 (0.96)	5.05 (0.67)	0.31 (0.03)	0.42 (0.05)	6.89 (0.72)
Low	5-15	0.02 (0.01)	0.78 (0.06)	1.26 (0.12)	0.16 (0.03)	0.59 (0.05)	1.52 (0.15)	3.69 (0.51)	4.01 (0.60)	0.10 (0.02)	0.21 (0.03)	3.94 (0.44)
	15-25	0.02 (0.01)	0.23 (0.02)	0.50 (0.03)	0.04 (0.01)	0.23 (0.02)	0.58 (0.07)	0.83 (0.08)	1.67 (0.14)	0.04 (0.01)	0.07 (0.01)	1.56 (0.13)
	0-5	0.02 (0.01)	0.78 (0.04)	1.27 (0.07)	0.22 (0.05)	0.47 (0.04)	1.77 (0.12)	5.53 (0.22)	3.32 (0.14)	0.30 (0.03)	0.27 (0.04)	4.35 (0.29)
Mid	5-15	0.03 (0.01)	0.88 (0.04)	1.26 (0.04)	0.20 (0.02)	0.47 (0.02)	1.98 (0.04)	3.20 (0.13)	5.90 (0.23)	0.11 (0.01)	0.22 (0.01)	3.92 (0.07)
	15-25	0.01 (0.00)	0.18 (0.02)	0.42 (0.03)	0.04 (0.02)	0.14 (0.01)	0.40 (0.04)	0.53 (0.01)	1.09 (0.07)	0.03 (0.00)	0.05 (0.01)	0.97 (0.05)
	0-5	0.02 (0.01)	0.95 (0.49)	1.72 (0.68)	0.31 (0.17)	0.36 (0.16)	1.97 (1.07)	6.66 (3.65)	3.83 (1.96)	0.34 (0.17)	0.27 (0.13)	6.12 (2.91)
High	5-15	0.02 (0.01)	1.09 (0.07)	2.09 (0.15)	0.34 (0.03)	0.62 (0.02)	1.92 (0.14)	7.57 (0.46)	6.07 (0.64)	0.23 (0.02)	0.34 (0.03)	6.35 (0.33)
	15-25	0.02 (0.00)	0.09 (0.03)	0.54 (0.19)	0.04 (0.01)	0.10 (0.03)	0.17 (0.05)	0.29 (0.08)	0.42 (0.14)	0.04 (0.02)	0.04 (0.01)	0.82 (0.23)

Values presented are sample means per depth (n = 4); AMF, arbuscular mycorrhizal fungi; SAP, saprotrophic fungi; standard deviations given in brackets.

Supplementary Table 8. Mean contents of microbial biomass C (MBC), total PLFA, fungal indices (fungal glucosamine (GlcN), fungal PLFA, ergosterol, saprotrophic fungi specific - PLFA 18:2ω6) and bacterial indices (muramic acid (MurN) and bacterial PLFA) at each elevation level in two study sites.

Elevation (m asl - level)	MBC* (mg g^{-1})	Total PLFA (nmol g^{-1})	Fungal GlcN (mg g^{-1})	Fungal PLFA (nmol g^{-1})	Ergosterol* (µg g^{-1})	PLFA 18:2ω6 (nmol g^{-1})	MurN (µg g^{-1})	Bacterial PLFA (nmol g^{-1})
Alkaline site (Hochschwab)								
Low	6.43 (1.23)	58.18 (18.89)	10.43 (1.41)	26.12 (10.27)	49.64 (20.47)	15.01 (7.51)	245.99 (40.34)	21.11 (6.40)
Mid	6.41 (2.35)	96.04 (12.71)	10.46 (3.53)	46.41 (8.77)	62.03 (47.74)	24.29 (5.91)	246.32 (70.76)	33.77 (4.36)
High	6.24 (1.74)	86.57 (32.52)	8.92 (2.65)	45.23 (20.11)	23.79 (16.53)	28.77 (15.05)	341.69 (59.51)	27.34 (8.80)
Acidic site (Rauris)								
Low	1.68 (1.76)	23.08 (13.06)	4.47 (5.26)	9.44 (5.64)	4.29 (5.80)	3.58 (1.55)	201.14 (176.23)	9.13 (4.86)
Mid	1.82 (2.12)	18.07 (9.38)	4.58 (5.45)	7.91 (4.36)	8.14 (10.74)	3.44 (2.06)	166.91 (97.47)	6.76 (3.33)
High	3.83 (3.40)	23.03 (16.57)	4.11 (3.46)	9.63 (7.50)	12.04 (13.58)	3.44 (2.66)	223.54 (120.91)	8.55 (5.76)

Values presented are sample means per elevation (n = 12) on per gram dry soil basis; standard deviations given in brackets.

Supplementary Table 9. Mean contents showing contribution of fungal PLFA as mole percent (mol%) total PLFA, fungal glucosamine (Fungal GlcN) as %total amino sugars, and Phosphatase (PHO) as well as for the oxidative enzymes Phenoloxidase (POX) and Peroxidase (POD) over all soil depths at each elevation level in two study sites.

Elevation levels	Depth (cm)	Fungal PLFA	Fungal GlcN	PHO	POX	POD
		(mol% total PLFA)	(% total amino sugars)	(nmol $h^{-1}g^{-1}$)	(µmol $h^{-1}g^{-1}$)	
Alkaline site (Hochschwab)						
	0-5	41.19 (0.24)	68.78 (0.57)	1485.83 (11.58)	2.92 (0.11)	0.00 (0.00)
Low	5-15	43.08 (0.33)	68.02 (0.45)	1580.51 (14.24)	1.44 (0.13)	0.30 (0.13)
	15-25	47.91 (0.05)	66.75 (0.46)	1418.08 (17.59)	2.61 (0.17)	2.19 (0.06)
	0-5	43.77 (0.15)	67.40 (0.30)	915.39 (203.03)	7.70 (0.27)	0.00 (0.00)
Mid	5-15	47.92 (0.06)	71.98 (0.20)	934.47 (232.23)	0.88 (0.07)	0.14 (0.10)
	15-25	52.33 (0.18)	72.23 (0.36)	647.11 (130.62)	0.49 (0.13)	0.87 (0.33)
	0-5	52.38 (1.52)	51.61 (0.24)	1519.98 (123.58)	1.32 (0.29)	1.32 (0.47)
High	5-15	50.31 (6.98)	53.85 (0.47)	1840.07 (99.28)	2.59 (0.03)	3.83 (0.23)
	15-25	50.51 (0.18)	46.87 (0.40)	1330.85 (254.14)	2.01 (0.11)	2.86 (0.39)
Acidic site (Rauris)						
	0-5	41.90 (0.55)	62.24 (0.41)	1812.40 (26.18)	3.82 (0.33)	4.89 (0.29)
Low	5-15	40.24 (0.59)	62.89 (4.41)	706.37 (15.18)	6.83 (0.19)	6.88 (0.23)
	15-25	37.81 (0.14)	48.73 (5.59)	292.77 (09.60)	5.20 (0.11)	6.38 (0.13)
	0-5	44.77 (0.65)	56.61 (0.89)	1667.50 (14.38)	1.98 (0.13)	3.52 (0.39)
Mid	5-15	44.25 (0.42)	60.02 (0.48)	490.35 (10.95)	8.09 (0.11)	5.92 (0.07)
	15-25	37.18 (0.76)	45.20 (1.02)	266.32 (20.13)	2.31 (0.06)	2.17 (0.13)
	0-5	41.24 (1.58)	58.64 (0.11)	2586.39 (37.85)	6.31 (0.12)	9.30 (0.11)
High	5-15	43.17 (0.50)	53.77 (0.52)	1278.90 (12.87)	10.27 (0.21)	11.17 (0.10)
	15-25	41.90 (0.55)	49.67 (0.43)	468.26 (14.56)	5.32 (0.14)	7.03 (0.26)

Values presented are sample means per depth (n = 4); standard error of means given in brackets.

Supplementary Table 10. Basic soil properties along depth gradients at each elevation in the two alpine ecosystem sites.

Elevation (levels)	Depth (cm)	Soil pH	SOC	TN	Soil C/N
			(mg g^{-1} soil)		
Alkaline site (Hochschwab)					
High (H1)	0-5	6.39 (0.14) bc	442 (11) bc	27 (1) a	16.2 (0.3) fgh
	5-15	6.23 (0.16) c	403 (13) cd	28 (0) a	14.5 (0.3) hi
	15-25	6.87 (0.20) b	270 (10) ef	20 (1) b	13.6 (0.1) i
Mid (H2)	0-5	6.05 (0.35) c	495 (16) ab	22 (1) b	23.0 (0.6) b
	5-15	6.51 (0.47) bc	422 (57) cd	21 (3) b	20.5 (0.5) cd
	15-25	7.55 (0.05) a	205 (23) f	7 (1) d	29.0 (1.1) a
Low (H3)	0-5	5.11 (0.14) de	512 (05) a	22 (0) b	23.7 (0.2) b
	5-15	4.60 (0.08) fgh	494 (02) ab	21 (0) b	23.5 (0.4) b
	15-25	5.09 (0.47) de	454 (25) abc	20 (1) b	23.1 (0.4) b
Acidic site (Rauris)					
High (R1)	0-5	4.58 (0.06) fgh	368 (41) d	18 (2) b	20.0 (1.2) de
	5-15	4.74 (0.22) efg	124 (25) g	8 (2) d	15.4 (0.6) gh
	15-25	5.06 (0.05) de	39 (05) h	2 (0) e	15.8 (0.7) gh
Mid (R2)	0-5	4.26 (0.02) hi	281 (63) e	12 (3) c	22.8 (0.9) bc
	5-15	4.48 (0.04) fghi	46 (13) h	3 (1) e	16.7 (0.1) fg
	15-25	5.32 (0.12) d	24 (04) h	1 (0) e	18.0 (0.8) ef
Low (R3)	0-5	4.09 (0.08) j	243 (37) ef	12 (1) c	20.3 (2.2) d
	5-15	4.32 (0.09) ghi	47 (15) h	3 (1) e	15.8 (0.1) gh
	15-25	4.86 (0.05) def	25 (02) h	1 (0) e	19.4 (1.5) de
CV (± %)		3	12	12	3

SOC, Soil organic carbon; TN, total nitrogen; Soil C/N, Soil organic carbon to nitrogen ratio; For each variable, values represent means across sites and sampling depths followed by different letters within a column indicating significant differences according to Tukey's HSD test ($p < 0.05$); CV = mean coefficient of variation between replicate profiles (n = 4).

Supplementary Table 11. Relative abundance of top 10 most abundant fungal classes at elevation gradients in the two alpine ecosystem sites.

		Elevations in alkaline site			Elevations in acidic site			
Class	Phylum	H1	H2	H3	R1	R2	R3	*p*-value
Agaricomycetes	Basidiomycota	23.2 bc A	34.6 a A	42.8 a A	13.2 c B	24.8 b A	19.5 bc A	< 0.001
Leotiomycetes	Ascomycota	19.9 ab A	13.6 c B	20.1 a B	17.9 bc A	8.8 d C	10.0 d BC	< 0.001
Tremellomycetes	Basidiomycota	13.6 a B	5.8 b D	6.8 b D	4.7 b BC	16.1 a B	18.5 a A	< 0.001
Mortierellomycotina incertae sedis	Zygomycota	8.2 ab C	5.3 b D	5.5 b DE	9.2 ab B	9.9 a BC	10.9 a BC	< 0.01
Eurotiomycetes	Ascomycota	3.5 c D	6.7 ab CD	10.0 a C	5.0 bc BC	9.7 a BC	2.9 c E	< 0.001
Other	Ascomycota	1.3 d E	1.5 cd E	1.3 d F	18.2 a A	7.7 b C	1.8 c E	< 0.001
Sordariomycetes	Ascomycota	9.4 a C	9.2 a C	4.1 c E	2.1 d C	2.1 d DE	4.7 bc D	< 0.001
Pezizomycetes	Ascomycota	3.1 bc DE	1.0 cd EF	0.3 d G	3.8 c C	4.6 ab D	9.0 a C	< 0.001
Saccharomycetes	Ascomycota	0.1 d G	0.5 c G	0.6 c G	4.0 b C	1.2 b E	14.0 a B	< 0.001
Archaeorhizomycetes	Ascomycota	0.5 d F	0.7 d FG	0.8 d F	4.7 b BC	9.5 a C	2.6 c E	< 0.001
p-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Values presented are sample means (n = 12) followed by lower case letters within a row indicating significant differences in relative abundances of one fungal class across all elevations; Upper case letters indicate significant differences among different fungal classes within respective elevation based on Kruskal-Wallis rank sum post-hoc test at significance level $p < 0.05$.

Supplementary Table 12. Fungal community diversity properties at soil depth gradients at each elevation in the two alpine ecosystem sites.

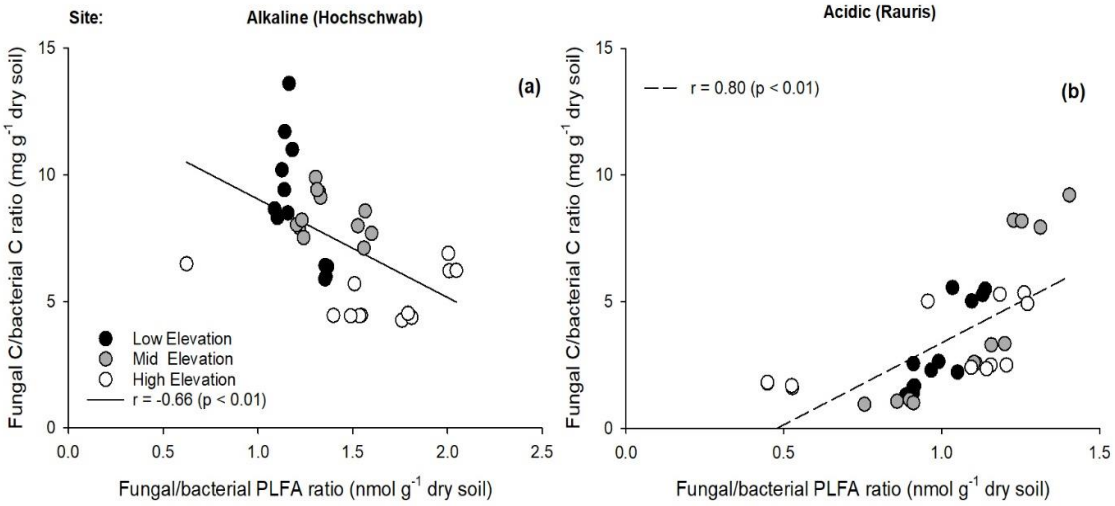
Elevation (levels)	Depth (cm)	No. of sequences	Richness	Evenness	Shannon index	Chao1	ACE
Alkaline site (Hochschwab)							
	0-5	40,768 abc	685 a	0.16 bcd	4.7 ab	800 abc	812 ab
High (H1)	5-15	52,606 a	455 cd	0.08 i	3.6 hi	562 def	561 de
	15-25	24.292 c	371 efg	0.14 cde	4.0 defgh	421 h	414 gh
	0-5	30,441 bc	750 a	0.13 de	4.6 ab	1017 a	1003 a
Mid (H2)	5-15	31,683 bc	576 b	0.09 ghi	3.9 efgh	723 bcd	731 bc
	15-25	44,847 ab	705 a	0.18 b	4.9 a	841 ab	833 ab
	0-5	40,391 abc	389 def	0.10 fghi	3.7 fghi	521 efgh	512 defg
Low (H3)	5-15	34,041 bc	321 gh	0.12 efgh	3.6 fghi	446 fgh	435 fgh
	15-25	30,857 bc	422 cdef	0.13 ef	4.0 defgh	557 defg	560 de
Acidic site (Rauris)							
	0-5	30,755 bc	493 bc	0.17 bc	4.4 bc	628 de	623 cd
High (R1)	5-15	31,180 bc	355 fg	0.22 a	4.3 bcd	424 h	413 gh
	15-25	40,637 abc	295 h	0.07 i	3.1 j	407 h	405 h
	0-5	31,510 bc	452 cd	0.12 efg	4.0 defgh	631 cde	609 cd
Mid (R2)	5-15	37,375 abc	319 gh	0.12 efgh	3.6 ghi	443 fgh	423 gh
	15-25	28,939 bc	428 cde	0.14 cde	4.1 cde	555 defg	542 def
	0-5	36,560 abc	391 def	0.09 hi	3.5 i	559 defg	543 def
Low (R3)	5-15	33,521 bc	313 gh	0.12 efgh	3.6 hi	463 fgh	440 fgh
	15-25	27,096 bc	322 gh	0.13 cde	3.8 efghi	436 gh	445 efgh

Values for diversity properties are means across sites and sampling depths followed by different letters within a column indicating significant differences according to Tukey's HSD test ($p < 0.05$). Data represented are replicate means (n = 4) per depth.

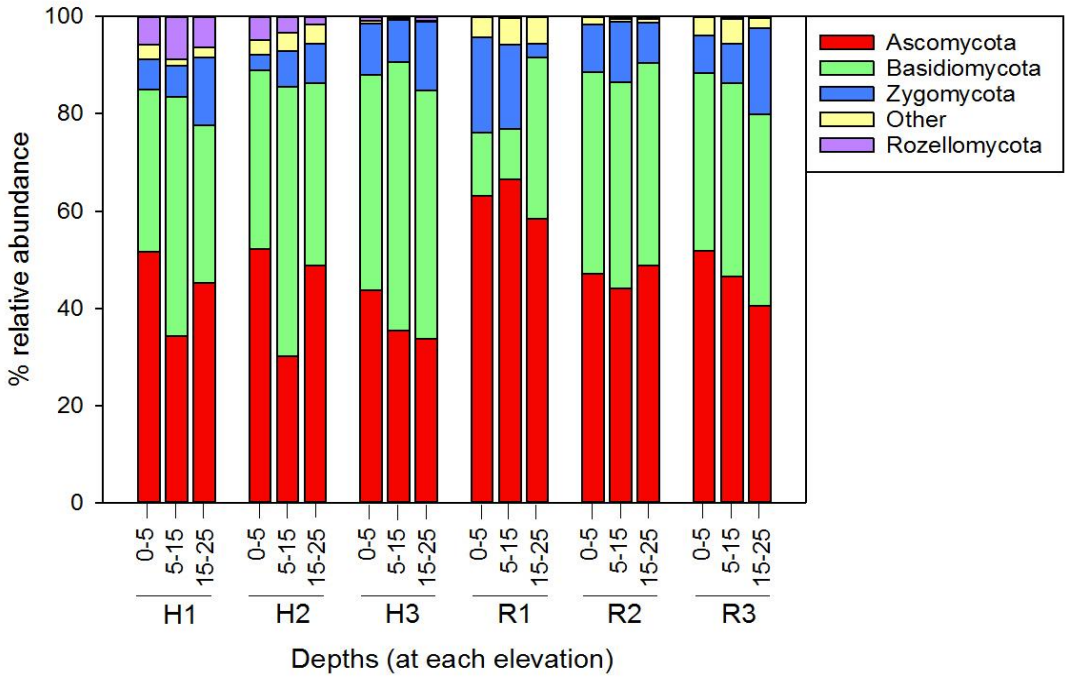
Supplementary Table 13. Multiple correlations between top 10 most abundant fungal classes and environmental and soil variables in the two alpine ecosystem sites.

Fungal class	Fungal Phylum	Elevation	Depth	MAT	MAP	pH	SOC	TN	C/N
					Alkaline site (Hochschwab)				
Agaricomycetes	Basidiomycota	0.66	ns	-0.66	0.66	0.38	-0.47	ns	-0.69
Leotiomycetes	Ascomycota	ns	ns	ns	ns	ns	ns	ns	ns
Tremellomycetes	Basidiomycota	-0.56	ns	0.56	-0.56	-0.58	0.51	ns	0.31
Mortierellomycotina incertae sedis	Zygomycota	-0.33	0.81	0.33	-0.33	ns	-0.30	-0.63	ns
Eurotiomycetes	Ascomycota	0.61	ns	-0.61	0.61	0.58	-0.49	ns	-0.36
Other	Ascomycota	ns	ns	ns	ns	ns	ns	ns	ns
Sordariomycetes	Ascomycota	-0.35	ns	0.35	-0.35	ns	ns	ns	0.65
Pezizomycetes	Ascomycota	-0.48	0.38	0.48	-0.48	ns	ns	-0.46	0.53
Saccharomycetes	Ascomycota	0.54	ns	-0.54	0.54	0.69	-0.66	ns	ns
Archaeorhizomycetes	Ascomycota	0.33	0.70	-0.33	0.33	0.44	-0.77	ns	ns
					Acidic site (Rauris)				
Agaricomycetes	Basidiomycota	ns	0.63	ns	ns	0.71	-0.56	-0.58	ns
Leotiomycetes	Ascomycota	-0.47	0.40	0.47	-0.47	ns	-0.40	-0.38	ns
Tremellomycetes	Basidiomycota	0.71	-0.33	-0.71	0.71	ns	0.47	0.44	ns
Mortierellomycotina incertae sedis	Zygomycota	ns	ns	ns	ns	ns	ns	ns	-0.33
Eurotiomycetes	Ascomycota	ns	-0.51	ns	ns	-0.58	ns	ns	ns
Other	Ascomycota	-0.92	ns	0.92	-0.92	-0.49	ns	ns	ns
Sordariomycetes	Ascomycota	0.60	-0.42	-0.60	0.60	ns	0.64	0.65	ns
Pezizomycetes	Ascomycota	0.48	-0.69	-0.48	0.48	-0.32	0.78	0.80	ns
Saccharomycetes	Ascomycota	0.54	-0.36	-0.54	0.54	ns	0.54	0.57	-0.36
Archaeorhizomycetes	Ascomycota	-0.33	0.56	0.33	-0.33	0.33	-0.70	-0.71	NS

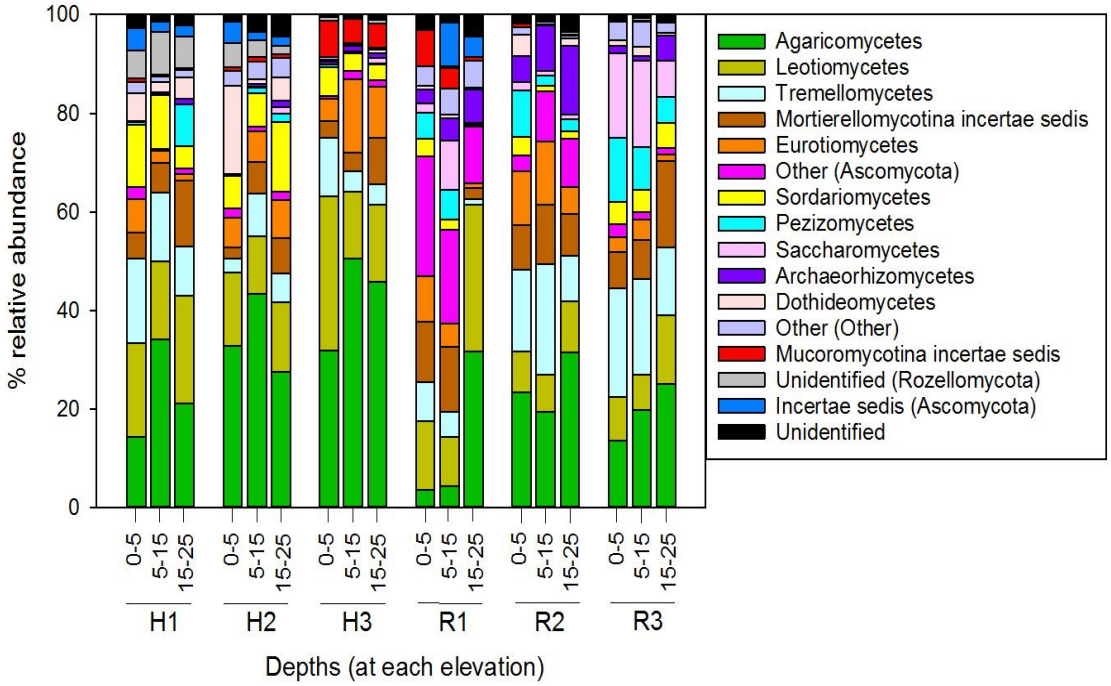
Values indicate statistical significance at $p < 0.05$ level; ns, not significant.



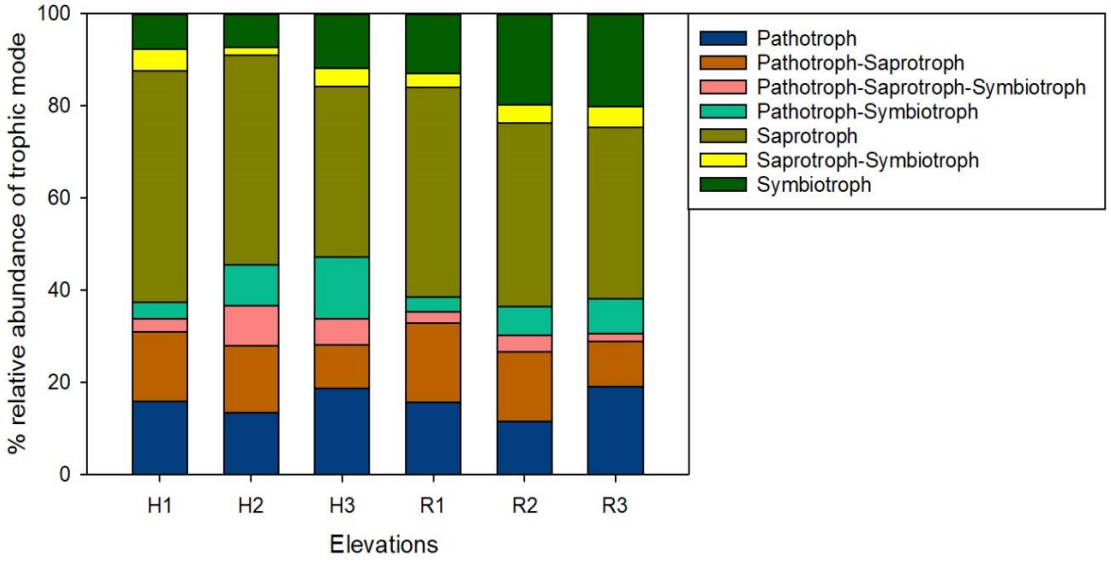
Supplementary Fig. 1. Relationships between PLFA derived fungal/bacterial (F/B) ratio and amino sugar derived fungal C/bacterial C ratio in the alkaline site (a) and in the acidic site (b). Relationships calculated using Spearman's non-parametric test for each site (n = 36 per site) in (a) Alkaline (Hochschwab) and (b) Acidic (Rauris) site, separately; low = 900, mid = 1300, and high = 1900 m asl in alkaline site, as well as low = 1300, mid = 1600, and high = 2100 m asl in acidic site.



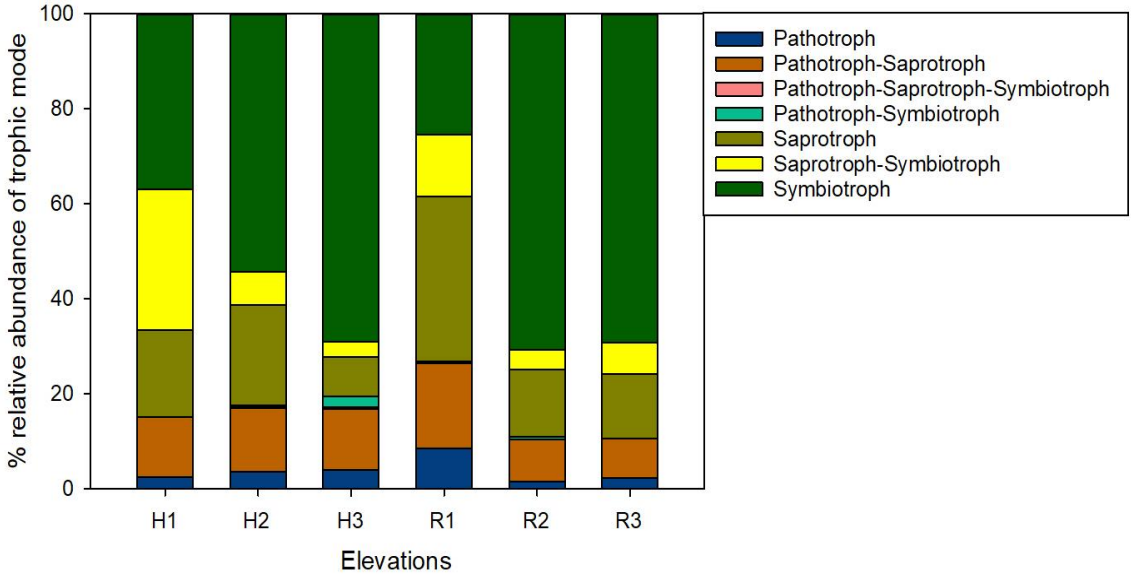
Supplementary Fig. 2. Relative abundance of top 5 fungal phyla at depth gradients (0-5; 05-15; 15-25 cm) in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl).



Supplementary Fig. 3. Relative abundance of top 15 fungal classes at depth gradients (0-5; 05-15; 15-25 cm) in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl).



Supplementary Fig. 4. Relative abundance of trophic mode of Ascomycota along elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl), excluding OTUs unassigned at trophic level.



Supplementary Fig. 5. Relative abundance of trophic mode of Basidiomycota along elevation gradients in alkaline site (Hochschwab) (H1, 1900; H2, 1300; H3, 900 m asl) and acidic site (Rauris) (R1, 2100; R2, 1600; R3, 1300 m asl), excluding OTUs unassigned at trophic level.

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