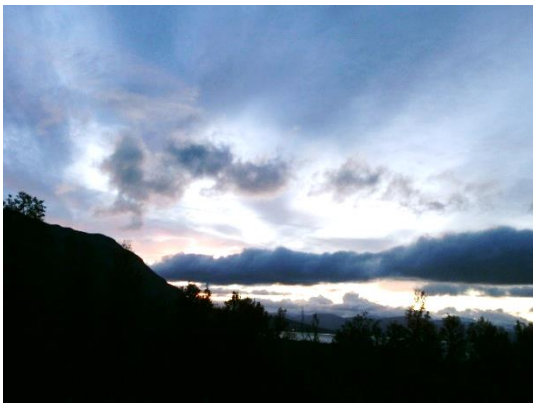
A study of plant-soil-microbe interactions across contrasting treelines in the Peruvian Andes and sub-arctic Sweden



Submitted by Jennifer Michel to the University of Exeter
as a thesis for the degree of
Doctor of Philosophy in Physical Geography
In September 2019

Supervisor at the Centre for Ecology & Hydrology: Dr. Jeanette Whitaker
Supervisor at the University of Exeter: Prof. Dr. Iain Hartley
Internal examiner: Dr. Angela Gallego-Sala
External examiner: Dr. Jens-Arne Subke

I certify that all material which is not my own work has been identified and that no material in this thesis has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……J. Michel……..

Title page includes photographs of (left) sunrise at the Peruvian field site Tres Cruces in the department of Cusco and (right) sunset at the scientific research station near Abisko in Sweden.

I Abstract

This dissertation addresses the question of how plant species shifts would impact carbon cycling in ecosystems, which are likely to soon and strongly be affected by climate change. Examples of such ecosystems are the high latitudes and the high altitudes, where the treeline ecotone can be an early indicator of changes in plant community composition. Biotic changes aboveground also modify belowground processes, particularly carbon (C) and nutrient cycling between plant roots and the assembled microbes. Plant-soil-microbe interactions were therefore studied across treelines in the Peruvian Andes and sub-arctic Sweden.

The first objective was to determine and compare the present soil C and nitrogen (N) stocks and vegetation characteristics through systematic study of a high altitudinal tropical and a sub-arctic treeline. This revealed higher soil C-stocks in the boreal region with potentially also higher microbial activity in summer. For both countries, organic soils were higher in C and N contents compared to the mineral soils. Soils were sampled from both soil horizons across respective treelines and taken to the laboratory to deepen the question of functionality. Microbial mineralisation of soil organic matter (SOM) was quantified in a microcosm soil incubation with addition of substrates of different C:N ratios. Treatment C:N had negligible effect on SOM-mineralisation, which was reduced following substrate addition in the majority of incubations (negative priming). Mechanistically, this questions the N-mining hypothesis and suggests preferential substrate use. For the final data chapter, efforts were made to bring together all three compartments of soils, plants and microbes *in vivo* and study how their interactions mediate carbon and nutrient cycling between them. Negative rhizosphere priming was measured in most soils during the course of the late growing season. This consistent result provides new insights to potential mechanisms of the finely tuned synchronisation of plant-soil-microbe interactions.

In the final discussion, these results were set into context to anticipate what could be done to further our understanding of ecosystem functioning at appropriate scales. Unravelling the interactions of plants, soils and microbes in more detail could help resolve the mechanisms of nutrient cycling and energy flows in different ecosystems and estimate the impact of climate change on the global carbon cycle with less uncertainty.

I.1 List of content

I Abstract **5**
I.1 List of content 6
I.2 List of tables 10
I.3 List of figures 12
I.4 Glossary 15

II Introduction **20**
II.1 Motivation 21
II.2 The global carbon cycle and the importance of soils 22
II.3 Direct and indirect effects of climate change on carbon cycling 24
II.3.1 Direct effects of climate change on soil carbon cycling 25
II.3.2 Indirect effects of climate change on soil carbon cycling 26
II.4 Vegetation shifts in high latitudes and high altitudes 27
II.5 Priming effects (PE) 30
II.6 Rhizosphere priming effects (RPE) 32
II.7 Study sites 33
II.8 Research questions and thesis overview 36

Chapter 1: Taking stock of soil C and N across contrasting treelines in the Peruvian Andes and sub-arctic Sweden **40**

1.1 Abstract 42
1.2 Introduction 43
1.3 Material and methods
1.3.1 Peru soil inventory 46
1.3.2 Sampling across Peruvian and Swedish treelines 48
1.3.3 N - mineralisation 50
1.3.4 Vegetation analysis 51
1.3.5 Soil analysis 51
1.3.6 Statistics 52
1.4 Results

1.4.1 Peruvian soil inventory 53
1.4.2 Comparison of Swedish and Peruvian treeline soils 57
1.4.3 Treeline plant communities 61
1.5 Discussion
1.5.1 Peruvian soil Inventory 65
1.5.2 Comparison of treeline characteristics in Peru and Sweden 67
1.5.3 N-mineralisation 70
1.6 Conclusion 71

Chapter 2: Soil carbon priming across contrasting treelines: The interplay of soil, substrate and microbial C:N ratios? 72

2.1 Abstract 74
2.2 Introduction 75
2.3 Material and methods
2.3.1 Study sites and field sampling 79
2.3.2 Pre-incubation analysis 80
2.3.3 Experimental design 81
2.3.4 Isotopic and source partitioning 84
2.3.5 Statistical analysis 85
2.4 Results
2.4.1 Soil and microbial characteristics 86
2.4.2 Soil respiration 88
2.4.3 CO_2 source partitioning 90
2.4.4 Soil carbon priming 93
2.4.5 The interplay of soil, substrate and microbial C:N ratios 95
2.4.6 Substrate-use and priming 96
2.5 Discussion
2.5.1 Revision of hypothesis 99
2.5.2 General mechanisms of priming effects 101
2.5.3 Potential implications for carbon cycling across treelines 103
2.6 Conclusion 104

Chapter 3: Plant induced rhizosphere priming and microbial mediators in contrasting ecosystems 105

3.1 Abstract 107
3.2 Introduction 108
3.3 Material and methods
3.3.1 Experimental set-up 113
3.3.2 RPE measurements 114
3.3.3 Post-harvest laboratory analysis 115
3.3.4 Calculation of RPE 118
3.3.5 Data uncertainty 119
3.3.6 Statistical analysis 121
3.4 Results
3.4.1 Data uncertainty 123
3.4.2 RPE amongst different soil types 125
3.4.3 Soil and microbial characteristics 128
3.4.4 Environmental and plant parameter 130
3.4.5 Microbial parameters 132
3.4.6 RPE and key soil and microbial parameter 136
3.4.7 Potential drivers of RPE 138
3.5 Discussion
3.5.1 Seasonal RPE *in vivo* (H1) 140
3.5.2 Distinct priming in organic and mineral soils (H2) 144
3.5.3 Microbial community composition (H3) 146
3.5.4 Plant-soil-microbe interactions 148
3.5.5 Data uncertainty 149
3.6 Conclusion 150

III Synthesis and conclusions 151

III.1 Carbon cycling in the Andes and the Arctic
III.1.1 Résumé 152
III.1.2 Patterns and potential mechanisms of carbon turnover 153
III.1.3 Microbial mediators of organic matter mineralisation 158

III.2 Challenges studying C-cycling across ecosystems
III.2.1 Comparing contrasting soils 159
III.2.2 Substrate vs plant experiments 161
III.2.3 Error propagation and uncertainties 165
III.3 Could positive and negative priming synchronise plant-soil-microbe interactions? 166

IV Acknowledgments 170

V Appendices 171
V.1 Methodological protocols
V1-P1: N-resin bag protocol 171
V1-P2: Irrigation protocols 172
V1-P3: PLFA protocol & FAME assignment 178

V.2 Supplemental data for each chapter
V2-C1-A1: Plant species inventory 186

V2-C2-A1: Nutrient solution and pH shifts 200
V2-C2-A2: Cumulative fluxes 202
V2-C2-A3: Case study: microbial priming in contrasting soils 203
V2-C2-A4: Magnitude of PE and substrate use amongst soils 204

V2-C3-A1: In vivo parameter 205
V2-C3-A2: RPE uncertainty in plant-soil incubations 206
V2-C3-A3: Further microbial parameter 209
V2-C3-A4: $\delta^{13}C$ values 211
V2-C3-A5: Detailed model outputs 212

VI Bibliography 216

I I.2 List of tables

Table II.1: Summary of treeline observations from arctic and tropical ecosystems

Table II.2: Key characteristics of the sampling areas in Peru and Sweden

Table 1.1: Introduction of the eight different soil types

Table 1.2: Results of analysis of variance in soil C and N stocks between treeline type (patchy or continuous)

Table 1.3: Comparison of five soil parameters amongst the different soil horizons.

Table 1.4: Edaphic parameters of organic and mineral soils from forest and adjacent uplands in Peru and Sweden.

Table 1.5: Results of three-way ANOVA comparing soil C and N contents (%), dissolved inorganic nitrogen (DIN) and in situ N-mineralisation rates (N-min).

Table 1.6: Plant genera primarily present along transects across Peruvian and Swedish treelines

Table 2.1: Rationale of the experimental set-up

Table 2.2: Composition of the substrate additions: Microbial biomass carbon (mbC, mean ± SE, n = 12) for the different soil types and C and N contents of the substrate additions.

Table 2.3: Key edaphic and microbial properties of the eight soil types studied.

Table 2.4 Comparison of sample means of substrate C respired and primed C for subsetted time intervals (0-7, 8-21 and 0-21 days of incubation).

Table 2.5 Results of analysis of covariance (ANCOVA) with primed C as dependent variable, substrate CN as categorical independent variable with four levels (treatments) and microbial and soil C:N as covariates for each soil type from each country respectively.

Table 2.6: Results of three-way ANCOVAs for the sum of eight individual soils types testing for similarities of sample means of observed priming effects under four different substrate treatments in soils with different soil and microbial C and N peculiarities.

Table 2.7: Modelled factors potentially determining substrate-use ((%), given different soil and microbial C and N properties in soils from Peru (A) and Sweden (B).

Table 2.8: Results of three-way ANCOVA for combined data of Peruvian and Swedish soils testing for differences of sample means of observed priming effects under four different substrate treatments in soils with different soil and microbial C and N peculiarities.

Table 3.1: Uncertainty and data accuracy of RPE estimates for the different incubations

Table 3.2: RPE amongst soil types.

Table 3.3: Key soil and microbial parameters of unplanted soils measured at the end of the experiment.

Table 3.4: Plant parameter determined on live plants: leaf area index (LAI) and photosynthesis (PS: µmol m^2-1 s-1) as measured corresponding to first and second plant-soil incubations to estimate RPE (1: September, 2: October), n =12 per soil type and analysis of leaf and root $^{12/13}$C and N and biomass (dry weight (dwt)) and biomass carbon (C) as determined post- harvest

Table 3.5: Microbial parameters: Left hand side (columns 2 – 4): Microbial C:N and ^{13}C shifts (Δ i.e. the change in value attributed to plant action) and % change in microbial biomass (mb) carbon (C) and nitrogen (N). Right hand side (columns 6 – 13): Change (Δ) to individual groups of assigned biomarkers in PLFAs (µg g^{-1} dwt soil).

Table 3.6: Pearson's product moment correlation of soil and microbial carbon, nitrogen and C:N and fungi to bacteria ratios (F:B) with each estimate of rhizosphere priming as measured during three consecutive plant-soil incubations (RPE 1 - 3),

Table 3.7: Potential drivers of RPE (part 1, 2) and soil respiration with (3a) and without (3b) plants, as determined by generalised linear models.

Table III.1: Summary of findings and their possible interpretations

Table V1-P2.1: Values of soil water retention force (pF) corresponding to the characteristic values of soil moisture

Table V1-P2.2: Summary table of the speed to be applied, for a given humidity measurement

Table V1-P3.1: Calculate the needed amounts before you start

Table V1-P3.2: Final assignment of biomarkers to functional groups and some of their uncertainty.

Table V2-C1.1: Peru: Ecotone plant species composition

Table V2.C1.2: Sweden: Ecotone plant species composition

Table V2-C2.1: Detailed composition of Hoagland's solution No. 2, prepared as 10x diluted stock solution

Table V2-C3.1: ^{13}C values of all plant and soil compartments and respiration measured on the different soil types during the course of the experiment.

Table V2-C3.2: part 1: average RPE

Table V2-C3.3: part 2: RPE 0

Table V2-C3.4: part 3a: Root-soil respiration (CO2)

Table V2-C3.5: part 3b: control soil respiration (CO2_control)

I.3 List of figures

Figure II.1: Direct and indirect effects of the main components considered under climate change (MAT, CO_2, PAR, MAP) on soil organic matter (SOM) mineralisation

Figure II.2: Determining soil and substrate respired-C reveals priming effects (PE): The amount of CO_2-C respired from four substrate-amended soil samples (treatments 1 - 4) and an unamended control soil (red), partitioned between soil (green) and substrate-derived (blue) C

Figure II.3: Location of the two sampling sites in A: Abisko National Park in northern Sweden and B: Manú National Park (Tres Cruces) in the Peruvian Andes (3300 m)

Figure 1.1: A: High latitudinal treeline in Northern Sweden and B: High altitudinal treeline in Southeast Peru

Figure 1.2: Details of soil sampling for the fine-scale inventory of soil C and stocks in the Peruvian Andes

Figure 1.3: Plots covering an area of 225 m2 each were established 15 m above and below treelines in Peru and Sweden (n = 6 each)

Figure 1.4: Peruvian soil inventory: sampling depth (A, D), top soil (O(h) horizon) compaction (B, E) and bulk density (C, F)

Figure 1.5: Peruvian inventory: relative (%) soil carbon (A,C) and nitrogen contents (B,D) of three soil horizons at five sampling locations along 30 m transects leading from forest (5) into adjacent grasslands (1)

Figure 1.6: Peruvian inventory: stocks (kg m -2) of soil carbon (A,C) and nitrogen (B,D) of three soil horizons at five sampling locations along 30 m transects leading from forest (5) into adjacent grasslands (1)

Figure 1.7: Edaphic C and N entities above and below Peruvian and Swedish treelines

Figure 1.8: Ecotone composition (relative cover abundance of plant functional groups) of treelines in the Peruvian Andes

Figure 1.9: Ecotone composition (relative cover abundance of plant functional groups) for three locations across treelines in Peru and Sweden

Figure 2.0: Venn diagram illustrating the experimental approach

Figure 2.1: Land scape at the study sites A: Boreal treeline 200km North the Arctic Circle at field site near Abisko, Sweden and B: high altitudinal treeline 3300 m asl in the Peruvian Andes at Tres Cruces, Peru

Figure 2.2: Fluxes of CO2-C (µg g C-1 hrs-1) from eight soil types under four treatments of glucose-C with increasing N and an untreated control soil (red line), measured during 504 hours (21 days) of controlled laboratory soil incubation

Figure 2.3: Cumulative respired CO2-C (µg g-1 soil C) partitioned between soil and substrate-C for eight soils incubated for 21 days, (presented for 0-7 and 8-21 days of incubation)

Figure 2.4: Primed carbon (µg C g-1 soil carbon) for four soil types from Peru (A) and Sweden (B) under four treatments, all of which included addition of glucose-13C at constant ratio to 30 % of microbial biomass C of respective soil types

Figure 2.5: Correlation between substrate-use (%) and magnitude of priming (relative PE (%))

Figure 2.6: Revision of hypothesis: Expected and observed priming effects

Figure 3.0: Plant-soil mesocosms in the greenhouse

Figure 3.1: Timeline

Figure 3.2: Example of how the uncertainty was calculated

Figure 3.3: Changes in rhizosphere priming effect (RPE) quantified as µg primed C g $^{-1}$ soil carbon in plant-soil incubations for the eight soil types studies at three timepoints

Figure 3.4: Rhizosphere priming effect (RPE 0) quantified as µg primed C g^{-1} soil carbon in root-soil incubations of upper 15 cm of soil columns, where aboveground plant material has been removed instantly before the incubation

Figure 3.5: Differences in microbial biomass carbon (mbC) and nitrogen (mbN, mg g^{-1} soil dwt.) between planted (_plant) and unplanted soils of each soil type in (A) Peru and (B) Sweden

Figure 3.6: PLFAs indicating relative proportions of key functional groups of soil microbes as assigned to biomarkers for fungi, actinomycetes, other gram positive and gram negative bacteria and remaining unspecified PLFAs

Figure III.1: When measuring soil pH, the medium matters

Figure III.2: Correlation of soil C:N and WHC, which is hypothesised to be reflected in the correlation of proportion of microbial biomass C and N extracted and soil physical properties

Figure III.3: (Rhizosphere) priming effects induced by substrate additions and plants / roots for the four Peruvian soil types

Figure III.4: (Rhizosphere) priming effects induced by substrate and plants / roots for the four Swedish soil types

Figure III.5: Exemplary mechanisms and processes in plant-soil-microbe systems and their potential impact on priming effects

Figure III.6: Conceptual idea of plant-soil-microbe synchronisation during plant growth

Figure V1-P1: Preparation of resin bags, cores with bags in the field

Figure V1-P2.1: ESPMe terminal and circuit diagram

Figure V1-P2.2: From top to bottom row: Individual valves, water outlets in the greenhouse and irrigation pins to measure water amounts, final set-up with pots

Figure V2-C2.1: pH shift of four soil types after 3(7) days of adding different test substrates as 1ml solution in water to 5 g soil each

Figure V2-C2.2: Cumulative fluxes (CO2-C ug g dwt) for Peruvian soils during 21 days of incubation

Figure V2-C2.3: Cumulative fluxes (CO2-C ug g dwt) for Swedish soils during 21 days of incubation

Figure V2-C2.4: Priming effects in two contrasting soils: Peru Puna Mineral (PPM) and Sweden forest organic (SFO)

Figure V2-C2.5: PLFAs of PPM and SFO indicating relative proportions of key functional groups of soil microbes as assigned to biomarkers for fungi, actinomycetes, other gram positive and gram negative bacteria and remaining unspecified PLFAs

Figure V2-C2.6: Magnitude of priming effects of the different soils, letters indicate similarities of sample means following ANOVA with post-hoc Tukey test, n = 6

Figure V2-C2.7: Substrate-use (%) of the different soils, letters indicate similarities of sample means following ANOVA with post-hoc Tukey Test, n = 6

Figure V2-C3.1: Greenhouse temperature August – November

Figure V2-C3.2: Adjusted soil moisture, ratio of plant:soil-derived CO2 in headspace and temperature during plant-soil incubations, left: RPE 2, right: RPE 3

Figure V2-C3.3: Uncertainty, theoretical d13C and their test of RPE1 (September)

Figure V2-C3.4: Uncertainty, theoretical d13C and their test of RPE 2 (October)

Figure V2-C3.5: Uncertainty, theoretical d13C and their test of RPE 3 (November)

Figure V2-C3.6: Correlations of microbial C:N and F:B: Different extraction efficiencies depending on soil type (presuming because of different mineral fractions and aggregate stability (clay/silt/sand))

Figure V2-C3.7: Correlation of total PLFAs and microbial C by fumigation

Figure V2-C3.8: Total quantities of PLFAs

Figure V2-C3.9: Potential supporters and antagonists of (negative) rhizosphere priming based on the models in Table 3.7

I.4 Glossary

List of abbreviations, acronyms and terms.

Term	Classification	*Meaning*	*Specification*
°C	unit	degree celsius	temperature
^{12}C	isotope	carbon atom with 6 neutrons and 6 protons	natural abundance: 98.93%, isotope mass: 12 u, M = 144.1284 g mol $^{-1}$ (Hayes, 2004)
^{13}C	isotope	carbon atom with 7 neutrons and 6 protons	natural abundance: 1.109%, isotope mass: 13.003355 u, M = 156.1391 g mol-1 (Hayes, 2004)
A		surface soil beneath O horizon	soil horizon
a.s.l.	abbreviation	above sea level	reference to historic mean
ANCOVA	acronym	analysis of co-variance	
ANOVA	acronym	analysis of variance	
approx.	abbreviation	approximately	
Av.	abbreviation	average	
B		subsoil beneath A horizon	soil horizon
BD	acronym	bulk density	(mass soil - mass roots/rocks) / (volume soil - volume roots / rocks)
C	element	carbon	
C:N	ratio of elements	carbon to nitrogen ratio	
C_3	acronym	molecule of three carbon atoms	used to describe the common type of PS, where the first molecule formed with CO_2-C has 3 atoms of C
C_4	acronym	molecule of four carbon atoms	used to describe a type of PS, where the first molecule formed with CO_2-C has 4 atoms of C
$CHCl_4$	molecule	chloroform	
cm	unit	centimetre	length, 10^{-2} m
cm^3	unit	cubic centimetre	volume, ml
CO_2	molecule	carbon dioxide	
CRDS	acronym	cavity ring-down spectroscopy	method used in determination of isotopic gas concentrations
CUE	acronym	carbon use efficiency	simplified: ratio of measures of microbial allocation of carbon to biomass (growth) and respiration (energy)
d	abbreviation	diameter	as in length
D-(+)	abbreviation	dextrorotatory	optical activity of molecule to rotate the direction of polarized light clockwise
d.a.s.	abbreviation	days after sowing	
d.o.i.	abbreviation	days of incubation	
DIN	acronym	dissolved inorganic nitrogen	NH_4^+ and NO_3^-

DOM	acronym	dissolved organic matter	soluble root exudates, simple sugars and decomposition by-products, less than 5 % of total SOM, < 45 µm (in solution) (E. Griffin)
DON	acronym	dissolved organic nitrogen	soluble organic N as in amino acids, proteins and humic substances
dwt	abbreviation	dry weight	
E	abbreviation	east	geographic direction
element	species of atom	same number of protons	as in periodic table of elements
ESM	acronym	earth system model	comprehensive mathematical representation of material and energy flows between the atmo-, bio-, cryo-, geo-, hydro-, litho- and pedosphere mainly (IPCC, 2013)
EtOH	abbreviation of molecule	ethanol	C_2H_5OH
extra.	abbreviation	extractable	
F	acronym	fumigated	used in method of extracting microbial biomass from soil (causes lysis of microbial cells)
FC	acronym	field capacity	% here: water content when soil is saturated
fwt	abbreviation	fresh weight	
g	SI-unit	gram	10^{-3} kg
GHG	acronym	greenhouse gases	
h	acronym	height	as in length
hrs	unit	hours	time
humus	word	“soil” (Latin)	Older, decayed organic compounds that have resisted decomposition, can be > 50% of total SOM, < 53 µm (E. Griffin)
isotope	isotope	variant of element	same number of protons, different number of neutrons
K	element	potassium	
K_2SO_4	molecule	potassium sulphate	
KCl	molecule	potassium chloride	
kg	SI-unit	kilogram	SI unit for mass
km	unit	kilometre	length 10^3 m
l	unit	litre	volume 10^{-3} m^3
LAI	acronym	leaf area index	leaf surface / ground surface m^2 m^{-2}
M	SI-unit	molar mass	M = m / n = g / mol
m	SI-unit	metre	SI unit for length
m^3	unit	cubic metre	volume 10^3 l
MAOM	acronym	mineral-associated organic matter	
MAP	acronym	mean annual precipitation	
MAT	acronym	mean annual temperature	
max.	abbreviation	maximum	

mb	acronym	microbial biomass	here: amount of lysed cells in soil samples against unlysed controls using the direct extraction method w/ $CHCl_3$ (liq.) and K_2SO_4 (Fierer, 2003)
mbC	abbreviation	microbial biomass C	carbon content of extractable mb
mbCN	abbreviation	microbial biomass C:N ratio	carbon to nitrogen ratio of extractable mb
mbN	abbreviation	microbial biomass N	nitrogen content of extractable mb
MC	acronym	moisture content	%, specified as volumetric or gravimetric
mg	unit	milligram	mass 10^{-3} g
min	abbreviation	mineral	in reference to soil horizon, sub soil
min.	abbreviation	minimum	
ml	unit	millilitre	volume, 10^{-3} l
mm	unit	millimetre	length, 10^{-3} metre
mol	SI-unit	mole	amount of a substance as number of discrete atomic-scale particles (n = 6.02214076×10^{23} particles)
N	element	nitrogen	
N	abbreviation	north	geographic direction
n	abbreviation	number	quantity, number of entities
NaOH	molecule	natriumhydroxide	
NF	acronym	non-fumigated	used in method of extracting microbial biomass from soil
NH_4^+	molecule	ammonium	part of DIN: mineral N
nm	unit	nanometre	10^{-9} metre
NO_3^-	molecule	nitrate	part of DIN: mineral N
NPP	acronym	net primary production	net amount of carbon captured by plants through PS
ns	abbreviation	non-significant	$p > 0.05$
NUE	acronym	nitrogen use efficiency	measure of microbial use of organic nitrogen for biomass (growth) and excess N released through mineralisation
O		organic top soil	soil horizon
Oh		humic organic top soil	soil horizon
OM	acronym	organic matter	as in SOM
org	abbreviation	organic	in reference to soil horizon, top soil
p	abbreviation	probability value	likelihood of an observed result assuming that the null hypothesis is true
P	element	phosphorous	
PAR	acronym	photosynthetic active radiation	solar radiation of 400 to 700 nm
PBD	acronym	Pee Dee Belemnite	carbon-13 standard based on a Cretaceous marine fossil, Belemnitella americana, from the Peedee Formation in South Carolina (Hayes, 2004)

PE	acronym	priming effect	modified rate of microbial mineralisation of SOM in presence of labile substrate
pF	acronym	soil water retention force	log of soil matric potential, used to determine PWP (Hartge, 1978)
PFM	acronym	Peru Forest Mineral	here: soil type
PFO	acronym	Peru Forest Organic	here: soil type
PFT	acronym	plant functional type	loose classification of plants by traits (morphology, physiology, life history, etc. depending on study)
pH	acronym	potential for hydrogen	measure to determine acidity or alkalinity
PKS	acronym	Phosphate-kalium-sulphur	Phosphate-potassium-sulphur
PLFA	acronym	phospholipid fatty acid	biomarker used to estimate functional groups of microbes
POM	acronym	particulate organic matter	fresh or decomposing plant and animal matter with identifiable cell structure, 2–25 % of total SOM, 53 µm–2 mm (E. Griffin)
PPM	acronym	Peru Puna Mineral	here: soil type
PPO	acronym	Peru Puna Organic	here: soil type
PS	acronym	photosynthesis	µmol CO2 m^2 s^{-1}
PWP	acronym	permanent wilting point	critical soil moisture content below which plant vigour is not sustained
R^2	abbreviation	correlation coefficient	here: Pearson product-moment correlation coefficient: measure of linear correlation between two variables
ROM	acronym	resistant organic matter	relatively inert material, such as chemically resistant materials or organic remnants (e.g. charcoal), can be up to 10 % of total SOM, < 53 µm - 2 mm (E. Griffin)
RPE	acronym	rhizosphere priming effect	PE in the rhizosphere
RSD	acronym	relative standard deviation	coefficient of variation, describes the dispersion of frequency distribution
S	abbreviation	south	geographic direction
S	element	sulphur	
SFM	acronym	Sweden Forest Mineral	here: soil type
SFO	acronym	Sweden Forest Organic	here: soil type
SI	acronym	International System of units	
SOC	acronym	soil organic carbon	organic carbon in living organisms and other compounds at different stages of decomposition
SOM	acronym	soil organic matter	complex mix of living, dead and decaying organic materials, can be subsetted into four fractions: DOM, POM, humus, ROM (E. Griffin)
STM	acronym	Sweden Tundra Mineral	here: soil type

STO	acronym	Sweden Tundra Organic	here: soil type
subCN	abbreviation	substrate C:N ratio	
u	unit	dalton	1/12 mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state and at rest
W	abbreviation	west	geographic direction
WHC	acronym	water holding capacity	%
α	abbreviation	alpha, defined significance level	here: 0.05
$\delta^{13}C$	ratio of isotopes	^{13}C:^{12}C of a sample relative to ^{13}C:^{12}C of PBD standard	‰
µg	unit	microgram	mass 10^{-6} g
µl	unit	microliter	volume 10^{-6} l

II Introduction **20**
II.1 Motivation 21
II.2 The global carbon cycle and the importance of soils 22
II.3 Direct and indirect effects of climate change on carbon cycling 24
II.3.1 Direct effects of climate change on soil carbon cycling 25
II.3.2 Indirect effects of climate change on soil carbon cycling 26
II.4 Vegetation shifts in high latitudes and high altitudes 27
II.5 Priming effects (PE) 30
II.6 Rhizosphere priming effects (RPE) 32
II.7 Study sites 33
II.8 Research questions and thesis overview 36

II.1 Motivation

Evidence of observed climate change impacts on terrestrial ecosystems is strongest and most comprehensive for high latitude and high altitude ecosystems. Particularly the Arctic and the Tropics have been shown to be vulnerable to a destabilisation of their ecosystem functioning following changes in climatic conditions in the past, and likely also in the coming decades (IPCC 2007, 2013; ACIA, 2005). In this context, the treeline ecotone is potentially an early indicator of such ecosystem responses. Significant recent vegetation shifts have for example been observed in the Arctic (Tape et al., 2006; Bardgett et al., 2013). Empirical evidence includes widespread warming related upwards dislocation of alpine plant species (Walther et al., 2005; Lenoir et al., 2008), northward expansion of boreal forest (Danby & Hik, 2007a,b) and shrub encroachment in arctic tundra (Wookey et al., 2008). Fewer changes have been documented in the Tropics, for example the up-slope migration of tree species in the Andes (Feeley et al., 2011, 2013). Such large-scale range shifts of aboveground species can impact on belowground processes. Plants take up nutrients from the soil, while they also release diverse cocktails of exudates to the rhizosphere (Walker et al., 2003). Roots and the associated soil microbes hence mediate the belowground cycling of carbon (C), which is manifested and measurable as the carbon dioxide (CO_2) respired by soil microbes. The role of soils and their diverse lifeforms in the global carbon cycle is still not fully understood. Determining their potential impact on global C dynamics is critical, as at present much more C persists in soils than in the atmosphere (IPCC, 2013). However, currently, it is not entirely known how the exchange of carbon between soils, plants and the atmosphere is arbitrated. Understanding the interplay between the physico-chemical properties of the soil and the biotic players above- and belowground is key to advancing our understanding of soil carbon dynamics. This will also improve our ability to make realistic predictions about the possible impact of future climate change on our planets' major biomes. Filling the knowledge gaps about the mechanisms that regulate carbon cycling in dynamic terrestrial ecosystems will help us make wiser decisions for the sustainable management of our natural world and gain a realistic picture of the changes that could occur following climate change in the ecosystems that collectively support our life on Earth.

II.2 The global carbon cycle and the importance of soils

The three main pools that form the global carbon cycle are the atmosphere, terrestrial reservoirs and the oceans (Post et al., 1990; Schlesinger & Andrews, 2000). The terrestrial compartment includes the terrestrial biosphere, as well as geological reservoirs. Within this dynamic, anthropogenic and natural land reformation significantly affect the major biogeochemical cycles on Earth (IPCC, 2013). Meeting the increased demands for energy and food heavily relies on the worlds' soils, which is why they are a key focus of present research (IPCC, 2019). Soils are a major carbon sink and play a crucial role in the global carbon cycle (Jobbagy & Jackson, 2000). Organic matter in litter and soil, together with permafrost soils and old carbon in wetlands and peat are the main repositories of carbon and exceed the amount of carbon stored in vegetation and in the atmosphere by up to three- to fourfold (IPCC, 2013).

The major natural losses of C from soils are through respiration by heterotrophs (fungi, bacteria, soil fauna), with additional losses through autotroph respiration (plants and some photosynthetic bacteria) and as volatile organic compounds (Soil Biology, 1967; Heimann & Reichstein, 2008). Land management practices and climate change induced species shifts can significantly impact on these losses through modifying land cover and the associated plant-soil interactions. Autotroph plants assimilate atmospheric CO_2, with sugars as the primary C-products. A fraction of carbon is fixed in structural molecules in plant tissues above- and belowground. Another fraction of C serves as intrinsic transmitter molecules and plants also excrete C. Aboveground, this can happen in form of plant respiration or excretion of nectar, while belowground root exudates introduce a mix of signal molecules, acids and sugars into the soil (dtv-Atlas zur Biologie, Bd. 2, 1990). This diverse cocktail of root exudates becomes available to soil microbes and mesofauna, together with the inputs from organic material decelerated during plant growth (deDeyn et al., 2011; Vidal et al., 2018). Underneath the earth surface, the soil microbiome is the key driver of carbon turnover, with living and dead organisms contributing to the formation of a considerable pool of carbon and nutrients in soils (Kramer et al., 2012; Kuzyakov & Blagodatskaya, 2015).

Yet, the biogeochemical processes of soil formation, particularly the role of microbial products and turnover, are not fully understood (Liang et al., 2011; Kallenbach et al., 2016) and the mechanisms which determine long-term fixation and stabilisation of organic matter in soil are a topic of ongoing research and scientific debate. Amongst the agreed factors in the process of soil C stabilisation are the inherent recalcitrance of substances, like humic or aliphatic plant residuals, which are unfeasible for microbial degradation, soil mineralogy, like physico-chemical protection of SOM via aggregation and binding on mineral surfaces, and microbial energy and / or nutrient limitations (Marschner et al., 2008; Schmidt, Torn et al., 2011). There is also an emerging understanding that microbial necromass is a considerable pool of C in soils (Liang et al., 2019; Pommer et al., 2019).

Because biological and biochemical processes are not in a steady state in natural ecosystems governed by live plants and a vivid microbiome, reliable modelling of the processes between soil C pools and CO_2-fluxes is a major scientific challenge (Ostle et al., 2009; Chapin et al., 2009; IPCC, 2013; Todd-Brown et al., 2014; Bradford et al., 2016; Bonan et al., 2019). This is particularly demanding in light of coupled carbon and nitrogen dynamics (Cheng et al., 2016; Qiao et al., 2016; Kyker-Snowman et al., 2019). C and N are closely interconnected in all biological life forms (Watson & Crick, 1953), however, in the atmosphere, both elements are uncoupled, as predominantly present in form of the gaseous molecules CO_2, CH_4, N_2O and N_2. The coupling of C and N is mediated by plants, but also through microbial activity, as plants per se are not able to fix atmospheric N (dtv-Atlas zur Biologie, Bd. 2, 1990). Hence, understanding the responses of terrestrial ecosystems to increased C and N concentrations in the atmosphere would benefit from better understanding microbial C and N cycling, as the coupling of both elements could be a rate limiting step in the transfer of C and N from atmosphere to biosphere, mediated by plants, soils and microbes.

A recent technical summary by the IPCC specifically addressed the interactions between climate change and soils (IPCC, TS Climate Change and Land, 2019). The authors underline the necessity to fill in the knowledge gaps remaining in our understanding of the mechanisms of carbon cycling and turnover in soils. The formation of stable soil organic matter is a function of several non-linear processes (Lehmann & Kleber, 2015; Basile-Doelsch et al., 2020). For an accurate

representation of above-belowground C dynamics in Earth system models (ESM), it is crucial to understand the interplay between plant roots and microbes in different soils. Integrating microbial dynamics into terrestrial C models was shown to improve their accuracy (Cotrufo et al., 2013; Wieder et al., 2015; Robertson et al., 2019) and integrating both physical and biological parameters has improved predictions of the temperature response of soil enzymes (Schipper et al., 2014; Arcus et al., 2016, Alster et al., 2016). However, some mechanisms are still widely absent from ESM, such as the effect of labile C inputs on microbial decomposition rates of SOM (priming effect). Unravelling the stochastic and deterministic components of these non-linear feedback processes, particularly for the exchange of carbon and nitrogen between plants, soils and microbes, is important to better understand and predict the effect of climate change on the global carbon cycle and investigate resilient ways of land management in the future.

II.3 Direct and indirect effects of climate change on carbon cycling

Global climate change describes the more rapid modification of weather patterns over decades on circumpolar scales (The International Encyclopaedia of Geography, 2017). The main variables measured to describe such changes are atmospheric CO_2 and other greenhouse gas (GHG) concentrations, temperature, precipitation and solar radiation, and their interrelations (Oreskes, 2004; Lockwood, 2009). Changes, for example in fluctuations of both temperature and precipitation, are unlikely to be globally uniform. Greater than average warming is predicted for high latitudes and high altitudes, together with more extreme weather events in dry lands and monsoon regions (IPPC, 2013), which will affect carbon and nutrient cycling between vegetation and soils on a global scale (IPCC, 2019). The direct effects of the main drivers of climate change on plants include changes to plant productivity and the distribution of individual plant species, plant functional types (PFT) or whole plant communities. The direct effect of climate change on the aboveground vegetation is promptly transferred to the soil beneath, as most plants obtain most of their nutrients through root-soil interactions and leave their litter on the top layers of the soil. These plant-soil interactions are driven by a diverse soil microbiome, which is hence also

subject to climate change (see Section II.4). The interaction of direct and indirect effects of climate change determines ecosystem carbon turnover.

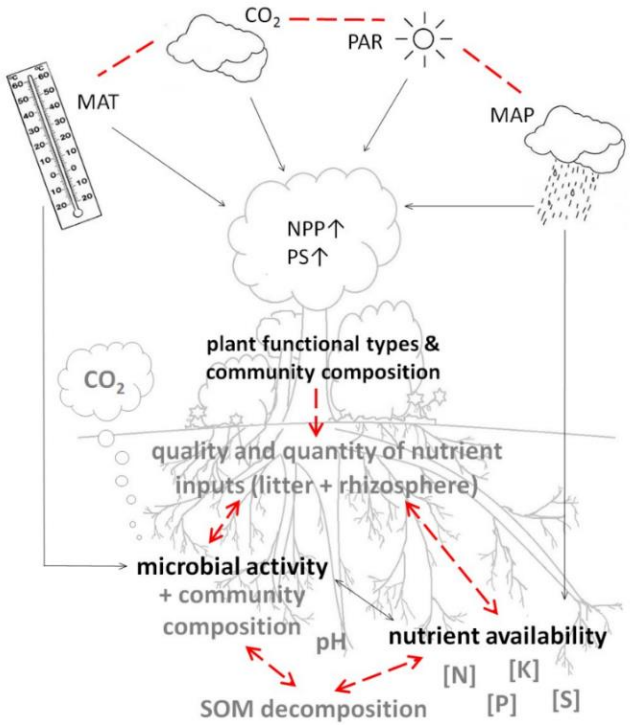


Figure II.1: Direct and indirect effects of the main components considered under climate change (MAT, CO_2, PAR, MAP) on soil organic matter (SOM) mineralisation; the interlinkage of main effects is indicated as red dashed line, direct effects in black with continuous lines, indirect effects in grey with red dashed arrows, Abbreviations: MAT: mean annual temperature,CO_2 : carbon dioxide, PAR: photosynthetic active radiation, MAP: mean annual precipitation, NPP: net primary production, PS: photosynthesis, SOM: soil organic matter, exemplary nutrients: N: nitrogen, P: phosphorous, K: potassium, S: sulphur

II.3.1 Direct effects of climate change on soil carbon cycling

The sum of all CO_2- effluxes originating from the soil is a combination of mainly root respiration (autotroph) and the metabolic activity of the (mainly heterotroph) soil microbiome. The latter comprises soil fauna, as well as bacteria and fungi, who respire CO_2 when metabolising plant litter, root decomposites and soil organic matter. These mineralisation processes are controlled by a set of environmental drivers, mainly mean annual temperature (MAT) and mean annual precipitation (MAP), both historically varying and likely altered in the near or further future (Fig.1; Raich et al., 2006; Heimann & Reichstein, 2008; Davidson & Janssens, 2006; Powers et al., 2009; Wood et al., 2012; Hopkins et al., 2012). Of recent interest are changes in fluctuations and extremes of both temperature and moisture and their impact on terrestrial ecosystems (IPPC, TS, 2019). As with any chemical reaction, the process of decomposition is to different degrees governed by temperature and as a matter of principle should follow kinetic theory. Therefore, temperature has the potential to substantially alter microbial activity in the future and this effect has extensively been

studied (Raich & Schlesinger, 1992; Lloyd & Taylor, 1994; Davidson et al., 1998; Bowden et al., 1998; Fang & Moncrieff, 2001; Rustad et al., 2001; Reichstein et al., 2005; Fang et al., 2006; Hartley et al., 2007; Bradford et al., 2008). Moreover, altered precipitation patterns can modify soils in terms of their physical structure, water content, pH and therewith nutrient availability (Pancotto et al., 2005; Luo and Zhou, 2006). This is likely to also affect the soil microbiome and consequently the processes governed by microbial mineralisation, such as decomposition of soil organic matter.

II.3.2 Indirect effects of climate change on soil carbon cycling

Besides the direct effects of climate change on soil processes, alterations of temperature, precipitation and solar radiation strongly influence vegetation. They can boost net primary productivity (NPP) and therewith litterfall, but also continuously change plant inputs to soils through different timing and composition of rhizodeposition throughout the growing season. Changes to the aboveground biosphere, like altered plant community composition and plant productivity, directly impact belowground nutrient allocation (Fig.II.1; Fitter et al., 1998; Raich et al., 2006; van de Weg et al., 2014). This influences soil microbes and soil C cycling through changes in the quantity and quality of C and nutrients in the soil. The chemical and physical composition and complexity of litter and rhizosphere inputs (e.g. C:N ratios, lignin, phenol) varies according to aboveground plant communities (Constantinides et al., 1994; DeLuca et al., 2002; Pancotto et al., 2005; Steward et al., 2015; Hobbie, 2015). Hence, different plants and plant functional types provide the belowground microbial community with different nutrient sources of different accessibility and suitability for different microbes. This will influence decomposition processes and nutrient cycling within the soil (Whitaker et al., 2014b) and between the above- and belowground compartments. Therefore, aboveground climate driven species shifts will also alter belowground carbon and nutrient cycling.

II.4 Vegetation shifts in high latitudes and high altitudes

An altitudinal formation of a more or less clearly defined border between the forest and the adjacent uplands, sometimes framed by a shrubby transition zone, can be observed in various ecosystems across all continents. After Berdanier (2010) the treeline is the area covered between the timberline, the border of “real” forest with dense tree cover, and the tree species line, the maximum range where individual trees are found. One of the first to describe this phenomena was Alexander von Humboldt, who related the elevational zonation of the vegetation to climate belts in the Andes in 1805 (Kosmos, pub.1845-62). At present, we observe changes in these species distributions on a global scale. As shown in the Arctic (Hartley et al., 2012; Parker et al., 2015), recent climate change has increased the habitability of high latitudinal areas for shrubs and trees. This caused the extension of their distributional range into wider areas in the recent past and was described as “greening” of high latitudinal ecosystems (Tape et al., 2006; Wookey et al. 2008). Many climate models today use the relationship of the location of the treeline and a common isotherm as basis for future predictions (e.g. Körner & Paulsen, 2014), but the consequences of changes in soil carbon storage following such treeline migrations have only recently gained attention. Such changes have the potential to amplify climate change by increasing atmospheric CO_2-concentrations through soil C release or to reduce rates of climate change by promoting C sequestration.

There is evidence for up-slope or northward extension of the treeline in both arctic and tropical ecosystems (Table II.1). Most studies examining upwards “movements” of vegetation were conducted in the northern Hemisphere (Harsch et al., 2009). For the arctic tundra, there is growing evidence of a recent upwards shift of shrubs and trees (Tape et al., 2006; Wookey et al., 2008; Harsch et al., 2009). The advance of the Arctic treeline was shown to have the potential to cause the release of older C previously stored in the soil, which was not fully compensated for by increases in aboveground C stocks (Kammer et al., 2009; Hartley et al., 2012). This is important in relation to global carbon fluxes, as heightened CO_2 effluxes from the soils of the Arctic could be amplified by other processes following warming, such as permafrost thawing and snow- and ice cover melting (Schädel et al., 2018; Turetsky et al., 2019). The observations made along tropical, and especially South American, treelines differ from findings in the northern latitudes. Evidence for upwards expansions is less

clear in the southern hemisphere (Feeley et al., 2011; Körner, 2012), with some studies also reporting downslope shifts of tropical treelines (Harsch et al., 2009; Rehm & Feeley, 2015a). Lutz et al. (2013) analysed 42 years of aerial forest surveys in Peru and found only very little evidence for ecotone upward shifts (18% of the treeline segments examined had shifted upslope), suggesting a barrier to migration for mid-and high-elevation species. The abrupt and pronounced “grass ceiling” that is formed by the relatively stable treeline in the tropics could thus be a possible threat to tropical biodiversity: If forest species cannot colonise upslope areas, they may lack a refugium when climate change renders lower areas uninhabitable or increases competition by invading lowland species (Rehm & Feeley, 2015b). Shifts in plant communities will result in a complex series of biotic cascades and feedbacks which are supplemental to the direct responses of ecosystem components to the primary global change drivers (Wookey et al., 2008).

The reasons why some vegetation types can invade the upper grasslands or tundra, while others cannot, are still being discovered. Climate factors affect the location of the treeline, such as temperature (Bader et al., 2007; Girardin et al., 2010; Conant et al., 2011; van de Weg et al., 2014), frost events (Rehm & Feeley, 2015) and solar radiation (Bader et al., 2007). But the success of seedling establishment in uplands depends not only on climate, but also on soil nutrient and water availability. The treeline can only shift upwards, if the conditions in the new location are favourable for enough components of the ecosystem to still maintain their individual metabolisms. Plant migration requires sufficient rates of growth and proliferation in the new habitat. When species lack the ability for competitive phenotypic plasticity in response to the new conditions, they cannot naturally shift their geographical ranges sufficiently fast to keep up with the rates of climate change (IPCC, 2014). The upward movement of an ecotone can thus be limited by the species with the narrowest tolerance spectrum, unless its ecosystem function can be replaced by another species (Greenwood & Jump, 2014). Hence, plant species types and vegetation community structure are important factors determining treeline extension (Wood et al., 2012; Bardgett et al., 2013; Greenwood et al., 2016). Moreover, herbivory (Metcalfe et al., 2014) and direct anthropogenic modifications, such as forestation, logging, fire, cropping or grazing, can shape the treeline (Gibbon et al., 2010).

Table II.1: Summary of treeline observations from arctic and tropical ecosystems

High latitudes of the sub-Arctic	High altitudes of the Andes
Many treelines advancing in alpine regions, meta-analysis with most study sites in Northern Hemisphere (Harsch et al., 2009)	SA tropical sites (n = 8): no upwards shift of treelines (Harsch et al., 2009)
Growing evidence for pan-Arctic 'greening' (reviewed in Wookey et al., 2008)	Tropic treeline stable/downward despite Climate Change (Körner, 2012; Lutz et al., 2013; Rehm & Feeley, 2015a)
Trees gradually colonising Tundra (Tape et al., 2006)	80% remained stable, 18% of the treeline shifted upslope (42 yr study period in Peru, Lutz et al., 2013)
Upward movement of alpine plant species (Walther et al., 2005; Lenoir et al. 2008)	Presence of tropical treelines at elevations lower than what is predicted, "grass ceiling" (Rehm & Feeley, 2015b)
Northward expansion of boreal forest (Danby & Hik, 2007a,b)	Up-slope warming-related migration of tree species in tropical Andes (Feeley et al., 2011, 2013)
Shrub encroachment in arctic tundra (Wookey et al. 2008)	

II.5 Priming effects (PE)

Microbial mineralisation of organic matter (OM) is a continuous process in all soils. Like all other lifeforms, soil microbes need energy (generally in form of C) and nutrients (N and others) to sustain their metabolism. Heterotrophic soil microbes obtain these from soil organic matter (SOM) and their metabolic activity can be measured as CO_2 emitted from soil. When they are supplied with an extraneous labile form of energy / nutrient this can change their reliance on the degradation of SOM to meet their nutritional requirements. Feeding on the labile substrate can either decrease their mineralisation of SOM or they can use the additionally provided substrate as fuel to degrade even more SOM. The latter case is termed a positive priming effect (PE). It's manifested in increased CO_2-emissions from soil, with carbon derived from both breakdown of added substrate and enhanced mineralisation of SOM ("**real positive priming**", Fig. II.2, Treatment 1). It is also possible, that microbial breakdown of added substrate-C enhances CO_2-respiration through changes in microbial turnover or pool substitution without affecting degradation of SOM ("**apparent priming**", Fig.II.2, Treatments 2 and 3) or with reduced microbial SOM- mineralisation ("**negative priming**", Fig.II.2, Treatments 3 and 4). Finally, supplying an extraneous energy and / or nutrient source can reduce not only soil derived CO_2-C emissions, but also overall microbial respiration ("**negative priming**", Fig.2, Treatment 4). The overlap between these definitions indicates that since the first description of priming effects by Löhnis (1926), Bingemann et al. (1953) and Jenkinson et al. (1985), who defined priming as "added nitrogen interaction", and despite many advances (reviewed in Kuzyakov, 2000, 2010), in-depth functional understanding is still pitted and underlying mechanisms remain unclear.

Mostly, priming effects are studied in controlled laboratory soil incubations as the modification of microbial soil organic matter mineralisation in response to labile C and / or nutrient inputs. Observations include positive and negative priming, but often substrate amendments result in mixed positive, negative and / or no priming (Hartley et al., 2010; Chen et al., 2014; Whitaker et al., 2014b; Qiao et al., 2016; Heitkötter et al., 2017; Hicks et al., 2019; Bastida et al., 2019). It is generally agreed that magnitude, direction and persistence of PEs are governed by the interplay between the identities of the added substrate, the microbial community and the soil itself.

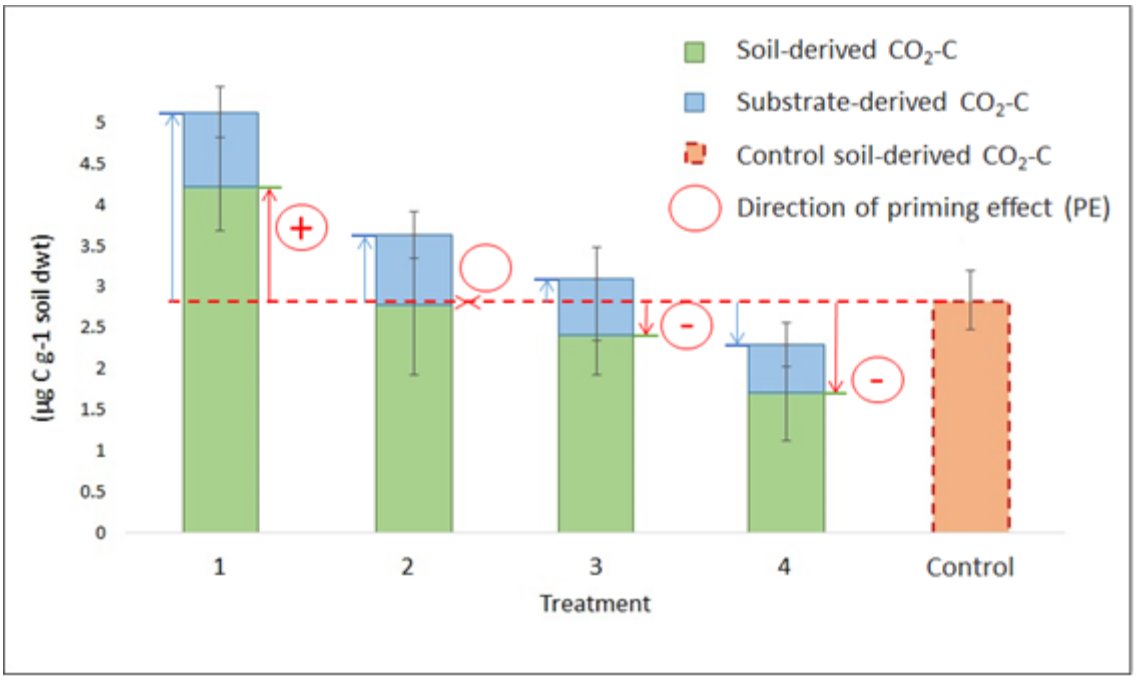


Figure II.2: Determining soil and substrate respired-C reveals priming effects (PE): The amount of CO_2-C respired from four substrate-amended soil samples (treatments 1 - 4) and an unamended control soil (red), partitioned between soil (green) and substrate-derived (blue) C. Blue arrows to the left of each bar indicate difference in total CO_2-C respired between treated soils and the control soil, red arrows to the right indicate the difference in quantity of CO_2-C respired that is derived from soil. The latter is the "priming effect" (PE), which is positive under treatment 1, absent in treatment 2 and negative under treatments 3 and 4

Amount, complexity and frequency of substrate additions were identified as central regulatory steps (Blagodatskaya & Kuzyakov, 2008; Qiao et al., 2014, 2016; Whitaker et al., 2014a; Wang et al., 2015). The structure and functional diversity of the soil microbiome has been addressed in manifold studies (Ayres et al., 2009; Pausas & Paterson, 2011; Fanin et al., 2013, 2015), where recent advance has been made to unravel the different roles of bacteria and fungi (Fontaine et al., 2003, 2011; Nottingham et al., 2009; Rousk et al., 2010; Silva-Sánchez et al., 2019) and the role of dynamic microbial carbon and nitrogen use efficiencies (Mooshammer et al., 2014a,b; Wild et al., 2014; Spohn et al., 2016; Silva-Sánches et al., 2019). Consensus exists, that the soil nutrient status as such is a key determinant for PEs (Jingguo & Bakken,1997; Azam, 2002; Craine et al., 2007; Chen et al., 2014; Wieder et al., 2015; Rousk et al., 2016). PE have been studied under a plethora of different substrate additions in different soils, but they are subject to dynamic microbial and environmental parameters, which together conduct the priming response. Therefore, the underlying mechanisms of soil carbon priming remain elusive, particularly on ecosystem scale, where multiple mechanisms might coexist.

The interaction between C and N availability can drive priming effects, but the proposed mechanisms are contradictory. On the one hand, the microbial (N) mining theory suggests that fresh carbon inputs are used by soil microbes in order to release nutrients previously locked in SOM, especially under low N availability (Moorhead & Sinsabaugh, 2006; Craine & Morrow, 2007; Fontaine et al., 2011). However, this can be hindered when microbes compete with plants for the limiting elements. PEs can even be reversed when plant consumption is outcompeting microbial decomposition (competition hypothesis Cheng, 1999; Meier et al., 2009; Dijkstra et al., 2010). Contrastingly, according to the stoichiometric decomposition theory, high N availability can enhance SOM decomposition, when C and N inputs match microbial nutrient demands (Hessen et al., 2004). However, in soils of high nutrient availability, a negative priming effect can result from the reduced need of microbes to mine nutrients from SOM as they can rely on easily accessible substrates for their carbon and energy requirements (Blagodatskaya et al., 2007; Guenet et al., 2010). Moreover, microbes can specialise on the degradation of distinct substrates complementary to their metabolisms, thus their processing of carbon is dependent on their metabolic preferences and their functional capacity. In such case, the addition of a certain substrate to a soil can have opposing effects independent from the nutrient status, but governed by the preferential substrate utilization of the present microbial community (Cheng, 1999; Blagodatsky et al., 2010; Whitaker et al., 2014b; Nottingham et al., 2015).

II.6 Rhizosphere priming effects (RPE)

Even more complexity accompanies the study of priming effects *in vivo*, when instead of artificial or natural substances, live plants are introduced to soils. When plant derived inputs to soil cause alterations of microbial SOM-degradation, rhizosphere priming effects (RPE) can be observed. In natural ecosystems, vegetation plays a key role in soil C cycling, as plants can alter both chemical and physical properties of the soil and directly interact with belowground biota. The main channels by which plants provide supplies to soil microbes are root exudates, mucilage, root decomposition and litterfall (Shahzad et al., 2012; Clemmensen et al., 2013; Dijkstra et al., 2013; Keiluweit et al., 2015; Carillo et al., 2017). The transfer of nutrients between the soil matrix, the microbial community and plant roots is limited

by a range of chemical and physical factors. These include soil moisture, structure and texture, complexation, aggregation, biochemical recalcitrance, temperature and pH (Lauber et al., 2008; Grandy et al., 2009; Fan et al., 2013; Cotrufo et al., 2013). Plant variables are just as diverse as soils per se, comprising primary production, resource allocation, root activity and species traits (Klumpp & Soussana, 2009; Girardin et al., 2010; Zhu & Cheng, 2011; Chen et al., 2014; Classen et al., 2015; Shahzad et al., 2015). Soil microbes are likely as diverse as plants, but their community composition and dynamics, and their role in soil carbon cycling, have only recently gained detailed attention (Fontaine et al., 2003; Nottingham et al., 2009; Rousk et al., 2010; Silva-Sánchez et al., 2019). Both plants and microbes act in a complex network of cooperation and competition amongst and between them (deBoer et al., 2005; Barea et al., 2005).

II.7 Study sites and soils

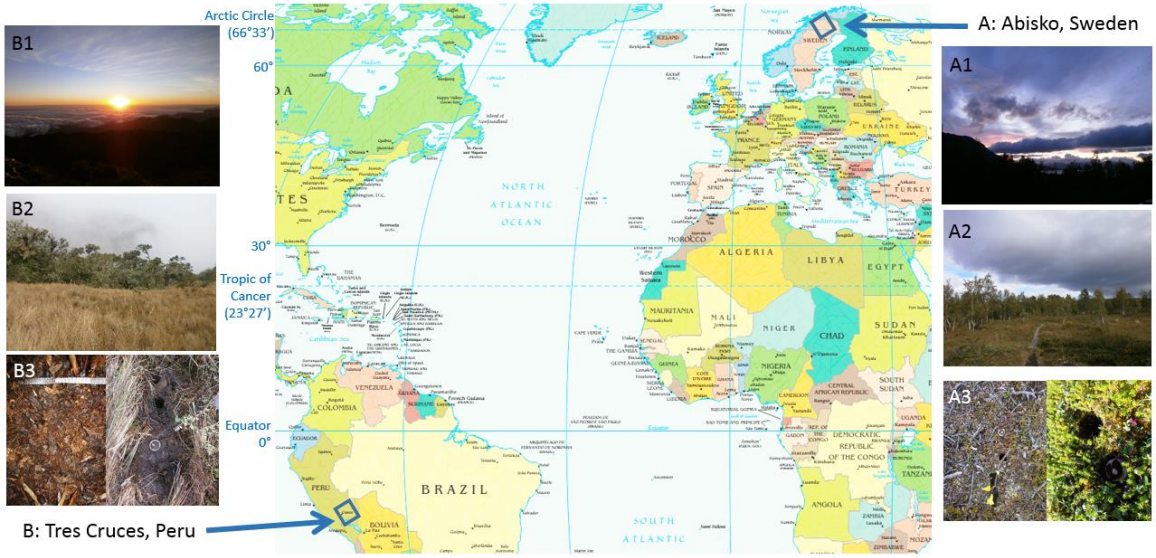


Figure II.3: Location of the two sampling sites in A: Abisko National Park in northern Sweden and B: Manú National Park (Tres Cruces) in the Peruvian Andes (3300 m) with featured sunrise / set (A / B1), ecotone transition from forest to tundra / grassland (A/B2) and characteristic land cover in boreal forest/tundra heath (A3) and high Andean tropical forest / Puna grassland (B3)

To obtain an insight to the carbon stocks and their vulnerability to priming from the ecosystems, that are most likely to be affected by climate change, both a high latitudinal and a high altitudinal field site were chosen for treeline and soil investigation and sampling (Fig. II.3). The high altitudinal study area was located at the upper end of the Kosñipata transect in the Andes in Manú National Park in

eastern Peru (13°07'13.8"S 71°36'41.0"W). Sub-arctic treelines were studied in the National Park near Abisko, approximately 200 km north of the Arctic Circle in northern Sweden (68º21’N, 18º49’E).

When comparing soils from different land covers, such as boreal forest, high Andean tropical forest, Puna grassland and tundra heath, it is important to consider that not only the bases and history of soil formation are very different in these ecosystems, but also the circumstances under which soil organic matter turnover takes place. While on the eastern side of the Andes annual temperature is relatively constant around 12 ºC, precipitation varies strongly throughout the course of the year, with a dry season and rainfall below 10 mm / month from November to March and a wet season peaking in June with rainfall of 140 mm / month. Temperature differences at Manú are distinct between day and night and between forest and Puna (Table II.2, left). In contrast, the sub-arctic climate in Abisko is characterised by a clear seasonality reflected in distinct summer and winter periods. The land receives constant rainfalls, which oscillate around 15 mm / month during the year, with July and August being wetter (60 mm / month). There is regularly snow on the ground until late May and while average temperatures may be a little over 10 ºC in July, by mid-August the average is already declining rapidly with frosts likely by early September. In winter, temperatures can drop down to - 34 ºC. This seasonality results in a clear growing season for most vegetation during the European summer (Table II.2, right).

Table II.2: Key characteristics of the sampling areas in Peru and Sweden

	PERU **Tres Cruces** **Manú National Park**	**SWEDEN** **Abisko** **Abisko National Park**
Land cover type	Montane tropical forest and Puna grassland	Sub-arctic birch forest and tundra heath
Grid reference	S 13°7'16.71" W 71°36'42.00"	N 68°20'4.41" E 18°48'9.92"
Elevation	3300 m asl	650 m asl
Average "summer" temperature	12.15 °C (annually rel. constant, summer every day and winter every night)	10.27 °C (strong summer - winter seasonality)
Average rain-fall / season	dry season Nov - Mar < 10 mm / month, wet season peaking in June ~140 mm / month	annually rel. constant ~ 15 mm / month, with more rain July - August ~ 60 mm / month
Forest soil horizons	Oh 20 – 30 cm with dense root network and partly decomposed plant litter, organic-rich humic Ah horizon ~17 cm and mineral B horizon ~ 45 cm, pH: 4.23 (organic), 4.5 (mineral)	Thin O horizon ~3 cm, Ae horizon ~4 cm, stony B/C horizon (glacial till), pH: 5.13 (organic), 5.7 (mineral)
Grassland / tundra soil horizons	Mainly dark, organic-rich A horizon with high clay content ~10 cm on stony B/C horizon ~ 15 cm, pH: 4.6 (organic), 4.74 (mineral)	O horizon 5-20 cm, Sandy mineral A horizon ~10 cm, coarse B/C horizon, pH: 4.79 (organic), 5.41 (mineral)
References	Nottingham et al., 2015; Oliveras et al., 2014 ; Zimmermann et al., 2010a,b ; Girardin et al., 2010	Rousk et al., 2016; Wayolle 2011; Hartley et al., 2010; Wookey et al., 2009; Akerman & Johansson 2008

II.8 Research questions and thesis overview

Even though climate change occurs gradually, it can have a sudden and strong impact on ecosystem function and stability. Particularly affected are those regions already in extreme states of temperature or humidity, like the Polar Regions and deserts (IPCC, 2013). The Arctic is agreed to be one of the ecosystems potentially approaching a “tipping point” in ecological stability, because too many of the environmental parameters are subject to recent change (Huntington et al., 2012). But climate change will also modify the continuous weather gradients along high altitudes and high latitudes (IPCC, 2013; ACIA, 2005). Such gradients arise from interacting climate, vegetation and soils and result in characteristic ecotypes, for example altitudinal zonation on mountain slopes (von Humboldt, 1845). Treelines can be an early indicator of climate change induced species shifts both in high altitudes and high latitudes. However, quantifying and predicting possible climate feedbacks on the carbon cycle is difficult, because of the limited understanding of the processes by which carbon and associated nutrients are transformed or recycled within ecosystems, in particular within soils. Understanding the processes of OM-mineralisation in soils across the treelines could help to estimate how modified vegetation can affect C and N allocation and the vulnerability of soil organic matter to priming.

The treeline ecotones in the Peruvian Andes and in sub-arctic Sweden were simultaneously studied to unravel details of their coupled C and N cycling and provide a unique and direct comparison of a high altitudinal and a high latitudinal ecosystem seeking answers to the following research questions:

A) What are the exact amounts of carbon and nitrogen potentially located across a high Andean tropical treeline? Do stocks change across the treeline ecotone and are there differences in C and N stocks between forest patches and the continuous treeline?

B) How do soils across treelines in the Peruvian Andes compare to Swedish sub-Arctic treeline soils in terms of their carbon and nitrogen stocks? How is the contrast between sub-arctic and tropical treelines reflected in their plant community composition and N-mineralisation rates?

C) Do different substrates with different carbon to nitrogen ratios induce different priming effects and are these effects consistent in the contrasting Arctic and Andean soils?

D) How does the introduction of live plants change SOM-mineralisation in these different soils? Can seedlings and their roots alter belowground carbon cycles and nutrient availability and how does the microbial community respond?

(A) Given the acute climatic pressure on Arctic ecosystems, numerous studies exist addressing their C and N reservoirs and dynamics on a circumpolar scale (Hobbie et al., 2002; Hinzmann et al., 2005; ACIA, 2007; Hartley et al., 2012; Buckeridge et al., 2013; Parker et al., 2015; Lynch et al., 2018). Detailed information about carbon and nitrogen stocks is comparably low for high elevational tropical treelines (Zimmermann et al., 2010a; Vásquez et al., 2014; Sylvester et al., 2017; Rolando et al., 2017). The first part of chapter one therefor explicitly addressed this knowledge gap and expanded previous soil inventories in Manú National Park in the Peruvian Andes from the high elevational mountain forests into adjacent Puna grasslands (Zimmermann et al., 2010a; Whitaker et al.,2014a; Hicks et al., 2019). The studied landscape consists of a fragmented treeline, where the natural boundary between montane forest and the adjacent Puna grasslands is not continuous and distinct forest patches spread into the uplands (Rehm & Feeley, 2013). Previous studies have not particularly addressed soil carbon stocks of these forest patches and how they edaphically compare to the continuous treeline at high spatial resolution. Thus, they do not account for the role of forest patches as shelter for some species under threat at lower latitudes due to increasing temperatures or their possible impact on future carbon cycling in the Andes. As some species shift their distribution ranges upwards in escape of the climatic changes in their previous habitats, they might be hindered to invade open Puna grasslands per se, but could be able to propagate in association with the existing patches (Forest Patches in Tropical Landscapes, 1996). This might have strong impacts on the distribution of carbon in these areas, including the possibilities to either increase carbon storage in biomass, or to release older carbon from SOM while new plant communities establish. A fine-scale soil inventory was therefore conducted to better understand the structure of this static tropical treeline. Differences in C and N stocks and plant community composition were

accessed to gain new insights to the edaphic parameters underlying the persistence of forest patches and continuous treeline in close vicinity.

(B) Estimates of soil C and N stocks vary, depending on sampling methodology and timing and naturally across geographic regions (Schrumpf et al., 2011; FAO, 2018). Therefore, comparing literature values for C and N stocks between arctic and tropical treelines is impeded by this variance. Applying similar methodology for soil sampling in Peru and Sweden, the first chapter also provides a unique and direct comparison of a high altitudinal and a high latitudinal treeline ecosystem. A particular focus was to study the coupled C and N dynamics across both ecotones. The availability of nitrogen (N) was expected to be higher in the forest (Reed et al., 2011, Hedin et al., 2009), while above the treeline N inputs via litter mineralization and biological N fixation can be lower due to lower temperatures and fewer legumes (Bruijnzeel et al., 2011). As cumulative litter decomposition in wooded areas can increase N-mineralisation and shift N from soil to vegetation (Melillo et al., 2011), carbon storage in vegetation can also increase through increased biomass production. Alternating the C:N ratio in Puna soils and vegetation has the potential to also change the fate of soil C, which could be released from previously latent SOM. It has been shown for arctic ecosystems that during growing periods in the summer, mining of older SOM can be induced (Hartley et al., 2012). If this becomes the case in tropical ecosystems, there is great potential for a future source of carbon from upper montane ecosystems, which has not been accounted for in detail yet. Besides gathering soil C and N stocks, the experimental set-up therefore also addressed soil N dynamics to determine the net N-mineralisation across the treeline and throughout the forest patches using a resin bag ion exchange approach.

(C) One consequence of treeline shifts is modified C and N inputs to soils, as different plant communities provide different quality and quantity of root exudates and litter to the ground. Labile organic inputs can increase or decrease microbial degradation of SOM, resulting in positive or negative priming effects (PE), and therewith increase (Hartley et al., 2012; Perveen et al., 2019) or decrease (Qiao et al., 2014; Liang et al., 2018) CO_2 released from soil. The susceptibility of the soils from Peru and Sweden to priming was therefore tested in chapter two. Eight soil types were differentiated according to soil origin (Peru, Sweden), land cover (high Andean tropical forest, Andean Puna grassland, boreal birch forest, tundra heath)

and soil horizon (organic and mineral). Taking advantage of this soil diversity, a mechanistic study was designed, to investigate the effect of soil, substrate and microbial C:N stoichiometry on magnitude and direction of PE. Four hypotheses were tested, centred around the different mechanistic concepts proposed for PE: the N-mining theory (Schimel et al., 2003; Craine et al., 2007), preferential substrate use (Blagodatskaya et al., 2011) and stoichiometric decomposition (Mooshammer et al., 2014a; Zhu et al., 2018).

(D) In natural environments, plants provide their associated microbes with a variety of organic inputs, therewith causing rhizosphere priming effects (RPE). Determining these effects is particularly challenging because of the influence of a varying nutrient status of the soil according to plant nutrient uptake and simultaneous root exudation. Amongst environmental parameter, microbial processing of carbon is mediated by the availability of nitrogen (N) and other nutrients (Dijkstra et al., 2013; Nottingham et al., 2015). Fresh carbon inputs can be used in order to release nutrients previously locked in SOM (Moorhead & Sinsabaugh, 2006; Craine et al., 2007; Fontaine et al., 2011; Keiluveit et al., 2015). But this can be hindered when microbes compete with plants for the limiting elements and reverse RPEs when plant consumption is outcompeting microbial decomposition (Cheng, 1999; Meier et al., 2009; Dijkstra et al., 2010). On the other hand, high N availability can enhance SOM decomposition when C and N inputs meet the microbes' nutrient demands (Hessen et al., 2004). However, in soils of high nutrient availability, a negative priming effect can result from the reduced need of microbes to mine nutrients from locked SOM as they can rely on easily accessible substrates for their carbon and energy requirements (Blagodatskaya et al., 2007; Guenet et al., 2010). To investigate the direct impact of live plants on the C-cycle of the different soils, a greenhouse experiment was conducted. In chapter three rhizosphere priming effects (RPE) were determined *in vivo* using a natural abundance labelling approach, introducing a C_4 grass to the set of C_3 soils (Blagodatskaya et al., 2011). RPE was measured three times during the late growing season and microbial biomass C and N and PLFAs were determined at the end of the experiment in planted and unplanted control soils. It was hypothesised that RPE would be higher in mineral soils, compared to organic soils with larger increases of microbial biomass C in organic soils and more positive priming in soils with higher fungal abundance.

Chapter 1:

Taking stock of soil C and N across contrasting treelines in the Peruvian Andes and sub-arctic Sweden

(Michel J, Buckeridge K, Hartley IP, Whitaker J)

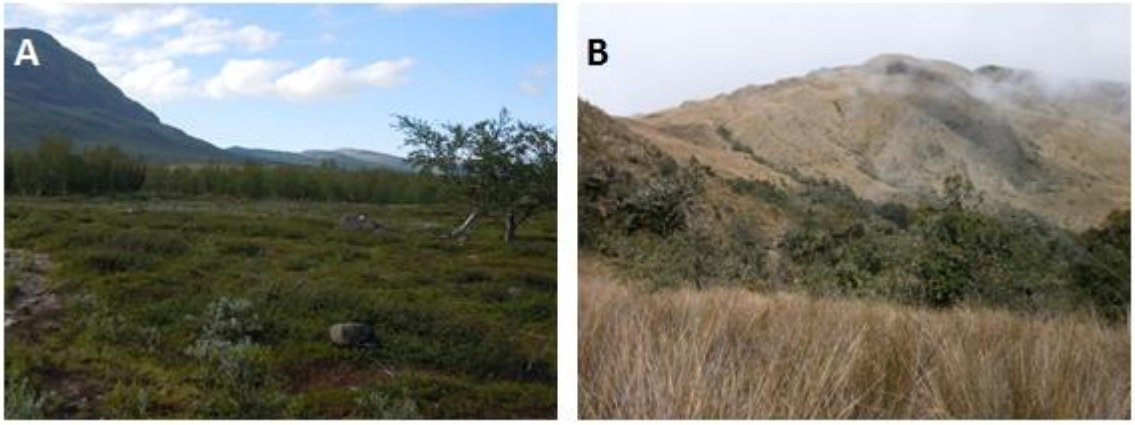


Figure 1.1: A: High latitudinal treeline in Northern Sweden and B: High altitudinal treeline in Southeast Peru

Chapter 1: Taking stock of soil C and N across contrasting treelines in the Peruvian Andes and sub-arctic Sweden 40

1.2 Abstract 42

1.2 Introduction 43

1.3 Material and methods

1.3.1 Peru soil inventory 46

1.3.2 Sampling across Peruvian and Swedish treelines 48

1.3.3 N – mineralisation 50

1.3.4 Vegetation analysis 51

1.3.5 Soil analysis 51

1.3.6 Statistics 52

1.4 Results

1.4.1 Peruvian soil inventory 53

1.4.2 Comparison of Swedish and Peruvian treeline soils 57

1.4.3 Treeline plant communities 61

1.5 Discussion

1.5.1 Peruvian soil Inventory 65

1.5.2 Comparison of treeline characteristics in Peru and Sweden 67

1.5.3 N-mineralisation 70

1.6 Conclusion 71

1.1 Abstract

Climate change is predicted to have stronger than average impact on ecosystems in high latitudes and high altitudes, where it can modify plant species distributions across landscapes. This includes treeline shifts, which alter carbon (C) and nutrient turnover in these regions. It has been shown in the arctic that this can lead to additional losses of C from soil. In contrast, the tropical treeline has as yet remained comparably stable, but might be subject to comparable changes in the near future.

This study explores the edaphic parameters of a shifting high latitudinal (Sweden) and a stable high altitudinal treeline (Peru). Identical sampling methodology was applied across Peruvian and Swedish treelines to enable for the first time a systematic comparison of their treeline soils. We hypothesised divergently pronounced N-limitation both across the treeline ecotones and even more in direct comparison of the two ecosystems. Therefore, we determined C and N stocks together with *in situ* N-mineralisation, dissolved inorganic nitrogen (DIN) and plant community composition.

The direct comparison of high altitudinal tropical treelines with boreal treelines in the high latitudes revealed potentially higher C-stocks in the boreal region. Swedish soils also had higher C:N ratios and DIN contents, and presented higher rates of N-mineralisation, which was higher in the forest compared to the tundra soils. For both countries, organic soils were enriched in C and N compared to the mineral soils, but the Peruvian top soils cover a greater depth both in forest and Puna compared to the Swedish forest and tundra soils.

These results indicate that plant-soil N-cycling can be an important factor determining the current position of global treelines. A better understanding of treeline C:N dynamics could provide valuable insights to growth limitations across landscapes. Future studies could address how this differentially affects different plant species and their microbial communities. This would help predict potential consequences of climate change induced species shifts on soil C stocks and the global carbon cycle.

1.2 Introduction

The treeline ecotone can be an early indicator of climate change induced species shifts. The forest border represents a spatially limited clash of contrasting, sometimes extreme, biomes. Over a short range, dense tree cover opens into adjacent uplands, where the abundance of trees eventually decreases to zero and shrubs and grasses prevail. Beyond the transition zone, the distribution of trees is limited by environmental factors, such as temperature and precipitation, but also fundamentally by soil nutrient richness (Bader et al., 2007; Girardin et al., 2010; Conant et al., 2011; van de Weg et al., 2014; Rehm & Feeley, 2015a). Changes to these parameters could be amplified in high latitudes and altitudes, which can indirectly affect position and composition of global treelines and treeline species (Heimann & Reichstein, 2008). Modified plant community composition can lead to changes in litterfall and also changes belowground allocation of labile carbon through rhizodeposition by roots and root exudates (Heath et al., 2005; Subke et al., 2006). This changes the allocation of carbon and nutrients in soils and the rates at which soil organic matter (SOM) is mineralised by microbes. Recent research shows, this can cause loss of soil C, particularly from warming arctic soils (Plaza et al., 2019; Philips et al., 2019), but also from tropical soils (Nottingham et al., 2019). However, the long-term impact of such modified SOM-mineralisation on the net ecosystem C balance remains uncertain (Schädel et al., 2018; Wieder et al., 2019).

Northwards expansion of the treeline and shrub encroachment have been observed in sub-arctic boreal ecosystems (Tape et al., 2006; Danby & Hik, 2007a,b; Wookey et al., 2008; Harsch et al., 2009). It has been demonstrated that this can significantly alter soil C cycling and microbial SOM-mineralisation in these high latitude ecosystems (Subke et al., 2009; Hartley et al., 2012; Parker et al., 2015). In Sweden, soil C stocks were shown to be twice as high in tundra soils, compared to the adjacent forest soils (Parker et al., 2015). These large C-stocks could become subject of microbial SOM-mineralisation when plant inputs change under treeline advance and significantly alter soil C-respiration. Treeline shifts have also been reported from temperate montane ecosystems (Walther et al., 2005; Lenoir et al., 2008). But many high altitudinal treelines in the tropics have remained widely unchanged over the last decades (Lutz et al., 2013; Rehm & Feeley, 2015a,b). As these regions are predicted to experience greater than average warming (IPCC,

2013), it is important to consider them in global ecosystem C models. Given the yet traceable effects of climate change on plant communities in the arctic, these environments are relatively well studied, including the here investigated Swedish region (Hartley et al.,2012; Parker et al., 2015, 2016, 2018; Friggens et al., 2020). On the other hand, the focus of studies in the tropics seldom expands above the treeline ecotone. Particularly the Puna grasslands on the here studied Andean mountain slopes received less attention than their tundra counterparts above the treelines in high latitudes (Zimmermann et al., 2010a; Feeley & Silman, 2011; Rolando et al., 2017). We unlocked this knowledge gap and provide detailed data on soil C and N stocks from a fine-scale soil inventory across the treeline ecotone in Manú NP in the Peruvian Andes.

The first part of this chapter focusses on this treeline system in the Andes. No upwards shifts have been recorded from this region so far (Rehm & Feeley, 2015a, b). However, one treeline peculiarity is the presence and persistence of several isolated forest patches above the treeline. It remains to determine, whether these patches are the early avant-garde of an upwards moving treeline or remnants of a previously higher treeline (Rehm & Feeley, 2013). Previous inventories of carbon stocks across the continuous treeline ecotone in Manú suggest that soil carbon stocks are not significantly different between high Andean forests and adjacent Puna grasslands (Zimmermann et al., 2010a), while soil respiration has been shown to be significantly higher in grassland soils than in montane forest soils (Hicks et al., 2019). In the presented soil C and N inventory, half of the studied transects (n = 5) were located across the treeline of forest patches, while the other set of five transects represented the continuous treeline. By this differentiation we tried to understand whether soil carbon stocks are larger within the continuous forest or the forest patches. This aimed to estimate the potential impact of a future treeline advance on the distribution of soil C in this area. It is not known if C accumulates underneath forest patches or if it is even more prone to enhanced rates of SOM-mineralisation?

Secondly, these high altitudinal treelines were compared with treelines of a high latitudinal reference site in Abisko, Northern Sweden. Therefore soils were sampled in plots each 15 m above and below respective treelines and composite soil samples were taken from organic and upper mineral soil horizons. The availability of mineral nitrogen (NH_4^+/NO_3^-) is expected to be higher in forests than in adjacent uplands

(Reed et al., 2011; Hedin et al., 2009), as above the treeline, N inputs via litter mineralisation and biological N fixation are reduced by low temperatures and fewer or no trees (Bruijnzeel et al., 2011). As cumulative litter decomposition in wooded areas increases N mineralisation and shifts N from soil to vegetation (Mellilio et al., 2011), carbon storage in vegetation is potentially going to increase through increased biomass production. In addition to determining soil C and N stocks, this study therefore also addresses soil N dynamics by determining net N-mineralisation rates across the studied treelines using a resin bag ion exchange approach. Soil nitrogen mineralisation, the transformation rate from organic to inorganic N, is a measure for system productivity and nutrient cycling (Schimel & Bennett, 2004). Nitrogen mineralisation and immobilisation varies with soil properties and climate (Risch et al., 2019) and can thus be an important factor determining treeline positioning. Edaphic parameters were complemented by assessing the aboveground vegetation and determining plant cover abundance of functional groups across the treelines.

In this study the following research questions were addressed:

1. In the Peruvian Andes (i) Do soil C and N stocks differ across 30m transects from forest into adjacent upland Puna grasslands? (ii) Are soil carbon stocks higher in forest patches or within the continuous forest? (iii) Is N-mineralisation limited in Puna grasslands due to the lack of leaf litter inputs and low temperatures?
2. Do soil C and N stocks differ between forest and tundra uplands on a comparable spatial scale in the Swedish sub-arctic? How do soil C and N stocks of Andean tropical forest, boreal forest, Puna grasslands and tundra heath directly compare?
3. Is N-mineralisation enhanced in *Ericaceae* dominated shrublands in Sweden and how do rates of N turnover compare between the different land cover types of a static high altitudinal treeline in Peru and a shifting high latitudinal treeline in Sweden?

1.3 Material and methods

Soils were collected during summer and autumn 2016 in the high altitudes of the Peruvian Andes in Manú National Park (Tres Cruces) in the department of Cusco, Peru at an average elevation of 3300 m a.s.l. (May / June), and in the sub-arctic at the Research Station of Abisko, 250 km north of the Arctic Circle in Northern Sweden (end of August). The sites provide a contrast in elevation, latitude, seasonality and land cover type. The Peruvian study area comprises a montane tropical forest with a short transition zone leading into Puna grasslands. In Sweden, the treeline ecotone was studied between mountain birch forest and tundra heath. Detailed site descriptions can also be found in the Introduction under section II.7.

1.3.1 Peru soil inventory

The tropical montane treeline ecotone in the Peruvian Andes is located at the high elevation sites on the far end of an established elevational gradient, where most studies have focused on comparing the lowland Amazon forests to the montane tropical forests (Nottingham et al., 2009; Whitaker et al., 2014a,b; Nottingham et al., 2015; Hicks et al., 2019). This study extends their research into the higher altitudes, complementing existing studies and respective soil surveys (Zimmermann et al., 2010, 2012; Gibbon et al., 2010).

For this soil inventory in Peru, ten 30 m transects were established across treelines including each five forest patches and five continuous treelines (n = 10). Each transect reached both 15 m into tropical montane forest and 15 m into adjacent Puna grassland (Fig. 1.2). Five sampling points were evenly spread along each transect (each 7.5 m apart, “1” to “5”, Fig.1.2). Position “1” was considered to represent Puna grassland, position “3” the main transition zone between grassland and forest and “5” the forest. At each sampling point (1 - 5), one PVC core (h = 15 cm, d = 5.5 cm), equipped with resin ion exchange bags, was placed in the upper soil layer. Cores and resin bags were collected 15 days after instalment to determine in-field N-mineralisation rates (see section 1.3.3). At each of the five sampling points along the transects, soil was also sampled with a soil corer (d = 5.5 cm, Fig. 1.2 B). At each sampling location, two replicate soil cores were taken, each sampling to the full depth of O(h), A and B horizons. For each soil core, the total depth of the hole was

measured, as well as the length of the obtained soil cores. Excess length was noted to account for soil compaction during the sampling procedure (Fig.1.4 B, E), which can falsify calculations of stocks when not accounted for (Measuring and modelling soil carbon stocks, FAO, 2018). Depth of visually distinguishable layers of soil horizons was measured. Compaction was assumed only for the upper horizon and excess length of sampling hole added to each top core to accurately calculate bulk density (BD) in the lab. All cores were separated into the distinguished horizons in the field, bagged and labelled accordingly. The organic layer was characterised as O in the Puna and Oh in the forests. This differentiation of the upper soil layer was maintained, in order to relate the different composition of top soil to the N-mineralisation rates studied in this layer *in situ.* At some sampling locations, stratification of the soil profile allowed differentiation of further mineral horizons. As they were not consistent amongst the ten transect, layers were combined to fit the general characterisation (O-A-B).

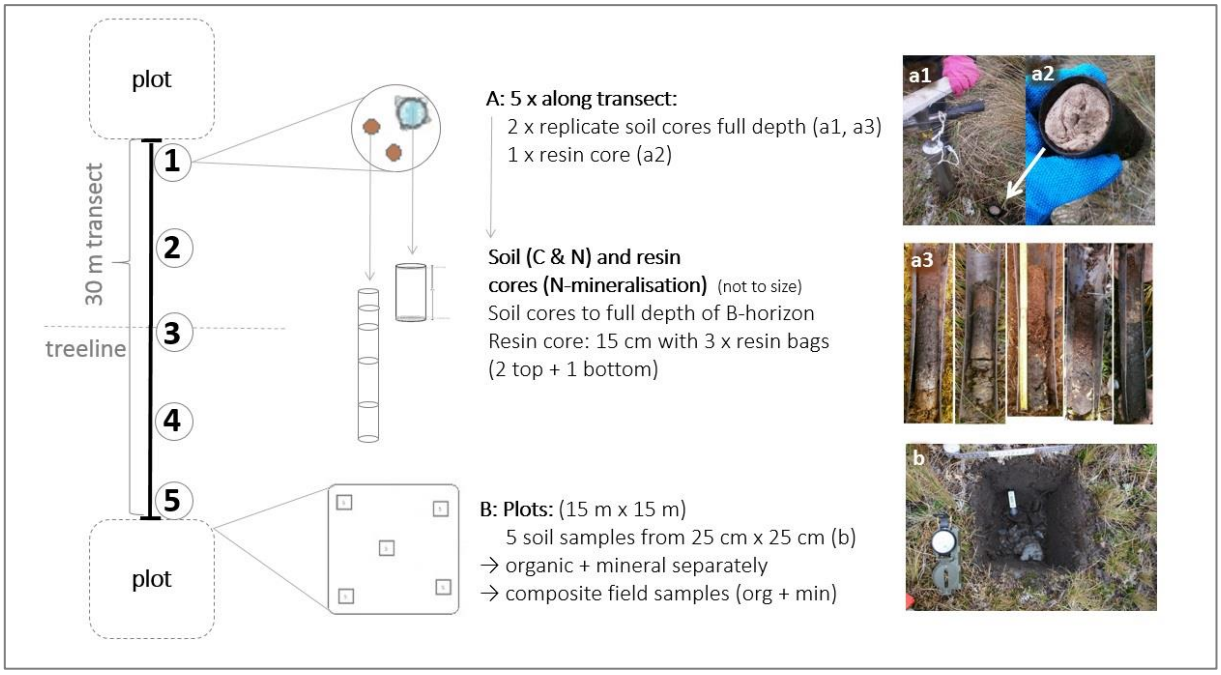


Figure 1.2: Details of soil sampling for the fine-scale inventory of soil C and stocks in the Peruvian Andes: positioning of transects (n = 10) with 30 m total length, each orthogonally traversing the treelines reaching 15 m each into forest and Puna from the forest border at position (3), with a total of five sampling points alongside (1 – 5). At each point two replicate cores were taken to full depth (a1, a3) and one small PVC core (a2) was placed in the field to determine N-mineralisation rates at positions 1 and 5. 15 m x 15 m Plots (b) were established at each end of each transect within Puna and forest to sample soil for the comparison of soil C and N stocks of these high Andean soils with sub-arctic soils from Sweden (Fig.1.3).

1.3.2 Sampling across Peruvian and Swedish treelines

For the comparison of treeline characteristics in Peru and Sweden, six of the ten Peru sites were selected. They comprised three continuous and three patchy treelines and excluded the shrubbiest field sites (Appendix V2-C1-A1). Six comparative transects were set up in Sweden and a similar sampling strategy was applied in both countries for N-mineralisation across the treelines and soil sampling in plots at each end (Fig. 1.3). To compare C and N characteristics between both countries, a plot (15 m x 15 m) was established at each end of each transect, one in the forest and one in the adjacent upland (Puna grassland in Peru and tundra heath in Sweden). Plots covered 225 m^2 each with five times sampling within each in a dice layout. At each sampling point, aboveground plant material and leaf litter were removed from squares of 625 cm^2 to firstly sample upper organic soil, excluding the densely rooted surface. The area was extended when the organic horizon was too shallow to obtain at least 10 l of soil for each replicate within one plot. When the mineral soil horizon was not reached after sampling of organic soil, sampling depth was increased to obtain the upper 10 - 15 cm of the mineral soil horizon, where 10 l of soil was then sampled accordingly.

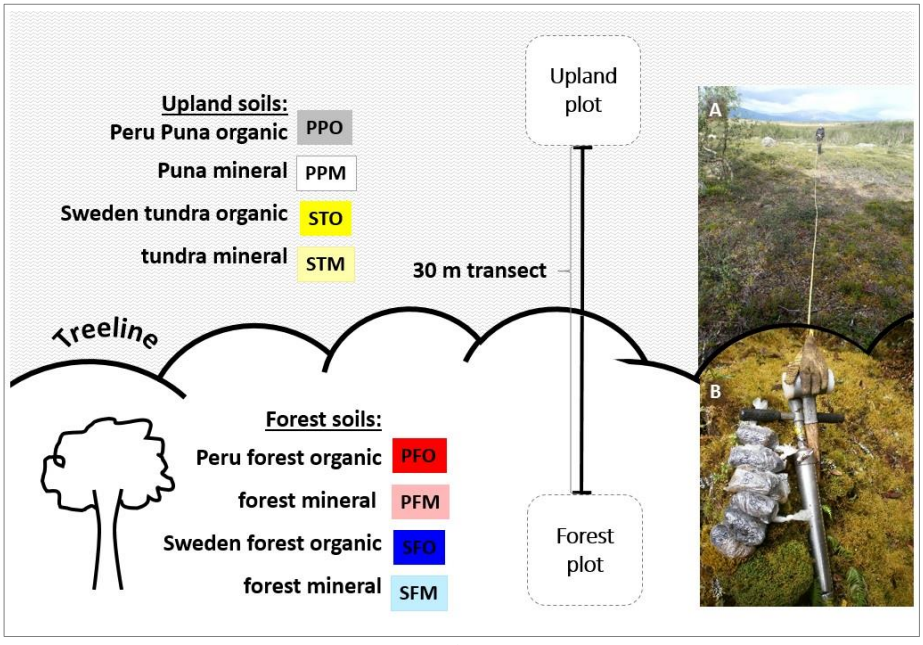


Figure 1.3: Plots covering an area of 225 m^2 each were established 15 m above and below treelines in Peru and Sweden (n = 6 each). Within each plot, soil was sampled at five replicate points in a dice layout, distinguishing the organic top soils and separately sampling the mineral sub soils. Respective samples were combined to a composite sample of 20l for each horizon in each plot. In situ N-mineralisation was measured at both endpoints of the 30 m transects. A: Transect measuring into tundra heath (Sweden), B: Bagged soil samples, coring equipment and mossy floor cover inside montane cloud forest (Peru).

Replicates of each horizon were combined to yield approximately 20 litres composite sample for both organic and mineral soils from each plot (n = 6 for each land cover type (forests and uplands) and soil horizon (organic and mineral) in both countries (Peru and Sweden)). BD was determined from 15 cm cores (d = 5.5 cm) taken in duplicate for each soil horizon at the five sampling points This resulted in a set of eight distinct soil types, for which a top down terminology was used scaling down from country, over land cover type to soil horizon: PFO: Peru Forest Organic, PFM: Peru Forest Mineral, PPO: Peru Puna Organic, PPM; Peru Puna Mineral, SFO: Sweden Forest Organic, SFM: Sweden Forest Mineral, STO: Sweden Tundra Organic, STM: Sweden Tundra Mineral (Table 1.1).

Table 1.1: Introduction of the eight different soil types whose edaphic and microbial properties were addressed in this thesis. PFO: Peru Forest organic, PFM: Peru Forest Mineral, PPO: Peru Puna Organic, PPM: Peru Puna Mineral, SFO: Sweden Forest Organic, SFM: Sweden Forest Mineral, STO: Sweden Tundra Organic, STM: Sweden Tundra Mineral.

Soil ID ("soil type")	Country	Land cover	Soil horizon
PFO	**P**eru	tropical mountain (cloud) **F**orest	**O**rganic (Oh)
PFM	**P**eru	tropical mountain (cloud) **F**orest	**M**ineral (A)
PPO	**P**eru	**P**una grassland	**O**rganic (O)
PPM	**P**eru	**P**una grassland	**M**ineral (A)
SFO	**S**weden	boreal birch **F**orest	**O**rganic (Oh)
SFM	**S**weden	boreal birch **F**orest	**M**ineral (A)
STO	**S**weden	**T**undra heath	**O**rganic (O(h))
STM	**S**weden	**T**undra heath	**M**ineral (A)

1.3.3 N - mineralisation

Net nitrogen mineralisation was assessed in situ at five sampling points along each of the six transects in Peru and Sweden (Fig. 1.3). Before the field campaign, resin bags were prepared in the laboratory, following the method described in DeMarco et al., 2011 (Appendix V1-P1). In brief, bags were made of nylon membrane from tights, which was soaked in 1.2 M HCl for 2 h, followed by repetitive washing with deionised water to remove any ions from the membrane. Individual bags were filled with 5 g ion exchange resin and formed to uniformly covered an area of 8.64 cm^3 each (the size of the cores, compare Fig. 1.2a2).

In the field, at each of the five sampling points along each transect, two short soil cores (PVC, h = 15 cm, d = 5.5 cm) were sampled from the top soil layer, after careful removal of aboveground vegetation and densely rooted top soil (Fig. 1.2). One core (initial) was then stored at 4°C until processed in the laboratory to assess the initial N status of the soil. The second core (final) was extracted from the soil and capped with one resin bag at the top and two at the bottom. The core equipped with resin bags was then returned to its original hole and left in the field for 15 days. At the end of the incubation period (15 days), final soil cores were collected and resin bags were removed from the cores, bagged, labelled and kept at 4°C until processed. Soil of initial and final cores was analysed in the same fashion to determine pools of dissolved inorganic nitrogen. DIN was measured by extracting 10 g of fresh soil with 50 ml of 0.5 M K_2SO_4. The soil slurry was agitated on a shaker table for 2 h, allowed to sit overnight in a cooler, and then vacuum filtered. Filtrates were frozen until all samples were extracted and then analysed colorimetrically on a segmented flow autoanalyzer.

Net N-mineralization was calculated as the difference between DIN in the initial soil core and DIN in the final soil core plus DIN accumulated in the middle resin bag. The purpose of the additional bags at the top and bottom of the core was to buffer the soil column inside the core against external N-inputs from above and leaching to the underside, to obtain spatially accurate comparison amongst all core (initial and final and replicates).

1.3.4 Vegetation analysis

Plant community composition was assessed within each plot (25 m x 25 m) at the distant ends of the transects corresponding to “forest” and “Puna grassland / tundra heath” and similarly in the middle of each transect considered the forest border (position “3”, Fig. 1.2). Vegetation was grouped into the four categories of 1. grasses, 2. cryptograms, 3. shrubs and angiosperms and 4. trees. Within each plot, firstly, the relative area covered was determined for each group. Then, within each group, the most abundant plants were identified to species level to represent species richness. This measure is not equal to α-diversity, as not all species of all groups were identified. Cover abundance for each individual group was calculated as the ratio of all species identified amongst all groups and the number of species identified for respective group, multiplied by the relative area covered.

1.3.5 Soil analysis

For the Peruvian soil inventory, where sampling was conducted to the full depth of the B horizon (Fig. 1.2 a3), soil layers of each core were separated in the field, individually bagged (Fig. 1.3 B) and depth was recorded (see section 1.3.1). In the lab, these individual soil samples were homogenized by hand and the larger than 2 mm fraction (roots, rhizomes, course woody debris, and rocks) was removed and the volume determined to subtract from the volume of the core to calculate the volume of soil in each sample. Bulk density was determined for the individual sections of the soil cores by dividing the difference of the mass of soil minus the mass of roots and rocks by the difference of volume of soil minus volume of roots and rocks (BD = g ml^{-1}). The volume of the light fraction was determined in small cylinders filled with water, using a spatula to ensure full submergence and avoid surface tension at the cylinder margins. Since soil samples were relatively small, the ratio of fine to coarse fragments might overrepresented the light fraction, potentially leading to sub-estimation of bulk density (Measuring and modelling soil carbon stocks, FAO, 2018). Soil water content was determined and bulk soil percent C and N measured on subsamples of the smaller than 2 mm soil fraction, dried at 60°C until constant weight, ground to a fine powder and then analysed on an elemental analyser (Elemental analyser Vario EL).

For the comparison of Peruvian and Swedish treeline soils (see section 1.3.2), the larger in volume samples from the organic and mineral horizons from the plots were treated the same way and BD determined as described before using the undisturbed (intact) core method (h = 15 cm, d = 5.5 cm). In addition, for each country three sub-samples were taken from the four different soils types (organic and mineral soils from forests and uplands) and soil texture was determined following standard field protocols (VD LUFA I, D 2.1, 1997) and further specified by analysis of stratification in suds solution ("jar test" as in Sitton & Story, 2006).

1.3.6 Statistics

Statistical analysis was conducted in R (version 3.2, The R core team, 2015).

For the Peruvian soil inventory, similarities in soil C and N stocks between forest patches and continuous forests, location along the transect and soil horizon were tested in three-way ANOVA. Variability of C and N contents and stocks through the soil profile was then further determined in one-way ANOVA followed by post-hoc Tukey test. Linear regression was applied to determine trends in total C stocks along the five locations of the transects across continuous and patchy treelines (from sampling point 1 to 5, Fig.1.6 A,C)

For comparison of soil C and N characteristics and N-mineralisation in Peruvian and Swedish soils, three-way ANOVA was applied to each factor to compare parameters between countries, land cover types and soil horizon. One-way ANOVA followed by Tukey HSD-tests was applied to determine variability amongst the eight soil type. Differences in relative vegetation cover of ecotones across the treelines were tested using Pearson's correlations.

1.4 Results

1.4.1 Peruvian soil inventory

Three-way ANOVA revealed no significant differences in carbon and nitrogen stocks (Table 1.2) along the transects (location: sampling positions 1 – 5) or between continuous (Fig. 1.6 A, B) and patchy treelines (Fig.1.6 C, D).

In spite of that, there was a steady decline in soil carbon stocks from forest into adjacent Puna grasslands for the continuous forest transects (R^2 = 0.84, Fig. 1.6 A). Such trend could not be distinguished for the forest patches (R^2 = 0.44, Fig. 1.6 C). Soil carbon and nitrogen contents (%, Fig. 1.5) and stocks (Fig. 1.6), as well as bulk density (BD, Fig. 1.4 C, F), were significantly different between the soil horizons (Table 1.3). The top organic O-horizon was specified by land cover type, which is Puna (O), transition zone (O(h)) and forest (Oh), in order to correlated possible differences with the N-mineralisation rates determined for these same upper soils in the second part of the study.

Table 1.2: Results of analysis of variance in soil C and N stocks between treeline type (patchy or continuous), loc: location along the transect (positions 1-5) and hor: soil horizon (O(h)-A-B). p-value with significance code for p < 0.001.

	C stock	**N stock**
type*loc*hor	ns	ns
treeline type	0.64	0.98
location	0.41	0.96
horizon	0.0005 ***	$7.53e^{-8}$ ***

Compaction of the upper soil horizon was higher at the two sampling locations within the forest (5 + 4, Oh) for both continuous and patchy treelines (Fig. 1.4 B, E). Bulk density increased down the soil profile (Fig. 1.4 C, F), while carbon and nitrogen contents (%) decreased (Fig. 1.5). Carbon and nitrogen stocks were lowest in the organic top soils, with particularly low C stocks in Puna soils (locations 1 + 2) adjacent to forest patches (Fig.1.6 A, C). Nitrogen stocks were mostly accumulated in the A horizon (Fig.1.7 B, D).

Table 1.3: Comparison of five soil parameters amongst the different soil horizons. For the purpose of the soil inventory, classification of mineral horizons was simplified into A and B for all sampling points along the transects, while the organic horizon was separated into O, O(h) and Oh to account for the differences in top soil composition (see also section 1.3.1). Letters indicate similarity of sample means following one-way ANOVA and post-hoc Tukey test. p-value with (sig.) significance code for p < 0.001.

Horizon (land cover)	C (%)	C stock	N (%)	N stock	BD
O (Puna)	b	c	b	b	b
O(h) (transition)	bcd	ac	b	ab	b
Oh (forest)	c	abc	b	ab	b
A	d	b	c	c	c
B	a	ab	a	a	a
p-value (sig.code)	$1.11e^{-09}$ ***	0.0005***	$8.45e^{-12}$***	$7.53e^{-08}$***	$5.41e^{-11}$ ***

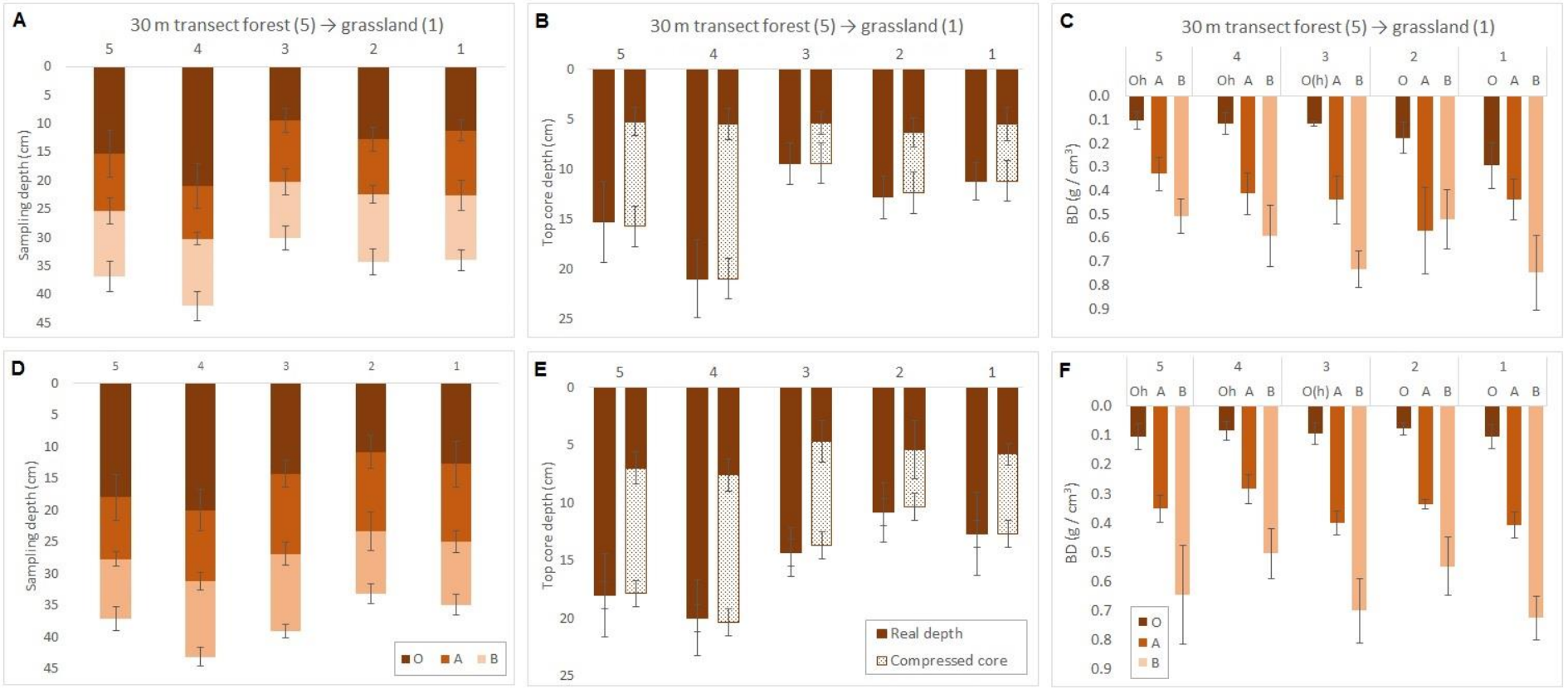


Figure 1.4: Peruvian soil inventory: sampling depth (A, D), top soil (O(h) horizon) compaction (B, E) and bulk density ("BD", panels C, F) of three soil horizons at five sampling locations along 30 m transects leading from forest (5) into adjacent grasslands (1). Top row: continuous treeline, bottom row: forest patches (n = 5 ± SE each).

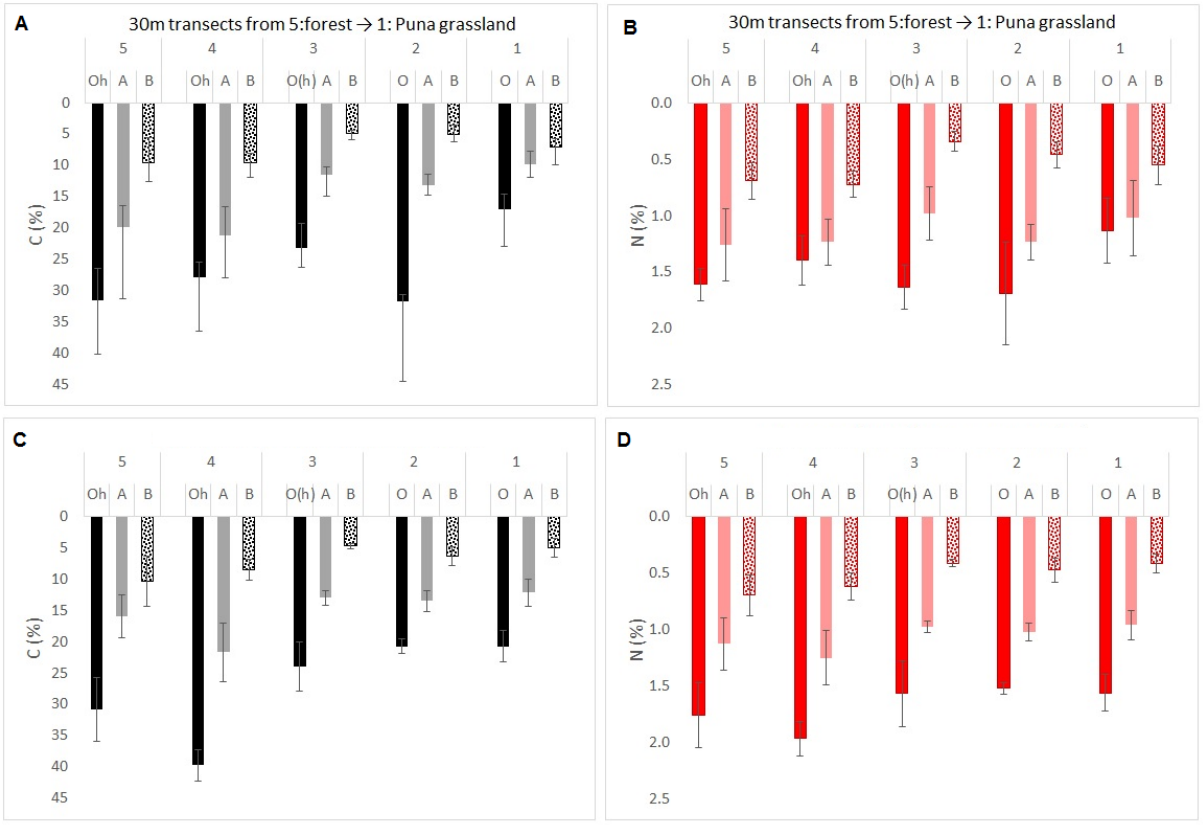


Figure 1.5: Peruvian inventory: relative (%) soil carbon (A,C) and nitrogen contents (B,D) of three soil horizons at five sampling locations along 30 m transects leading from forest (5) into adjacent grasslands (1). Top row: continuous treeline, bottom row: forest patches (n = 5 ± SE each).

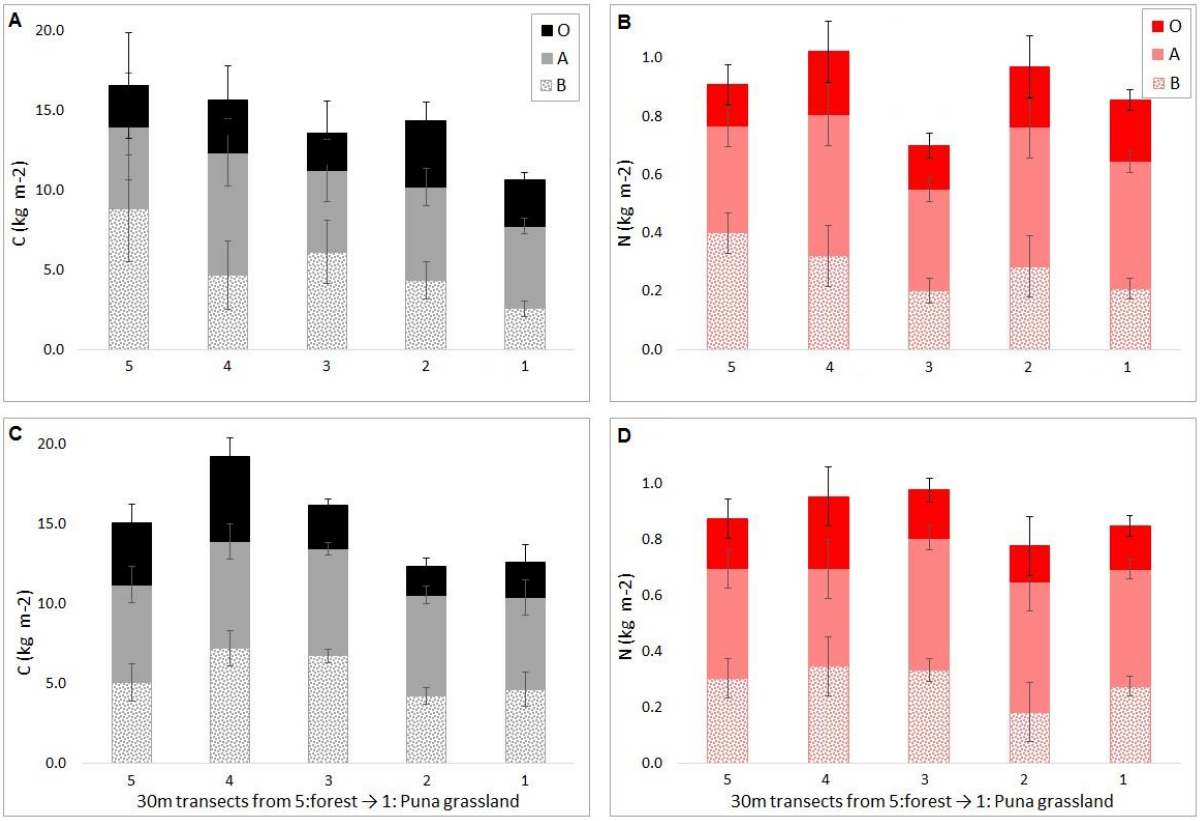


Figure 1.6: Peruvian inventory: stocks (kg m $^{-2}$) of soil carbon (A,C) and nitrogen (B,D) of three soil horizons at five sampling locations along 30 m transects leading from forest (5) into adjacent grasslands (1). Top row: continuous treeline, bottom row: forest patches (n = 5 ± SE each).

1.4.2 Comparison of Swedish and Peruvian treeline soils

For the second part of this study, the question was addressed whether soil C and N and N mineralisation rates across treelines differ between high altitude (Peru) and high latitude (Sweden) ecosystems. Therefore, eight soil types were differentiated to represent these ecosystems (Table 1.1) and several edaphic parameters were determined (Table 1.4). The organic tundra soils (STO) had the largest C stocks amongst the here compared samples (17.2 kg m^{-2}). N-mineralisation rates however were higher in Sweden, where they were measured at the end of a warm summer (Fig. 1.7 D). Soil C:N ratios were highest in the tundra soils (29), similar for the Swedish and Peruvian forests (20-25) and lowest in the Peruvian Puna soils (14). The Swedish soils provided a large contrast of C and N contents, with mineral soils very limited in C and N (< 5 / 1 %) and very CN-rich organic soils (> 45 / 1.5 %), while all Peruvian soils ranged between them. The C contents in the Peruvian organic soils were half as high as in the Swedish organic soils, while N contents were comparable and only slightly lower (Table 1.4).

Table 1.4: Edaphic parameters of organic and mineral soils from forest and adjacent uplands in Peru and Sweden. Values are mean ± SE *for n = 6*, n = 5 for N-mineralisation in Sweden, *p-value and significance code (sig.code: p < 0.001***, 0.01**) given for analysis of variance of sample means between soil types, post-hoc Tukey test results provided in following figure 1.7. Abbreviations: soil types: Peru Forest Organic (PFO) and Mineral (PFM), Peru Puna Organic (PPO) and mineral (PPO); Swedish Forest Organic (SFO) and mineral (SFM) and Swedish tundra organic (STO) and mineral (STM); soil horizons: Oh: humic organic, A: upper mineral, O: organic; OM: organic matter, C: carbon, N: nitrogen, DIN: dissolved inorganic nitrogen, dwt: dry weight*

Soil ID	**Soil texture**	**C** (%)	**C stock** (kg m $^{-2}$)	**N** (%)	**N stock** (kg m $^{-2}$)	**Soil C:N**	**DIN** (µg g $^{-1}$ dwt)	**N-mineralisation** (%)
PFO	OM (+silty clay)	32.36 ± 6.89	13.28 ± 3.21	1.67 ± 0.35	0.70 ± 0.10	22.18 ± 4.67	9.23 ± 0.15	0.12 ± 0.004
PFM	(Silty) Clay loam	9.54 ± 2.48		0.68 ± 0.18		20.67 ± 3.1	8.72 ± 0.03	na
PPO	OM (+silty clay (loam))	20.57 ± 1.03	10.78 ± 2.21	1.43 ± 0.12	0.73 ± 0.05	14.21 ± 1.99	8.82 ± 0.13	0.12 ± 0.005
PPM	(Silty) Clay loam	6.70 ± 1.63		0.45 ± 0.10		14.22 ± 1.03	8.87 ± 0.16	na
SFO	OM (+silt)	45.21 ± 1.81	15.95 ± 1.24	1.83 ± 0.20	0.24 ± 0.06	25.63 ± 3.01	14.83 ± 1.3	0.2 ± 0.04
SFM	Sand	2.09 ± 0.24		0.10 ± 0.03		21.88 ± 2.73	10.84 ± 0.34	na
STO	OM (+silt)	50.73 ± 1.24	17.20 ± 3.54	1.53 ± 0.15	0.60 ± 0.15	29.13 ± 3.99	13.26 ± 1.16	0.16 ± 0.035
STM	(Loamy) Sand	3.59 ± 0.98		0.08 ± 0.02		29.73 ± 3.19	12.06 ± 1.58	na
p-value (sig.code)		$<2e^{-16}$***	0.001**	$<2e^{-16}$***	$5.57e^{-5}$***	$4.72e^{-5}$***	$2.82e^{-7}$***	$4.97e^{-6}$***

*Table 1.5: Results of three-way ANOVA comparing soil C and N contents (%), dissolved inorganic nitrogen (DIN) and in situ N-mineralisation rates (N-min). p-values given and significance code given for p < *** 0.001, ** 0.01, * 0.05, . 0.1. df: degrees of freedom, sum sq: sum of the squares of the deviations of all the observations from their mean, mean sq: mean square (sample variance)*

	C (%)	N (%)	DIN	N-min
Country	**$3.91e^{-10}$*** **	**0.014***	**$5.28e^{-09}$*** **	**$8.69e^{-07}$ *** **
Land cover	0.20	**0.02***	0.73	0.09 .
Horizon	**< $2e^{-16}$*** **	**< $2e^{-16}$*** **	**0.007****	na
Country:LandCover	**$4.75e^{-07}$*** **	0.42	0.98	**0.04 ***
Country: Horizon	**$1.62e^{-15}$*** **	**0.0005*****	**0.03***	na
LandCover:Horizon	0.18	0.14	0.10	na
Country:LandCover:Horizon	**0.005****	0.20	0.28769	na
df, sum sq, mean sq	36, 2.023, 0.056	36, 367, 10	38, 114.02, 3.00	18, 0.007, 0.0004

Soils across the treelines in sub-arctic Sweden and the Peruvian Andes were significantly different in their C and N contents, as well as DIN and N-mineralisation rates. Carbon and nitrogen contents were significantly higher in the organic soils in both countries (Table 1.5). DIN showed little variation amongst the Peruvian samples, with little difference between organic and mineral soils in both forest and Puna. In Sweden, DIN in organic forest soils was significantly higher (Fig. 1.7 C). N-mineralisation was twice as high in Sweden compared to Peru and higher in the forest soils than in the tundra soils (Fig. 1.7 D).

In Peru, no significant difference was observed in N-mineralisation rates between the different land cover types or soil horizons and all soils had less than 10 µg DIN per gram soil dry weight. N-mineralisation was analysed for the organic top soils in both countries. Along the transects from forests into uplands, no consistent trend was found in net N-mineralisation rates. However, comparing the most distant samples of the transects in both countries revealed that N-mineralisation was higher in the forest than in the adjacent uplands, but significantly only in Sweden (Table 1.5, Fig.1.7 D). Comparing the two countries, the magnitude of N-mineralisation rates reflected the measured soil DIN contents.

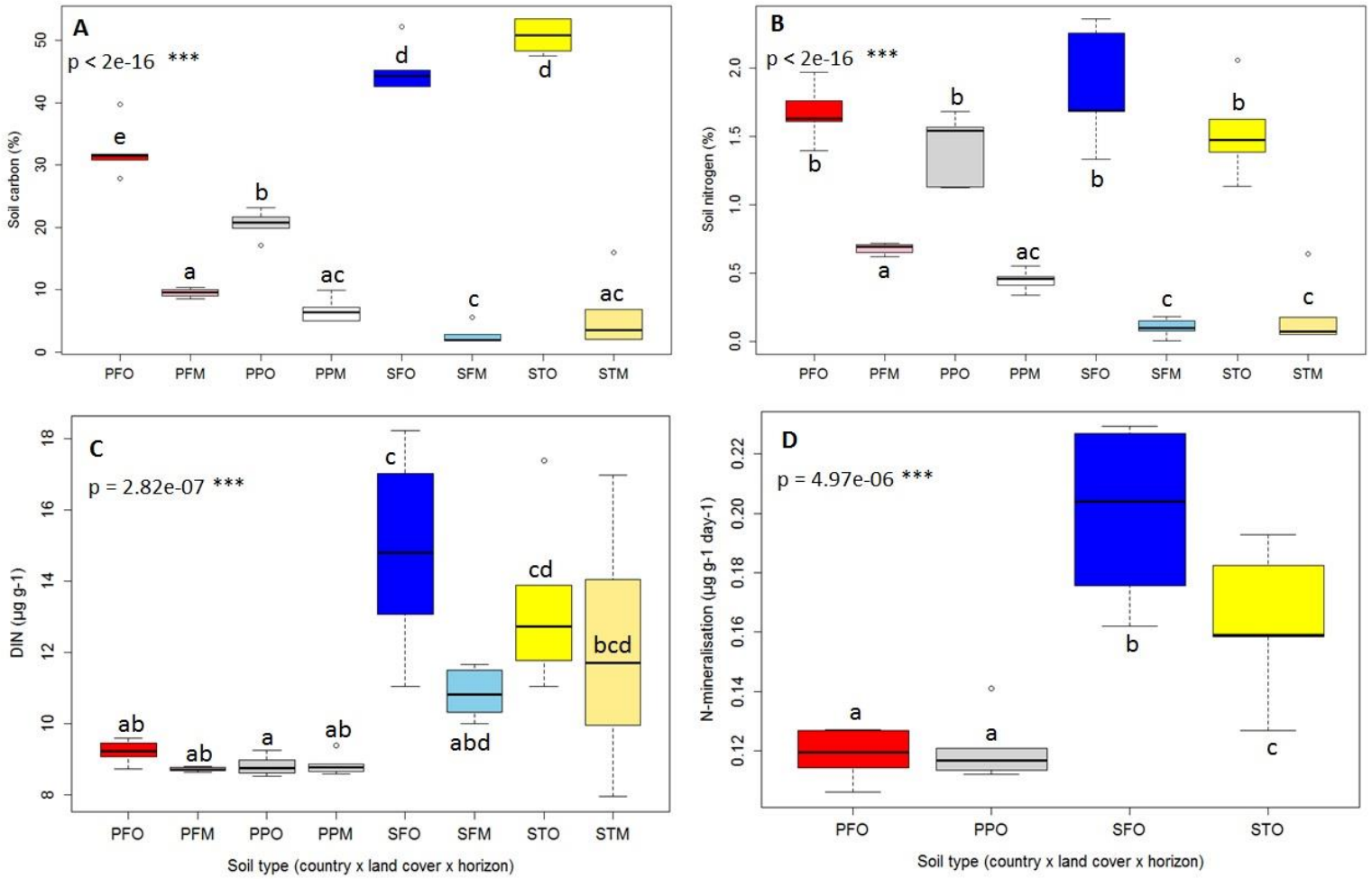


Figure 1.7: Edaphic C and N entities above and below Peruvian and Swedish treelines. A) carbon content (%); B) nitrogen content (%); C)Dissolved inorganic nitrogen (DIN) content; D) N-mineralisation in organic top soils. P-value and significance code given for differences between soil types. Letters indicate similarity of sample means according to Tukey-post hoc tests following ANOVA. Boxes shows median lines and interquartile ranges for n = 6 (A-C), in Fig. D n = 5 for Swedish samples

1.4.3 Treeline plant communities

Plant cover abundance was determined along the transects transversing the treelines in patchy and continuous treelines in Peru and also across the Swedish treelines.

In Peru, continuous and patchy treelines were compared in terms of cover abundance of major plant functional groups. Across the continuous treelines, the change from mainly tree-dominated forest to Puna grassland was pronounced and abruptly occurred between sampling points 3 and 5 (Fig.1.8A). The relative abundance of cryptogams was two-fold higher in the forests, than in the transition zone or the grasslands. Across the forest patches, plant community composition changed gradually, while the relative proportion of cryptogams at respective locations remained unchanged (Fig. 1.8B). Notably, less trees and more grasses were found in forest patches, compared to continuous forest. Plant community composition in forest patches was thus composed by equal proportions of trees, shrubs and angiosperms, cryptogams and grasses.

As expected, the treeline ecotone was differently composed in the Andean and the sub-arctic ecosystems (Fig. 1.9). While the Swedish tundra heath was composed of mainly cryptogams and shrubs, in absence of grasses (apart from a few *Carex,* Appendix V2-C1-A1), the Peruvian Puna is a real grassland with a range of different grasses present (Table 1.6). The significant change between forest and upland plant communities was located closer to the dense forest in Peru (between sampling points III and V) and further into the open upland in Sweden (between sampling points I and III). In the forests, cryptogams were more abundant in Peru, while a larger cover abundance of trees was measured for Swedish forests.

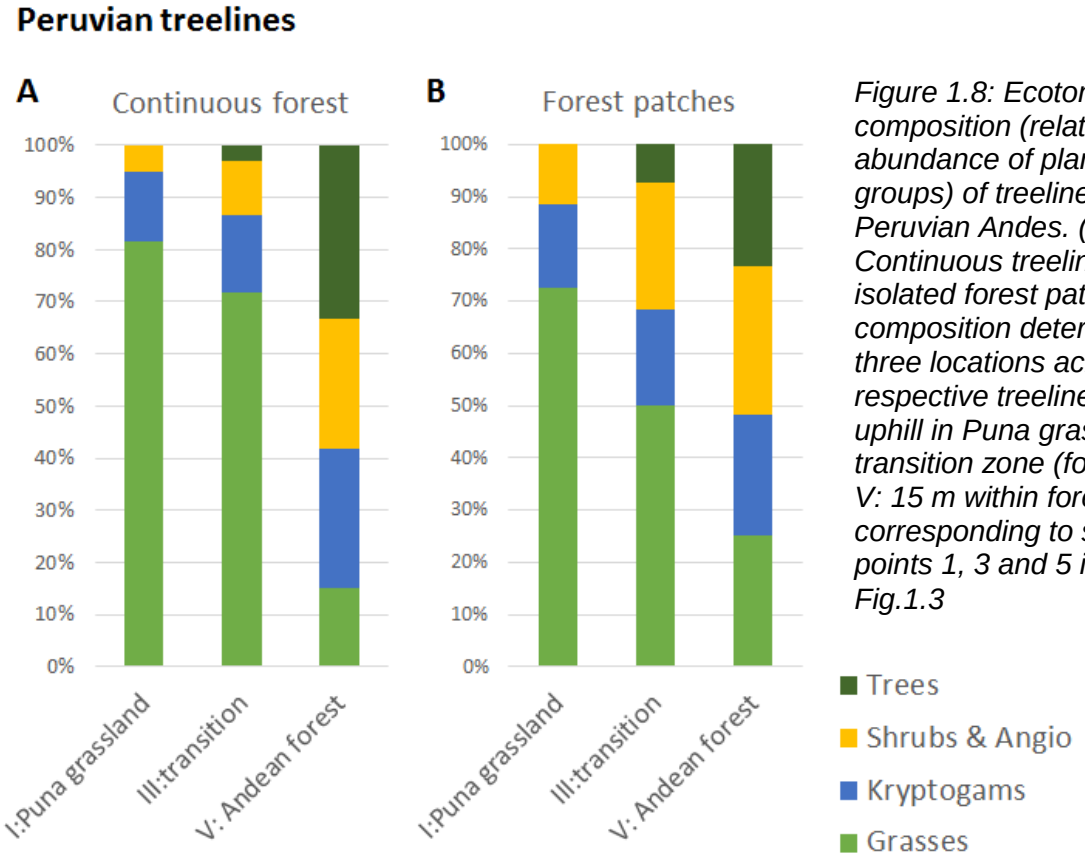


Figure 1.8: Ecotone composition (relative cover abundance of plant functional groups) of treelines in the Peruvian Andes. (A) Continuous treeline and (B) isolated forest patches. Plant composition determined at three locations across respective treelines: I: 15 m uphill in Puna grassland, III: transition zone (forest border), V: 15 m within forest. Locations corresponding to sampling points 1, 3 and 5 in Fig.1.2 and Fig.1.3

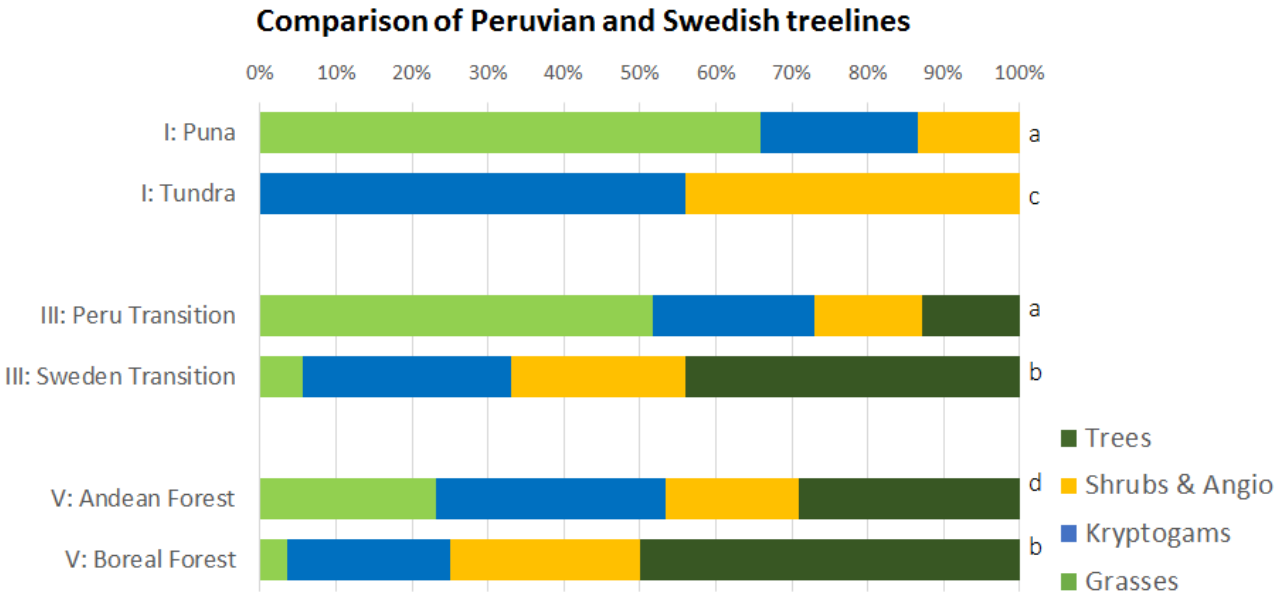


Figure 1.9: Ecotone composition (relative cover abundance of plant functional groups) for three locations across treelines in Peru and Sweden. I: upland (Puna / tundra), III: transition zone (forest border), V: forest, letters indicate similarity of ecotone composition with $R^2 > 0.95$

Plant community composition was estimated by identifying most abundant species. In Peru, the most characteristic tree species were identified as *Weinmannia mircrophylla* (Kunth), P*olylepis pauta* (Hieron.) and G*ynoxys induta* (Cuatrec.). The Swedish birch forest lived up to its name with high abundance of B*etula nana* (L.) and *Betula pubescence* (Ehrh.), while at some sites *Salix glauca* (L.) and Juniper sp. were also present. The adjacent Puna grasslands were dominated by species of the genera Festuca, hypericum and Carex. Shrubs and cryptogams where highly represented in the forests. The genus Miconia in the family Melastomataceae (unresolved and either classified as a large shrub or a small tree) *was* a dominant plant from the forest border into deeper forest. Two Ericaceae were further abundant down from the forest border: *Glautheria vaccinoides* (Wedd. var. humilis Wedd) and *Vaccinium floribundum* (Kunth). Amongst the cryptogams, several species of Cladonia and Usnea were highly abundant lichens and Blechnum sp. the dominant fern.
The Swedish tundra heath was dominated by ericaceous plants, including *Empetrum nigrum* (L.), *Vaccinium myrtillus* (L.), V*accinium vitis-idea* (L.) and *Vaccinium uliginosum* (L.) and also occasionally cloudberries *Rubus chamaemorus* (L.). Abundant cryptogams included species of the lichen *Cladonia* and the mosses *Dicranium* and *Polytrichum*. True grasses were widely absent, but the grass-like genera of *Lycopodium* and *Equisetum* were commonly present at low abundance. Although the presence of at least three further different species of Graminoides was noticed, they contributed less than 3 % to total vegetation cover (Appendix V2-C1-A1).
Overall, species diversity was found higher in the tropical, compared to the arctic ecosystem. This could be applied to most plant functional groups, but shrub abundance was higher at the Swedish field sites. Despite the geographic and climatic contrast between the tropical and arctic ecosystems, some genera were positively identified across both Peruvian and Swedish treelines. These cosmopolitans included the moss *Cladonia* and different species of *Vaccinium*.

Table 1.6: Plant genera primarily present along transects across Peruvian and Swedish treelines, with particular plant peculiarities provided for distinct functional groups, and suggestions for potential roles in respective ecosystem functioning. P: Peru, S: Sweden.

Most abundant plant genera in ecotone	Peru	Sweden	Particular plant functional characteristics
Peru: Puna grassland **Sweden: Tundra heath**	*Festuca, Hyperium, Cladonia, Blechnum, Huperzia, Lycopodium, Escallonia, Gaultheria*	*Cladonia, Betula, Empetrum, Vaccinium*	P: grasses: AMF can enhance plant N-uptake S: ericaceous shrubs can slow N-cycling
Transition zone	*Bromus, Festuca, Hyperium, Carex, Cladonia, Blechnum, Usnea, Huperzia, Leucobryum, Vaccinium, Gaultheria, Miconia, Weinmannia, Polylepis*	*Cladonia, Dicranium, Polytrichum, Lycopodium, Rubus, Betula*	P: abundance of cryptogams, liana and epiphytic ferns: high aboveground biodiversity & species density: disentangled bioactivity from direct soil contact S: ericaceous shrubs
Peru: Tropical mountain (cloud) forest **Sweden: Sub-arctic birch forest**	*Carex, Luzula, Cladonia, Usnea, Blechnum, Vaccinium, Gaultheria, Miconia, Diogenesia, Weinmannia, Polylepis, Gynoxys*	*Dicranium, Cladonia, Juniper, Salix, Sorbus, Betula*	P: diverse species composition, including *Miconia*, ferns S: *Betula*, EMF increased under C, mediate plant N-uptake

A full list of plants as identified by genera and / or species across the treelines can be found in Appendix V2-C1-A1. Species names were matched with entries in The Plant List of the World Flora Online (www.theplantlist.org) and the World Checklist of Selected Plant Families of the Kew Royal Botanic Gardens (https://wcsp.science.kew.org/ advanced.do) and last updated on 25/08/2019.

1.5 Discussion

1.5.1 Peruvian soil Inventory

Soil carbon and nitrogen were determined along 30 m transects traversing ten treelines in the tropical Andes. No significant differences were identified for C and N stocks across continuous and patchy treelines. However, a constant decline in C socks was identified from continuous forest into Puna grasslands (Fig. 1.6 A), which was not reported for C-stocks across the ecotone in forest patches (Fig. 1.6 C). The linear fit (R^2 = 0.84) across the continuous treeline could reflect belowground parameters, such as the fading presence of tree roots and their microbial symbionts across the forest border (Friggens et al., 2020). It also suggests that differences in carbon stocks can persist within small geographical scales as here above and below the Manú treeline. In a previous large-scale inventory of soil carbon stocks within Manú National Park, Zimmermann et al. (2010) identified no significant differences in C-stocks between forests and Puna. They also report no difference in C-stocks through the soil profile (0 – 80 cm) for forest soils, while in the transition zone and Puna grasslands, carbon stocks were lower at greater depth (> 30 cm). This study focused on one of their sampling locations (Tres Cruces) and applied a randomised sampling design at higher spatial resolution. In this study, carbon stocks were found to decrease from continuous forest into Puna and were higher at greater depth (> 15 cm). Moreover, C contents were lower in Puna top soils (O) than in forest top soils (Oh) across both continuous and patchy treelines (Fig. 1.5. A, C; Table 1.3), which is in accordance with Hicks et al. (2019), who also report lower C contents for the here studied Puna grasslands in Manú, compared to the lower forest soils. They also reported significantly increased soil respiration in incubations of Puna soils, but, when amended with different substrates, SOM-mineralisation was reduced. This could indicate that microbial metabolic activity is restricted in Puna grasslands, which would correspond to the low C:N ratios reported and the slightly lower N-mineralisation rates measured for Puna soils compared to the adjacent forests in this study *in situ* and also reported in Hicks et al. (2019). Previous studies in this region reported no significant differences in total C and N stocks across the treeline in the Peruvian Andes, (Zimmermann et al., 2010; Gibbon et al., 2010). Despite the apparent consistency of soil C contents across the forest to Puna ecotone, the treeline has remained relatively stable (Lutz et al., 2013; Rehm & Feeley,

2015a), which raises the question of what limits the treeline in the tropics at present? As relatively few long-term studies have observed treeline advance in direct relation to soil C:N, it remains an open research question, whether present soil C:N is the result of, or the cause for, current treeline position (Mayor et al., 2017; Müller et al., 2017). It is possible, that treeline advance is facilitated by microbial communities (Thébault et al., 2014) and plant genera (Hewitt et al., 2018), which could modify soil parameters before larger shrubs and trees can establish roots. It is agreed that tree growth and establishment can only be successful under a positive carbon balance. That is, when in the long term plant photosynthetic carbon gains exceed respiratory losses (Körner, 1998). In the high Andes, temperature has been shown to restrict treeline extension (Rehm & Feeley, 2015b), as short growth periods limit carbon gains through photosynthesis and also impede seed dispersal and establishment (Körner, 1998). In addition to the environmental controls on treeline limitation, our study indicates that edaphic characteristics could possibly set limits to upslope tree expansion in the Andes as well. Lower soil carbon and nitrogen stocks in the Puna top soils, a thin organic horizon and low N-mineralisation rates could restrict nutrient cycling and restrain seedling establishment above the current treelines. The absence of shrubby vegetation could be another factor limiting forest expansion in the high altitudes of the tropics at present. Higher abundance of cryptogam and epiphytic species in the tropical forests could function to protect border trees against cold clouds blowing in from the mountains. Climate change could potentially alter these effects in the future, but this study did not address the functional role of different plant functional groups in detail. One peculiarity of the treeline in Manú is its inconsistency and presence of defined forest patches. In this Andean mountain system, hill exposure and landslides are factors that likely contribute to treeline formation. Hill slope and exposition affect the local weather conditions in mountains to a larger extend than they do in open-plan. This affects in particular humidity and cloud formation (Holtmeier & Broll, 2005). Increased inputs of water can destabilise soils and, especially in the steep mountains of the Andes, cause landslides. This could be one factor contributing to the uneven treeline in this area of the Andes, and could potentially also be the cause for the formation of forest patches (Forest patches in tropical landscapes, ed. Schelhas & Greenberg, 1996).

1.5.2 Comparison of treeline characteristics in Peru and Sweden

Soil carbon and nitrogen were determined across treelines in the Andean mountains and sub-arctic Sweden. For some of the installed transects, the classification of the ecotones as “forest” and “Puna/tundra” might be better represented as “forest edge” and “upland-edge”. However, this has the benefit that the impact of other parameters, as for example large temperature differences between land cover types, was reduced along the 30 m transects. Overall, the transition between forest and uplands was smoother in Sweden. Over the distance of 15 m from the centre of each transect, shrubby vegetation commonly reached into the forests. In Peru, the shift from mainly trees to mainly grasses was more pronounced, but the density of cryptograms and liana and other epiphytes gradually changed over the area covered with the transects. This affected prevailingly the lower 15 m sections into the forests, while the Puna ecotone was, at least in terms of aboveground plant cover, well represented within the upland plots. Rehm & Feeley (2015b) also showed that seed dispersal was limited across the treeline over an even shorter range (± 10 m from the transition zone). Hence, the transects established for this study (± 15 m from the transition zone) covered a suitable area to represent significant changes of plant-soil interactions across treelines. In terms of the edaphic characteristics, C and N contents were higher in organic soils compared to the mineral soils in both countries. Carbon contents of the Swedish organic soils were above all sample mean and carbon contents of the Swedish mineral soils were below all sample mean (Fig. 1.7 A). The here reported C stocks in Abisko are higher than those reported in other studies (e.g. Parker et al., 2016), probably due to differences in site selection and methodology. For example, bulk density for this study was only determined in small cores and might therefore not be as accurate as determination of BD in larger samples (FAO, 2018). However, one possible explanation for the amplified distinction between organic and mineral soils in Sweden is the fact that in the sub-arctic ecosystem real summer and winter seasons occur. During the summer months, plant productivity peaks, so nutrient cycling is enhanced intensifying plant-soil interactions (Subke et al., 2012). However, nutrient inputs accumulate predominantly in the top soils, as seasonality sets limits to their microbial mineralisation. In winter, nutrient cycling can, but must not, be limited for example by snow cover on top soils (Grogan et al., 2001) and by freezing of sub

soils (Clein & Schimel, 1995). When periods of warm temperature and photosynthetic peak productivity are prolonged in these ecosystems, nutrient cycling between plants and soils is likely to be accelerated and plant nutrients can potentially reach greater depths. Moreover, mineral soils can be highly compacted in Sweden (BD up to 2.1).

In the here studied tropical mountain forests and grasslands, temperature does not fluctuate as much on an annual scale. The system is however subject to diurnal fluctuations, with low, sometimes near- zero, temperatures at night and an average of 12°C in daylight, or even doubled under full sun light. Mean annual temperatures are higher in the forest compared to grasslands. In lieu of pronounced annual temperature fluctuations, the studied high Andean mountain system is subject to a very rainy season December - February (Zimmermann et al., 2010; Nottingham et al., 2012; Whitaker et al., 2014a). The greater rates of precipitation at that time could alleviate profound differences in nutrient availability both between land cover types and soil horizons, as could fog deposition. Nutrient re-distribution between soil horizons could be facilitated by the fact that the soils are relatively well drained (Fig. 1.4 C, F). The combined effect of increased water input in the rain season, relatively low BD and presence of clay and silt (Table 1.4) could impact C and N allocation and concurs with the accumulated N-stocks in the A horizon of the Peruvian soils (Fig. 1.6 B, D; Table 1.3). It is therefore possible to hypothesis that C and N can accumulate in sub-soil beneath the weathered top soils and above the bedrock as a result of these conditions in the high Andean forest.

The here studied different land cover types are shaped by different plant communities and therewith also host characteristic mycorrhizal symbiont. In boreal forests, such as the here studied birch forest in Sweden, ectomycorrhiza (ECM) prevail (Parker et al., 2016), while some ericoid mycorrhiza (ERM) can also be found at lower abundance (Dickie, 2006). ERM are also the dominant mycorrhizal symbionts amongst ericaceous plants in tundra heath (Dickie, 2006), where additionally some orchid mycorrhiza and dark septate root endosymbionts (DSE) can occur (Newsham et al., 2009). Compared to the boreal forest, mycorrhizal symbionts in high Andean forests are more diverse, comprising ECM, ERM and also arbuscular mycorrhizal fungi (AMF) (Dickie, 2006; Smith & Read, 2002). AMF are also the prevailing symbionts in Andean

Puna grasslands (Dickie, 2006), where similarly to the tundra heath some orchid mycorrhiza and dark septate root endosymbionts (DSE) can occur (Lugo & Menoyo, 2019).

For the here studied Swedish treeline ecotone, Parker et al. (2015) found indication for decreasing abundance of ECM from forest into adjacent shrub heath. Increasing ECM coincided with decreasing SOC stocks from heath to forest, indicating that enhanced release of SOM-degrading enzymes by ECM could amplify soil C turnover (Parker et al., 2015). This is supported by our results showing lower C stocks in forest than in tundra soils (Table 1.4). While ectomycorrhizal plants can accelerate nutrient turnover, ericaceous plants have been shown to have the potential to slow nutrient turnover in sub-arctic soils (Parker et al., 2018), which is in accordance with the lower N-mineralisation rates identified here for the Swedish tundra soils compared to the forest soils (Fig. 1.7D; Table 1.4). The functional role of mycorrhizal plants could also play an important part in the Peruvian montane system, where AMF are present in roots of some Andean forest species and many Puna grassland plants (Lugo et al., 2012; Lugo & Menoyo, 2019; Haug et al., 2019). Like other forms of mycorrhiza, AMF are important symbionts for plants aiding their nutrient uptake (Marschner & Dell, 1994). But as endosymbionts, they release less enzymes to the rhizosphere (Read & Perez-Moreno, 2003) and in contrast to ECM and ECR, they do not sequester carbon into significant amounts of fungal biomass outside the plant root (Treseder & Holden, 2013). However, it has been shown that increased presence of AMF can result in augmented CO_2-losses from soil (Cheng et al., 2012). AMF may accelerate rhizosphere C turnover indirectly by altering plant exudation (Shahzad et al., 2015) and by influencing community composition of the associated soil bacteria (Frey-Klett et al., 2007). In addition to mycorrhiza, fungal:bacterial ratios (F:B) can therewith be an important factor determining soil C-turnover across treelines. F:B capture all saprotrophic fungi and gram positive and gram negative bacteria (Zelles, 1997,1999). For a tropical treeline ecotone in Puerto Rico, Smith et al. (2014) demonstrated that F:B ratios were comparable for pasture and early secondary forests, while late secondary and primary forests had fewer fungi and lower F:B ratios. This could indicate an adaptive change in belowground microbial communities following changes in land cover. Fungal to bacteria ratios have also been shown to

impact N limitation (Thébault et al., 2014) and soil carbon stocks (Friggens et al., 2020) across treelines and might thus play an important role for the early stages of treeline shifts.

1.5.3 N-mineralisation

In this study, the N-mineralisation rates of the Swedish soils were significantly higher than those determined in Peru. The rates reported for Peru are also lower than previously determined N-mineralisation rates in soils from the same field site in Peru (Hicks et al., 2019). N-mineralisation is strongly determined by local environmental conditions at the time of season / sampling / measurement (Rothstein et al., 2009) and is specific on small scales down to root level (Parker et al., 2016), which could explain differences amongst studies. The high availability of DIN in Swedish forest soils, especially the organic horizon, is in accordance with other studies of soil characteristics in the Swedish sub-arctic (Rousk et al., 2016; Parker et al., 2016). Lower rates of N-mineralisation in the tundra soils could be caused by seasonal inputs of recalcitrant litter with high C:N or lignin:N ratios or higher concentration of phenolic compounds, which can enhance immobilization of inorganic N and thus reduce net N-mineralisation (DeLuca et al., 2002; Keeney 1980; Scott and Binkley, 1997). Therefore, the here presented measurements only represent a snap-shot of nitrogen dynamics measured in autumn 2016 and generalisations are restricted. Moreover, the approach with resin bags in PVC cores involves significant disturbance of the soils when cores are hammered into the soil and equipped with resin bags. While this method ensures that N-mineralisation is determined for a defined and comparable volume of soil, the rhizosphere within is no longer intact and elevated N-mineralisation rates could be a result of decomposing roots and particularly EMF-activity in forest soils.

1.6 Conclusion

Significant differences were identified between Peruvian mountain treelines and Swedish treelines in the sub-arctic. C stocks were higher in the sub-arctic ecotone, as well as DIN and N-mineralisation rates measured in autumn 2016. At the Peruvian field sites, the ecotone transition from Andean forest to Puna grasslands was clearly differentiated, across the here established transects (30 m). However, the contrast in vegetation cover was not consistently represented in N-mineralisation rates or soil C or N stocks, which only gradually changed across the continuous treelines. No significant difference in edaphic parameters was reported between continuous and patchy treelines. At the Swedish field sites, vegetation cover from forest into tundra changed gradually and shrubs were often present al all sampling points along the installed transects. However, clear distinction could be made for edaphic parameters, both between landcover types (forest and tundra) and soil horizons (organic and mineral).

Chapter 2:

Soil carbon priming across contrasting treelines:

The interplay of soil, substrate and microbial C:N ratios?

(Michel J, Whitaker J, Hartley IP)

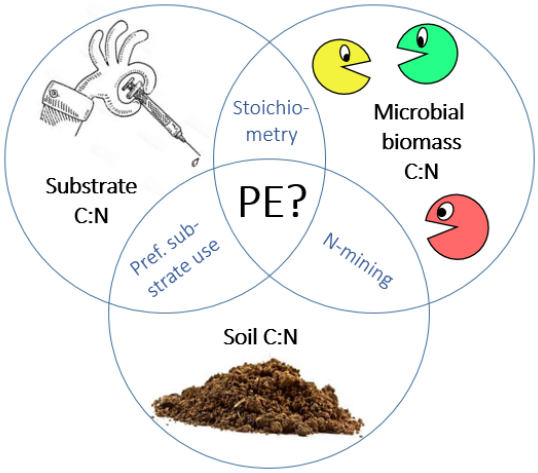


Figure 2.0: Venn diagram illustrating the experimental approach

Chapter 2: Soil carbon priming across contrasting treelines: The interplay of soil, substrate and microbial C:N ratios? 72

2.1 Abstract 74

2.2 Introduction 75

2.3 Material and methods

2.3.1 Study sites and field sampling 79

2.3.2 Pre-incubation analysis 80

2.3.3 Experimental design 81

2.3.4 Isotopic and source partitioning 84

2.3.5 Statistical analysis 85

2.4 Results

2.4.1 Soil and microbial characteristics 86

2.4.2 Soil respiration 88

2.4.3 CO_2 source partitioning 90

2.4.4 Soil carbon priming 93

2.4.5 The interplay of soil, substrate and microbial C:N ratios 95

2.4.6 Substrate-use and priming 96

2.5 Discussion

2.5.1 Revision of hypothesis 99

2.5.2 General mechanisms of priming effects 101

2.5.3 Potential implications for carbon cycling across treelines 103

2.6 Conclusion 104

2.1 Abstract

Climate change can modify the composition and productivity of plant communities, potentially resulting in treelines shifts at high latitudes and altitudes. Thus altered plant communities provide different carbon (C) and nitrogen (N) inputs to soils. This can affect microbial mineralisation of soil organic matter (SOM) with the potential to raise the amount of CO2 released to the atmosphere (positive priming effect (PE)) or to promote C-sequestration through reduced rates of SOM-mineralisation (negative PE). The mechanisms proposed to cause PEs remain controversial, particularly the role of C and N availability. Therefore, predictions of the potential impacts of modified nutrient inputs to soils on ecosystem C cycling remain uncertain.

To investigate how changes in the stoichiometry of C and N inputs might affect the mineralisation of SOM in high altitudes and high latitudes, organic and mineral soils from above and below treelines in sub-arctic Sweden and the Peruvian Andes were incubated for 21 days with 13C-labelled glucose combined with a pH-neutral nutrient solution. Treatment C:N ratios mimicked organic components of ecological relevance (LMW-sugar of root exudates, leaf litter, SOM, microbial biomass) and were added at a constant ratio of 30 % substrate-C to microbial biomass-C.

Positive, negative and no PE were measured across soils, irrespective of substrate C:N. We demonstrate that negative priming effects can occur across diverse soils following the addition of labile substrates. Root exudation of LMW-sugars could thus significantly reduce microbial SOM-mineralisation. This calls for a new perspective on negative priming as a persistent feature in plant-soil-microbe interactions. Mechanistically, we provide evidence for (preferential) substrate-use determining the magnitude of (negative) PE in both study ecosystems. Preferential substrate-use was controlled by microbial stoichiometry (C:N), while soil C:N further determined PE across all soils.

2.2. Introduction

The undisturbed ecosystems in high altitudes and high latitudes share two features, which are of particular importance in the context of climate change: their soils have relatively large carbon stocks (Yang et al., 2018; Rolando et al. 2017; Saatchi et al., 2011; Zimmermann et al., 2010a,b; Jobbágy & Jackson, 2000) and these biomes are predicted to experience greater than average increases in temperature (Wang et al. 2016; Classen et al. 2015; Wookey et al. 2009; Cramer et al. 2004). Hence, these current carbon sinks are vulnerable to future changes of climatic conditions, with the risk of becoming carbon sources. The direct effects of warming, increased carbon dioxide concentrations and altered precipitation and snowfall patterns on plants and soils have been widely studied in several of those regions (Parker et al., 2018; Rehm & Feeley 2015; Harsch et al. 2009; Heimann & Reichstein, 2008), but less is known about the indirect effects. Climate change can alter plant community composition and species distributions (van der Putten et al., 2010; Harsch et al., 2009), which modifies plant-soil-interactions and how nutrients, particularly carbon and nitrogen, are distributed between the atmosphere and soils. Such changes are likely to be manifested soonest and most dramatically at the treelines in high altitudes and high latitudes. This may push these ecosystems closer to a tipping point in ecological stability, with unknown consequences for the global carbon cycle.

In the sub-arctic region, there is evidence for changes in vegetation and species distributions (Hinzmann et al., 2005; Beniston, 2003) and associated increased amounts of CO_2 released to the atmosphere (Lynch et al. 2018; Bernal et al. 2016; Parker et al. 2015; Hartley et al. 2012). In contrast, there is limited observations of treeline shifts in the mountainous tropics (Fajardo & Piper, 2017; Girardin et al., 2010), where ecosystems are also affected by grazing and fire (Rolando et al., 2017; Oliveras et al., 2014). Plant upward migration in high altitude Puna grasslands is at present also limited by colder temperatures above the treeline (Rehm & Feeley, 2015; Lutz et al., 2013; Körner, 2012; Harsch et al., 2009). However, the carbon stocks in these grasslands could become accessible to microbial degradation when plant input chemistry changes following plant upward expansion, potentially catalysed under a

climate warming scenario (Soong et al. 2018; Razanamalala et al. 2018; Nottingham et al. 2018; Whitaker et al. 2014a).

Treeline shifts can have contrasting effects on the carbon cycle and SOC stocks. Increased above- and belowground biomass and different recalcitrance of litter from different species can increase the potential for new SOM formation (Rolando et al., 2017; Lange et al., 2015). But counterintuitively greater nutrient inputs do not always result in greater C and N storage, as plant litter inputs and root exudation can enhance SOM mineralisation by microbes and significantly increase the amount of CO_2 released to the atmosphere resulting in a negative ecosystem C-balance (Hartley et al. 2012; Parker et al. 2015). The phenomenon of altered degradation of SOM through modified microbial activity following the addition of a labile energy source is known as ‘priming effect’ (PE) (Löhnis 1926; Bingemann et al. 1953; but see Kuzyakov, 2000).

The concepts proposed to explain the mechanisms of PE are centred around soil and microbial nutrient availability and acquisition strategies mainly:

a) microbial N-mining (Jingguo & Bakken, 1997; Fontaine et al., 2011; Chen et al., 2014) or N-limitation (Craine et al., 2007; Schimel et al., 2003), where the addition of labile C in the absence of sufficient available N (high C:N) enhances microbial activity and hence the degradation of SOM (positive priming) as microbes strive to meet their nutritional requirements
b) preferential substrate use (Magasanik et al., 1961; Cheng et al., 1999; Wang et al., 2015), where the addition of labile C in sufficient amounts decreases the mineralisation of soil C (negative priming) when microbes shift from mineralizing SOM to using substrate-C as their primary energy source
c) microbial stoichiometric decomposition (Mooshammer et al., 2014a; Carrillio et al., 2017; Zhu et al., 2018), where the addition of labile C together with sufficient N can trigger an imbalance of resource C:N and enhance microbial degradation of SOM (positive priming)

A number of recent studies reported contrary effects, and also negative priming following the addition of labile substrates (Rousk et al. 2016; Heitkoetter et al.,

2017; Hicks et al., 2019). Particularly the N-mining hypothesis has been challenged (Mason-Jones et al. 2018; Wild et al., 2019). Adding to the contradiction, even similar experimental set-ups have led to inconsistent results in different studies, especially in low-nutrient environments, with both positive and negative priming occurring (Hicks et al., 2019; Qiao et al., 2016; Whitaker et al., 2014b; Guenet et al., 2010).

Notable contradiction of predicted PE under mechanisms a), b) and c) is why in this study the concepts were applied to soils of contrasting C and N characteristics to separate PE in light of their different soil and microbial C:N ratios (Table 2.1). Depending on the ecosystem, N-constrains in soils can be accompanied or substituted with other nutrient limitations, such as phosphorous or potassium (Fanin et al., 2014; Nottingham et al., 2015; Soog et al., 2018). In this study a nutrient solution was used to annul such micro-nutrimental constrains and to fairly compare tropical and sub-arctic soils regarding their C and N dynamics. This study focusses on two ecosystems experiencing current or prospective plant species migrations: treelines in northern Sweden and in the Peruvian Andes. To examine how soil, substrate and microbial biomass C:N interact to determine the magnitude and direction of priming effects in soils from these ecosystems, organic and mineral soils were collected across the treelines and amended with ^{13}C-labelled glucose in combination with N to obtain substrates of different C:N ratios. To unravel and test the underlying mechanisms of PEs, four predictions were made (Table 2.1).

According to the N-mining theory, the addition of C only in a high C:N soil (low N) will cause positive priming, because microbes need to degrade additional SOM in order to access N from SOM (hypothesis 1).

If they are provided with sufficient C and N, SOM mineralisation will be reduced, as microbes preferentially utilise substrate C and N, rather than investing into SOM-degradation (hypothesis 2).

In contrast, in soils of low C:N, when microbes are unlimited in N, the addition of labile C will reduce SOM degradation, as microbial metabolism can be fully sustained by the current availability of N resource (hypothesis 3).

According to the microbial stoichiometry theory, addition of sufficient nutrients in low C:N soils would cause positive priming when microbes become C-limited (hypothesis 4).

For all substrate additions in this study, microbes were provided with comparable amounts of energy in form of labile C from isotopically labelled glucose. Therefore, the limiting factor to SOM-degradation was either the N-component, both in its availability / accessibility and as central building block of SOM-degrading enzymes, or the imbalance of C and N of the three compartments of soil, microbes and substrate. Hence, considering the treatments for within each soil type individually, the only variable that changes was substrate-N content. Therefore, the magnitude of priming should be proportional to substrate-N content.

Table 2.1: Rationale of the experimental set-up: Hypothesis are based on the interplay of substrate additions with varying C, and C and N, contents to soils with high or low C:N ratios. Hypothesis 1 predicts positive priming (+ PE) under addition of C-source only (+C "glucose") in soils with high C:N ratio (↑C:N) based on microbial N-mining and hypothesis 2 predicts negative priming (-PE) under addition of a combined C and N source (+C +N "glucose with N and nutrients") in soils with high C:N ratio (↑C:N) based on preferential substrate-use. In contrast, hypothesis 3 predicts negative priming (-PE) based on preferential substrate use under addition of C-source only (+C "glucose") in soils with low C:N ratio (↓C:N) and hypothesis 4 predicts positive priming (+PE) under addition of a combined C and N source (+C +N "glucose with N and nutrients") in soils with low C:N ratio (↓C:N) based on microbial stoichiometry.

Hypothesis	Substrate addition	Soil C:N	Predicted PE	Proposed mechanism
1	+C	↑C:N	+PE	N-mining
2	+C +N	↑C:N	-PE	Preferential substrate use
3	+C	↓C:N	-PE	Preferential substrate use
4	+C +N	↓C:N	+PE	Microbial stoichiometry

2.3 Material and methods

2.3.1 Study sites and field sampling

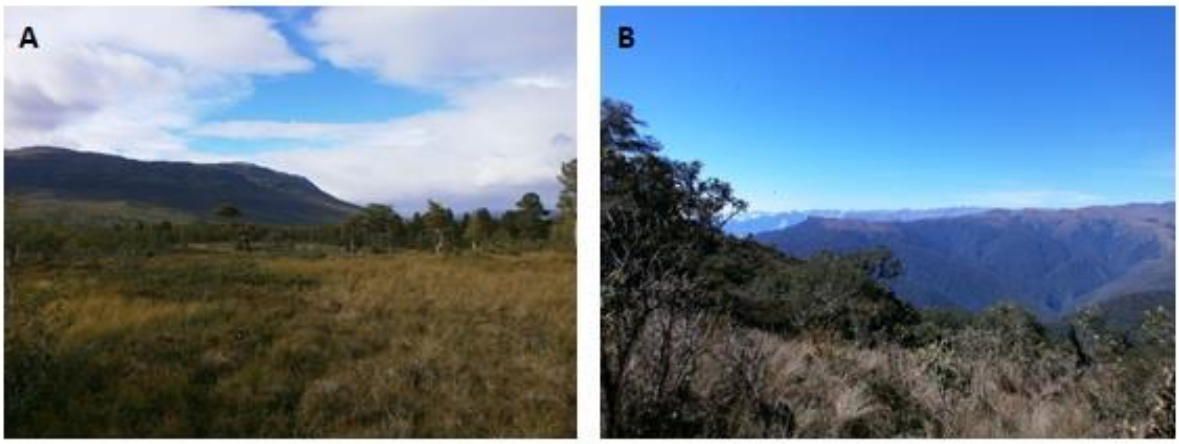


Figure 2.1: Land scape at the study sites A: Boreal treeline 200km North the Arctic Circle at field site near Abisko, Sweden and B: high altitudinal treeline 3300 m asl in the Peruvian Andes at Tres Cruces, Peru

Soils were collected during summer and autumn 2016 in the high altitudes of the Peruvian Andes in Manú National Park (Tres Cruces) in the department of Cusco, Peru at an average elevation of 3300 m, and in the sub-arctic at the Research Station of Abisko, 250 km north of the Arctic Circle in Northern Sweden. These locations in the two countries served as model ecosystems to represent high altitudinal and high latitudinal treelines. The sites provide a contrast in elevation, latitude and land cover types. The Peruvian study area comprised a montane tropical forest with a short transition zone leading into Puna grasslands at around 3300 m a.s.l., while in Sweden the studied ecotone diverges from mountain birch *forest* to tundra heath. In each country, 30 m transects were established across the treelines (transition zones between the timberline and the tree species line (Berdanier, 2010)). On each end of these replicate transects 15 m x 15 m plots (225 m^2) were established, i.e. one plot within the forest and one plot in the adjacent upland (Puna grassland / tundra heath). Within each plot, five soil replicates were taken individually for organic soils and top 15 cm of mineral sub soils. Samples from each plot were combined into one field compound sample for each horizon in each plot, from which approximately 20 litres were sealed in plastic bags and stored at 7°C. This resulted in a set of eight distinct soil types: PFO: Peru Forest Organic, PFM: Peru Forest Mineral, PPO: Peru Puna Organic, PPM; Peru Puna Mineral,

SFO: Sweden Forest Organic, SFM: Sweden Forest Mineral, STO: Sweden Tundra Organic, STM: Sweden Tundra Mineral.

2.3.2 Pre-incubation analysis

From each country, samples from six transects were chosen for this experiment (n=48 soils: 2 land cover types x 2 soil horizons x 6 transects x 2 countries). For these, the following basic soil characteristics were determined: pH, field water content, maximum water holding capacity (WHC), bulk density, total carbon and nitrogen contents and extractable nitrogen. Soil pH was determined using a Hanna HI-111 pH/ORP Metre according to Emmett et al. 2008. For each sample, 10 g of field moist soil was mixed with 25 ml deionised water, stirred and allowed to settle overnight, before the pH was recorded. Dry matter and water content were determined by drying soil samples at 105 °C until constant weight (Schlichting and Blume, 1966). Maximum water holding capacity was defined as the difference in weight of completely saturated soil (field capacity) and dry soil. The mass and volume of rocks and roots was determined and subtracted from soil mass and volume to calculate bulk density (BD). Soil texture was determined following standard field protocols (VD LUFA I, D 2.1, 1997) and detailed on three composite samples for each soil type by analysis of stratification in suds solution ("jar test" as in Sitton & Story, 2006). Total soil C and N concentration were analysed on 5 g oven-dried (105 ^{o}C) sub-samples, which were ground and analysed via combustion and thermal conductivity detection (Elemental analyser Vario EL). Sub-samples of 5 g soil each were extracted with 0.5 M K_2SO_4 for analysis of mineral nitrogen (extractable ammonium (NH_4^+) and nitrate (NO_3^-)). Extracts were colometrically analysed on an autoAnalyser (Bran and Luebbe, Northampton, UK). Microbial biomass carbon and nitrogen were analysed on fresh soils, using the direct extraction method after Fierer & Schimel, 2003: A K_2SO_4-salt solution was used as extractant and liquid EtOH-free chloroform ($CHCl_3$ stabilised with amylene)) directly added to release C and N from microbial cells. All soils were analysed as duplicates, where one rep was extracted with salt solution only ("NF": 5 g fwt soil + 25 ml 0.5 M K_2SO_4 (pH adjusted to 6.8-7 /w NaOH)) and an equal sample was additionally treated with $CHCl_3$ ("F": 5 g fwt soil + 25 ml 0.5 M K_2SO_4 (pH

6.8-7 /w NaOH) + 0.5 ml $CHCl_3$). C and N of all samples (F and NF) was analysed using a TOC/TN analyser (5000A, Shimadzu, Milton Keynes, UK) and extractable microbial biomass C and N were calculated by subtracting the non-"fumigated" (NF) from the "fumigated" (F) contents.

2.3.3 Experimental design

This study investigated how soil, substrate and microbial C:N ratios interact to determine priming effects in contrasting ecosystems. Therefore, soils from six transects covering two land cover types (forest, upland) and two soil horizons (organic, mineral) each in two countries (Peru, Sweden) were amended with four substrate treatments or a water control and incubated for 21 days. The C-component of all treatments was 10 atom% - enriched ^{13}C glucose. Glucose itself has ecological importance, as the dominant soluble carbohydrate in the rhizosphere (Gunina & Kuzyakov, 2015). It was administered as glucose-only addition (treatment 1), and in combination with Hoagland's solution as nutrient-source at three different C:N ratios (treatments 2-4, Table 2.2). The amount of nitrogen in the C:N treatments was varied to obtain final C:N ratios of 7:1, 17:1 and 71:1, mimicking C:N of microbial biomass, SOM and leaf litter respectively (Mooshammer et al., 2014a). The C-content of all additions was maintained at a constant ratio of 30 % substrate-C to microbial-C for each soil type from both countries. Isotopically labelled material was prepared as a 10%-enriched $^{12/13}C$-glucose blend of D-glucose (U-13C6, 99 %, Cambridge Isotope Laboratories) and D-(+)-Glucose (99.5 %, Sigma Aldrich). Resulting substrate additions were significantly lower in C and N compared to many other studies (e.g. Hartley et al., 2010; Whitaker et al., 2014b; Wang et al., 2015). The constant C-addition allowed to separate potential effects of N and nutrient limitation from energy (C) limitation without substrate-saturation. Further, this moderate addition rate reduced the risk of enhancing microbial biomass turnover and therewith 'apparent' priming (Blagodatskaya & Kuzyakov, 2008). That is, when increased turnover of microbial biomass contributes to CO_2 release from soil without affecting soil carbon itself. It is therefore important to avoid apparent priming, as it impedes the quantification of PEs based on measurements of total soil respiration.

The buffered nutrient solution was chosen as nitrogen compound to minimise the confounding factor of pH shifts caused by substrate chemistry, a problem caused when adding substances like oxalic acid or mineral nitrogen. After testing a range of nitrogen sources present in natural environments, including alanine and urea, Hoagland's solution (H2395, Sigma Aldrich) was chosen, as it has a moderate effect on soil pH compared to those other N-sources (Appendix Fig.V2-C2.1). Even though pH changes do also occur in natural rhizospheres (Hinsinger et al., 2003), it is difficult to disentangle their effect in soil manipulation experiments, as pH itself is closely correlated with PEs (Aye et al., 2018; Rousk et al., 2010) and changing the pH in soil solution also affects extracellular enzymes, and hence SOM degradation, without necessarily affecting soil microbial activity (Frankenberger & Johanson, 1982). Hence, the causality between such substrate additions and soil carbon priming cannot purely be attributed to substrate stoichiometry or microbial metabolic processes, but may be biased by a pure chemo-physical change in the soil matrix and thus modifying the charge of extracellular enzymes, nutrient binding to aggregates (Burns, 1982). However, the here used solution also contained salts and phosphorous (at N:P 6:1, detailed composition Table C2-A1.1), which remained at traces in the administered substrate solutions. Therefore, the N-gradient is in fact also a proportional gradient of micro-nutrients at marginal concentrations.

From each composite soil sample, 40 g fwt of soil was taken and moisture content adjusted to 75% of maximum WHC using deionised water (accounting for the additional liquid added as substrate solution). Soils were kept in the incubation chamber at 13 °C for five days pre-incubation and weighted before substrate addition. Aliquots of each soil (5 g fwt) were then placed in 250 ml Kilner jars (48 soils x 5 treatments, n = 240). The experiment was conducted in two batches of 120 jars, with a randomized selection of soils in each batch. All substrate additions were administered as 1 ml liquid solution to the soils. After uniformly distributed pipetting of substrates to the soil samples, jars were flushed with compressed air for 40 seconds, then sealed with lids and rings and over-pressurised by injecting 40 ml compressed air through stoppers in the lids. Soils were incubated for 21 days at 13 °C. Gas sampling for total CO_2 analysis (5 ml) was conducted at t_0=0 hours, t_1 = 24 hours, t_2 = 48 hours, t_3 = 7 days, $t_{3.2}$ = 8 days, t_4 = 14 days, t_5 = 21 days) after starting the experiment, with samples

for ^{13}C analysis (20 ml) taken at t1, t3, t3.2 and t5. Samples were kept in evacuated exetainers (Labco, UK), which were over-pressurised with sample-air by injecting 5/20 ml sample into 3/12 ml exetainers for subsequent $^{12/13}C$ analysis (Perkin Elmer Autosystem Gas Chromatograph, Speck & Burke, UK) followed by cavity ring-down spectroscopy (CRDS) using Picarro G2201i with a multiplexor (Picarro Inc., USA). To keep CO_2 headspace concentrations in the jars below 10000 ppm to avoid related feedbacks on soil respiration, the jars were opened after sampling at t_3, then flushed, over-pressurised and sampled again as at the beginning of the experiment.

Table 2.2: Composition of the substrate additions: Microbial biomass carbon (mbC, mean ± SE, n = 12) for the different soil types and C and N contents of the substrate additions. All treatments included addition of C corresponding to ≈ 30 % microbial biomass in form of ^{13}C-labelled glucose (LMW-sugar as in exudates (Jones et al., 2009)). Glucose-addition only in treatment 1, treatments 2 to 4 additionally included a nutrient solution, with the nitrogen (N) content adjusted to provide solutions of C:N ratios mimicking biological counterparts of ecological relevance (Mooshammer et al., 2014a). Abbreviations: Swe: Sweden, org: organic, min: mineral, dwt: soil dry weight, fwt: soil fresh weight, LMW: low molecular weight, SOM: soil organic matter.

Soil type	**Microbial biomass C** (µg g^{-1} dwt)	**Substrate addition** (µg 5 g^{-1} fwt, 75 % WHC)			
		C (≈30% mbC)	**N**		
Swe org	410.53 ± 114.78	138.67	1.95	8.16	19.81
Peru org	152.18 ± 39.9	75.65	1.07	4.45	10.81
Swe min	27.85 ± 11.21	27.71	0.39	1.63	3.96
Peru min	65.03 ± 25.8	41.63	0.59	2.45	5.95
Treatment		1	2	3	4
C:N of added solution		1:0	71:1	17:1	7:1
Representing		LMW-sugar (exudate)	leaf litter	SOM	microbial biomass

2.3.4 Isotopic and source partitioning

The amount of primed carbon in soil respiration (R_{PE}) was specified as the organic matter-derived CO_2-C respired from soils amended with glucose solution (R_G) relative to the total amount of CO_2-C respired from untreated control soils (R_T). The ^{13}C labelling of the added solutions allowed the separation between respiration from soil-C (R_S) and glucose-C (R_G) using mass balance:

$$R_S + R_G = R_T \quad (Eq.1)$$

$$R_S \times {}^{13}C_S + R_G \times {}^{13}C_G = R_T \times {}^{13}C_T \quad (Eq.2)$$

$$R_{PE} = R_G - R_T \quad (Eq.3)$$

The amount of primed C was then expressed as µg CO_2-C per g soil C, in order to normalise for the differences in soil type and carbon contents and increase comparability amongst the soils and their priming potential. The magnitude of priming (%) expresses the amount of primed carbon relative to the amount of carbon respired from untreated control soils. In order to set the observed PE into ecosystem context, the amount of primed C was upscaled to respective soil C stocks and expressed as magnitude (‰) change to stock. As substrate C:N had no significant effect on the observed PE, values were averaged across the four treatments for each soil type. Substrate use (%) was calculated as the amount of added substrate-C detected in soil respiration, divided by the initial amount of substrate-C added to the soil and multiplied by 100.

Substrate use (%) was calculated as the amount of added substrate-C which was detected in microbial respiration, divided by the initial amount of substrate-C added and multiple by 100. As in this experiment, the ratio of substrate-C to microbial biomass-C was kept at a constant ratio (30 %) for all soils and treatments, and substrate was added only once at the beginning of the experiment, increased substrate-C in respiration is a result of increased substrate utilisation (and not from higher substrate availability). This approach does, however, not include the incorporation of substrate-C into microbial biomass. The low ratio of substrate-C to microbial-C and the incubation time of 21 days were chosen to minimise microbial turnover and omit apparent priming (Blagodatskaya & Kuzyakov, 2008; Blagodatskaya et al., 2009, 2011; Chen et al., 2014).

2.3.5 Statistical analysis

Statistical analysis was carried out using R 3.4.1. (R Core Team, 2017). Similarities between substrate C respired, primed C and total CO_2-fluxes amongst soil types were tested by two-way ANOVA followed by Tukey tests for the different intervals of incubation (0-7, 8-21 and 0-21).
Analysis of covariance (ANCOVA) was applied to data subsetted as above, as well as to a combined dataset for the full incubation period for both countries, to compare mean PE under different treatments and given different intrinsic soil and microbial biomass C:N ratios in the different soil types. In each model, primed C was the dependent variable, substrate C:N a categorical independent variable with four levels (each treatment) and microbial and soil C:N were covariates. Moreover, Person's square correlations were used to test for linearity between substrate-use and the magnitude of priming for each soil type. Beta regression via maximum likelihood using parametrization with mean and logit link (Cribari-Neto & Zeiles, 2010) was applied to statistically determine potential drivers of substrate-use for the data sets of each country respectively (Peru and Sweden).

2. 4 Results

2.4.1 Soil and microbial characteristics

The soils studied in this experiment had contrasting edaphic and microbial properties (Table 2.3). Soil texture and soil horizon depth varied between countries and land cover type, as well as soil moisture content and maximum water holding capacity (WHC). All soils studied were slightly acidic, the Peruvian more than the Swedish soils, ranging from 4.2 (PFO) to 5.7 (SFM). The soils represented a broad range of soil characteristics and soil and microbial biomass C:N ratios. For the Peruvian soils, soil C contents of both organic and mineral layers were higher in the forests compared to the adjacent Puna grasslands, while microbial biomass C was higher above the treeline. Microbial biomass N (mbN) was higher in the organic layer of the forest compared to the organic layer of the Puna, while this interaction was reversed for the mineral soils (i.e. high microbial N above the treeline). For the Swedish soils, C and N were higher in the mineral Tundra soil compared to mineral forest soil, while soil N in the organic horizon was higher in forest soils, and soil N in the mineral horizon was higher above the treeline. For microbial biomass C, the same pattern as for the Peruvian soils emerged: elevated microbial C in both organic and mineral soils above the treeline. All Peruvian organic and mineral soils had lower soil C:N ratios (12.3 - 15.5) and lower microbial biomass C:N ratios (2.9 - 6.2) than the comparative Swedish soils, where soil C:N ranged from 25.5 - 34.7 and microbial biomass C:N from 5.9-16. Microbial C:N reflected soil C:N for all soils, with stoichiometric imbalances between 2.08 (PPO) to 5.7 (PFM). For all soils, microbial C:N of the organic horizon was approximately twice as high as microbial C:N compared to corresponding mineral horizons. Extractable nitrogen was comparable for all Peruvian soils, while in the Swedish soils, extractable N was higher in the organic compared to the mineral soils. The contrast between organic and mineral horizons was pronounced for most of the analysed characteristics in the Swedish soils, where notably the total amount of C and N was up to 16 x higher in the organic soils (e.g. for microbial biomass C (mbC)). The highest soil carbon contents were located in the shallow O-layers of the Swedish soils, while total soil nitrogen content was comparable between Swedish and Peruvian soils.

Table 2.3: Key edaphic and microbial properties of the eight soil types studied. Values are mean ± SE for n =6. Abbreviations: C: carbon, N: nitrogen, mb: microbial biomass, Max WHC: maximum water holding capacity, dwt: dry weight, Org: organic, Min: mineral, OM: organic matter

Soil ID	**PFO**	**PFM**	**PPO**	**PPM**	**SFO**	**SFM**	**STO**	**STM**
C ($g\ g^{-1}$ dwt)	0.404 ± 0.105	0.24 ± 0.07	0.27 ± 0.04	0.18 ± 0.04	0.56 ± 0.07	0.08 ± 0.02	0.52 ± 0.2	0.19 ± 0.21
N ($g\ g^{-1}$ dwt)	0.026 ± 0.006	0.015 ±0.004	0.021 ±0.002	0.015 ±0.003	0.022 ±0.005	0.003 ±0.002	0.015 ±0.004	0.006 ± 0.009
soil C:N	15.5	15.3	12.9	12.3	25.5	26.7	34.7	31.7
mb C ($\mu g\ g^{-1}$ dwt)	152.01 ± 55.1	48.91 ±28.26	152.4 ±30.86	66.36 ±33.54	328.9 ±84.95	20.96 ±11.13	492.1 ±75.82	28.74 ± 14.75
mb N ($\mu g\ g^{-1}$ dwt)	36.06 ± 14.59	16.9 ± 4.26	24.48 ± 3.2	18.47 ± 7.36	30.1 ±18.74	3.52 ± 1.43	30.74 ± 10.4	3.49 ± 2.2
mb C:N	4.2	2.9	6.2	3.6	10.9	5.9	16	8.2
NO_3^- ($\mu g\ g^{-1}$ dwt)	4.94 ± 0.14	4.82 ± 0.07	4.82 ± 0.04	4.91 ± 0.18	7.94 ± 1.34	6.3 ± 0.33	7.13 ± 1.18	5.86 ± 1.32
NH_4^+ ($\mu g\ g^{-1}$ dwt)	1.12 ± 0.22	1.36 ± 1.14	1.48 ± 0.22	1.74 ± 2.08	1.79 ± 0.54	0.83 ± 0.14	1.58 ± 0.35	2.05 ± 2.13
pH	4.2 ± 0.36	4.5 ± 0.36	4.6 ± 0.18	4.7 ± 0.33	5.1 ± 0.63	5.7 ± 0.78	4.8 ± 0.91	5.4 ± 0.8
Horizon depth (cm)	15 ± 3.89	48 ±20.62	10 ± 4.51	34 ± 7.86	4.8 ± 2.18	35 ± 6.96	7.5 ± 2.34	28 ± 5.77
Max WHC (%)	294.7 ± 72.78	184.3 ±29.29	236.5 ±38.08	160.9 ±32.13	463.2 ±84.52	50.3 ±10.83	615 ±58.25	78.2 ± 65.71
Soil compo-sition	OM (+silty clay)	(Silty) Clay loam	OM (+silty clay (loam))	(Silty) Clay loam	OM (+silt)	Sand	OM (+silt)	(Loamy) Sand

2.4.2 Soil respiration

All soils responded to substrate additions with a peak in respiration (48 hrs after substrate addition, but Swedish mineral soil respiration peaked earlier at 24 hrs) and then plateaued at basal respiration of control soils. Control soils were amended with 1 ml water at the beginning of the experiment in the same manner as substrate solutions were applied. Moderate peaks in control soil respiration were observed within the first 48h of incubation (not for organic forest soils PFO, SFO). Respiration rates from Peruvian soils were 4 - 20 lower than fluxes from Swedish soils (Fig. 2.2).

No significant differences between the fluxes were reported under four different substrate additions. Total soil respiration was significantly different between soil types (Table 2.4). Within both countries, a similar gradient of increasing soil respiration through the ecotones was identified (Fig. 2.2): mineral soils from above the treeline (PPM, STM) presented the lowest fluxes, followed by two-fold higher respiration from corresponding organic soils (PPO, STO). Similarly, mineral forest soil respiration (PFM, SFM) was half as high as fluxes measured from organic forest soils (PFO, SFO). The greatest peak amplitude was observed in soil respiration of organic Tundra soils (STO), where substrate addition tripled the flux rates at 48 hrs after substrate addition compared to basal control soil respiration.

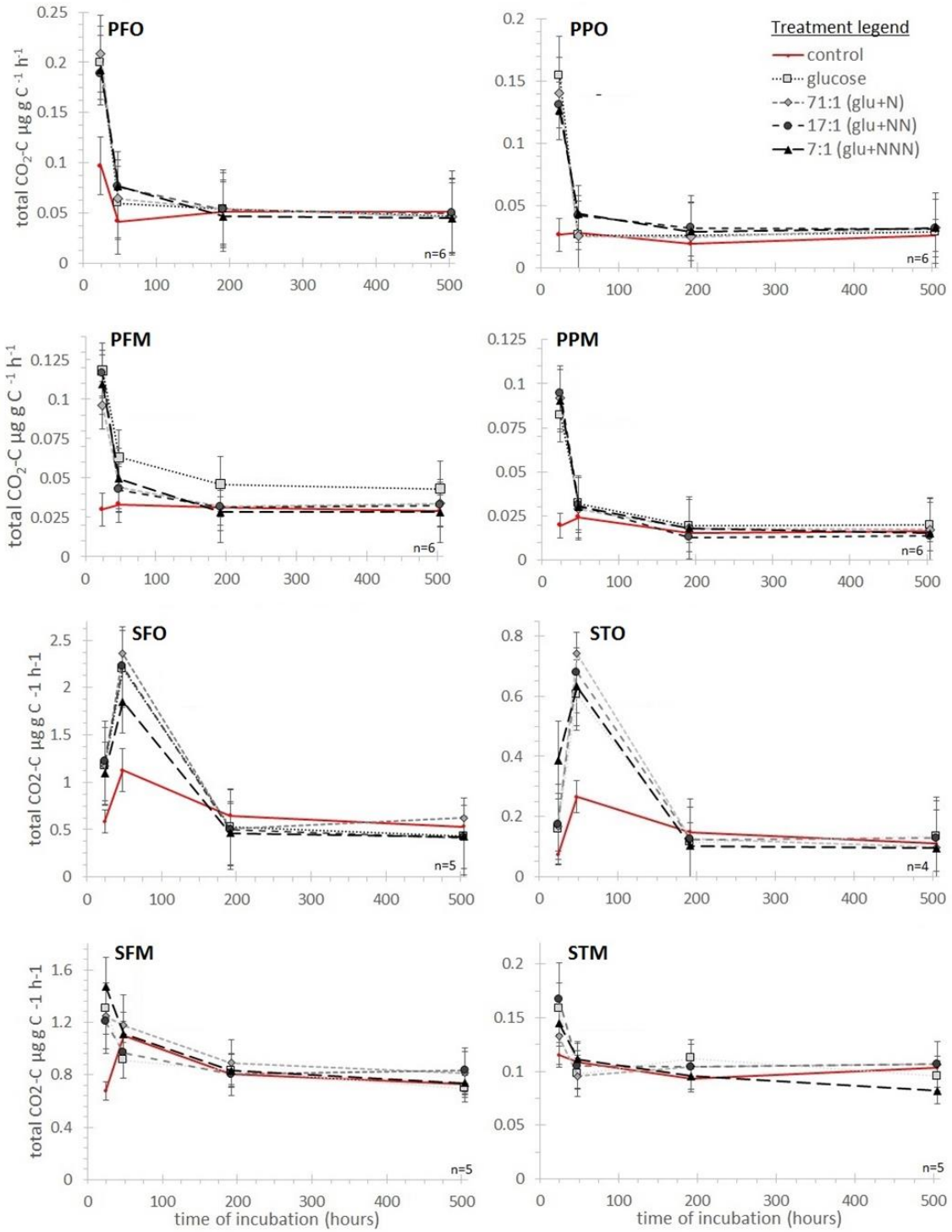


Figure 2.2: Fluxes of CO_2-C (µg g C^{-1} hrs^{-1}) from eight soil types under four treatments of glucose-C with increasing N and an untreated control soil (red line), measured during 504 hours (21 days) of controlled laboratory soil incubation: Soil types: PFO: Peru Forest Organic, PFM: Peru Forest Mineral, PPO: Peru Puna Organic, PPM; Peru Puna Mineral, SFO: Sweden Forest Organic, SFM: Sweden Forest Mineral, STO: Sweden Tundra Organic, STM: Sweden Tundra Mineral.

2.4.3 CO_2 source partitioning

Using isotopic source partitioning the contribution of soil- and substrate-derived carbon to respired CO_2 was determined from respiration of each treated soil both for the initial period of 0 - 7 days of incubation (d.o.i.) and the final period of 8 – 21 d.o.i. (Fig. 2.3). While the contribution of substrate-C to overall CO_2-C was significantly higher within the first week of incubation, compared to the second period of incubation, the amount of primed C did not differ significantly between the two time intervals (Table 2.4). Treatment (substrate C:N ratio)had no significant effect on the amount of C primed from SOM or on substrate C respired (Table 2.5).

Table 2.4: Comparison of sample means of substrate C respired and primed C for subsetted time intervals (0-7, 8-21 and 0-21 days of incubation). Letters indicate similarity of sample means following two-way ANOVA and post-hoc Tukey tests. p-value for differences between initial (0-7 days) and final periods (8-21 days) of incubation. Last column shows letters for similarity of fluxes of different soil types. A: Peru, B: Sweden

A		substrate C			primed C			Flux
		0-7	8-21	0-21	0-7	8-21	0-21	0-21
Soil type	PFO	c	ab	c	b	a	b	b
	PFM	ab	a	ab	ab	a	ab	ab
	PPO	ac	b	ac	a	a	a	ab
	PPM	b	b	b	a	a	a	a
PER	p-value (time)	1.16 e^{-5} ***			0.323			0.004**

B		substrate C			primed C			Flux
		0-7	8-21	0-21	0-7	8-21	0-21	0-21
Soil type	SFO	b	b	b	b	b	b	a
	SFM	a	a	a	a	a	a	a
	STO	a	a	a	a	ab	a	b
	STM	a	a	a	a	ab	a	b
SWE	p-value (time)	1.13 e^{-8} ***			0.145			3.94e^{-14}***

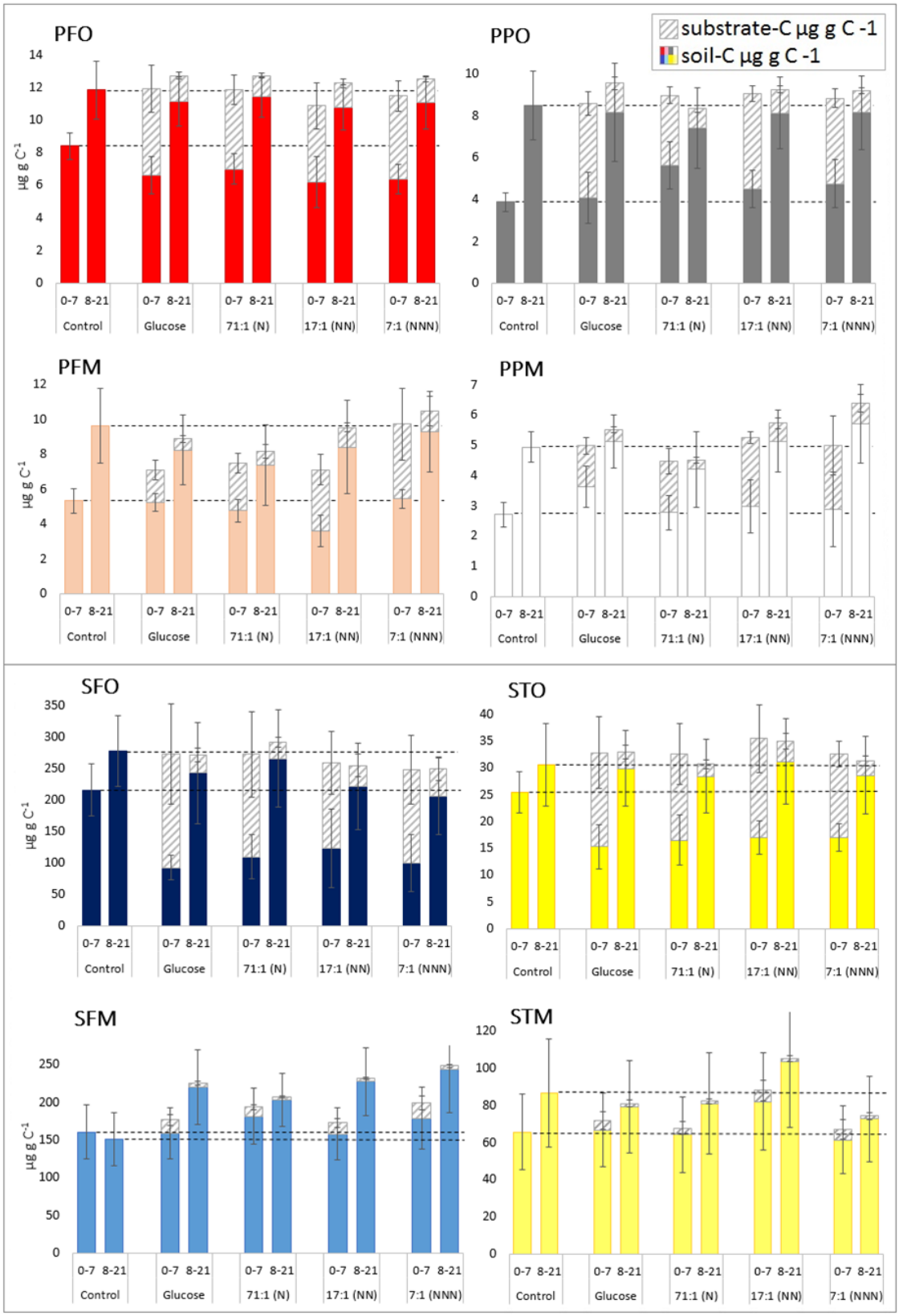


Figure 2.3: Cumulative respired CO_2-C (µg g^{-1} soil C) partitioned between soil and substrate-C for eight soils incubated for 21 days, (presented for 0-7 and 8-21 days of incubation). Bars represent mean ± SE (n=(5)6 (*SFM)) with coloured parts representing soil-derived C and upper grey hatched parts substrate-derived C. Dotted lines indicate level of control soil respiration for each incubation period. Where amounts of SOM-derived C are higher (lower) compared to untreated control soil at corresponding time, a positive (negative) PE is reported.*

*Table 2.5: Results of analysis of covariance (ANCOVA) with primed C as dependent variable, substrate CN as categorical independent variable with four levels (treatments) and microbial and soil C:N as covariates for each soil type from each country respectively. Analysis performed for initial and final periods of incubation (0-7 and 8-21 days) and for cumulative priming during full period of incubation (0-21 days), significance codes for p < 0.001***, 0.01**, 0.05*. Where no numbers are given, variables were non-significant (ns) with p > 0.1. A: Peru, B: Sweden*

A	**Peru Forest Organic (PFO)**			**Peru Puna Organic (PPO)**		
	0-7	8-21	0-21	0-7	8-21	0-21
subCN						
soilCN	0.028*		0.045 *	$8.23e^{-5}$***	0.002 **	0.021*
mbCN						
subCN:soilCN						
subCN:mbCN						
soilCN:mbCN				0.0006***	0.0004***	
subCN:soilCN:mbCN						
Country x land cover type x soil horizon	Peru Forest Mineral (PFM)			Peru Puna Mineral (PPM)		
days of incubation	0-7	8-21	0-21	0-7	8-21	0-21
subCN						
soilCN			0.005**			0.028*
mbCN						
subCN:soilCN			0.014 *			
subCN:mbCN		0.008**				
soilCN:mbCN			0.0006 ***			
subCN:soilCN:mbCN						

B	**Sweden Forest Organic (SFO)**			**Sweden Tundra Organic (STO)**		
	0-7	8-21	0-21	0-7	8-21	0-21
subCN		0.046 *	0.026 *			
soilCN	0.0009***		0.001**	0.006**	0.003**	$7.63e^{-5}$***
mbCN						
subCN:soilCN						
subCN:mbCN						
soilCN:mbCN						
subCN:soilCN:mbCN						
Country x land cover type x soil horizon	**Sweden Forest Mineral (SFM)**			**Sweden Tundra Mineral (STM)**		
days of incubation	0-7	8-21	0-21	0-7	8-21	0-21
subCN						
soilCN	0.002**	0.007**	0.003**			
mbCN						
subCN:soilCN	0.005**		0.042 *		all ns	
subCN:mbCN						
soilCN:mbCN	0.0002***		0.005**			
subCN:soilCN:mbCN						

2.4.4 Soil carbon priming

Across the different soils and treatments, both positive and negative PEs were measured, with varying direction under different C:N treatments. In addition, the variance amongst replicates within a treatment and soil type often spanned both positive and negative PEs. The amplitude of quantitative amounts of primed C ranged between – 10 and + 5 µg C g^{-1} soil C for the Peruvian soils and from -150 to + 40 µg C g^{-1} soil C for the Swedish soils. Significantly lower priming compared to the other soils was observed for the Swedish organic forest soil (SFO), which showed strong negative priming. There was no significant treatment effect (Table 2.5), but PEs were characteristic for the different land cover types in Peru and soil horizons in Sweden (Fig. 2.4).

For both countries, the observed pattern of priming amongst the different landcover types was consistent under all treatments. Apart from treatment with C:N 71:1, the lowest N-addition, PE increased for soils according to the treeline gradient: Lowest priming (negative) was reported in organic forest soils and highest priming (positive) in mineral upland soils.

The impact of the PEs measured in this 21-day soil incubation on soil C stocks would have been small. For the Peruvian soils, upscaled PEs would have depleted overall soil C stocks by 0.004 ‰ in organic forest soils and 0.001 ‰ in mineral forest soils and increased soil C stocks by 0.0001 ‰ and 0.0004 ‰ in organic and mineral Puna soils respectively. For the Swedish soils, upscaled PE would have increased soil C stocks by 0.05 ‰ in organic forest and by 0.009 ‰ in organic tundra soils. In the Swedish mineral soils, PE would have depleted soil C stocks by 0.009 ‰ in forest and by 0.001 ‰ in tundra soils.

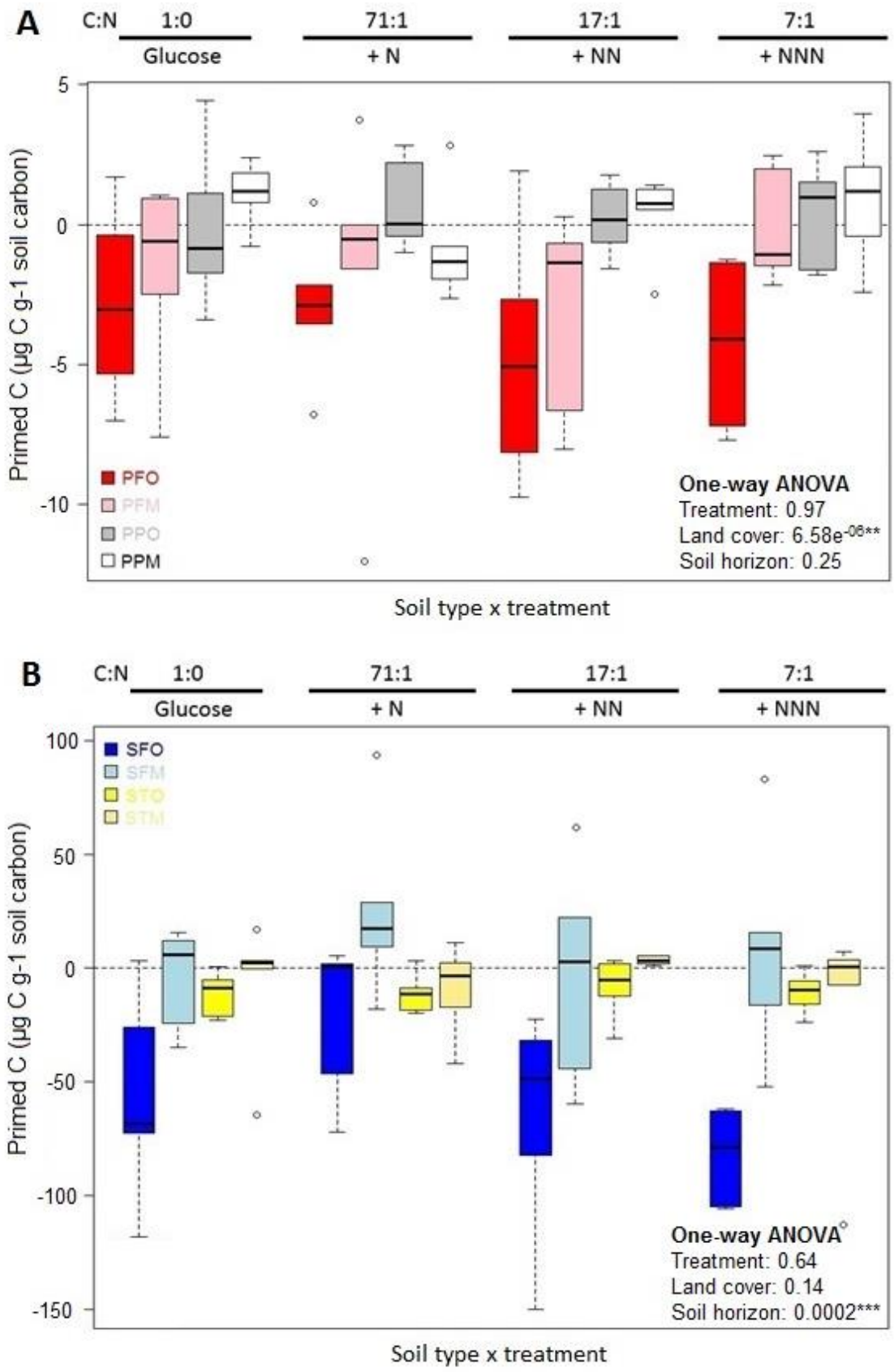


Figure 2.4: Primed carbon (µg C g^{-1} soil carbon) for four soil types from Peru (A) and Sweden (B) under four treatments, all of which included addition of glucose-^{13}C at constant ratio to 30 % of microbial biomass C of respective soil types. First addition is glucose only, the following three contained a nutrient solution at different concentrations, to obtain a gradient of substrate C:N ratios. Boxes show median lines and interquartile ranges for n = 6.

2.4.5 The interplay of soil, substrate and microbial C:N ratios

Analysis of covariance was performed to test for differences of PE under the four substrate additions, while statistically controlling for the effects of soil and microbial biomass C:N. There was large consistency amongst all soil types. Soil C:N was a significant covariate in all analysis (apart from SFM, where ANCOVA was non-significant). In the mineral forest soils from both countries (PFM, SFM) the imbalances between the C:N ratios of soil and substrate and soil and microbial biomass were significant, however not their three-way interaction. Substrate C:N, as well as the imbalance between substrate and microbial biomass C:N, were non-significant in all but one soil (SFO, Table 2.4).

*Table 2.6: Results of three-way ANCOVAs for the sum of eight individual soils types testing for similarities of sample means of observed priming effects under four different substrate treatments in soils with different soil and microbial C and N peculiarities. p-values to significance levels: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' where no numbers are given, variables were non-significant (ns) with p > 0.05.*

Country x land cover type x soil horizon	**PFO**	**PPO**	**SFO**	**STO**
subCN			0.026 *	
soilCN	**0.045 ***	**0.021 ***	**0.001 ****	**7.63e^{-5} *****
mbCN				
subCN:soilCN				
subCN:mbCN				
soilCN:mbCN				
subCN:soilCN:mbCN				
Country x land cover type x soil horizon	**PFM**	**PPM**	**SFM**	**STM**
subCN				
soilCN	**0.005****	**0.028 ***	**0.003 ****	
mbCN				
subCN:soilCN	0.014 *		0.042 *	all ns
subCN:mbCN				
soilCN:mbCN	0.0006 ***		0.005 **	
subCN:soilCN:mbCN				

2.4.6 Substrate-use and priming

The amount of substrate respired was analysed relative to the amount of the initially added substrate (substrate-use (%)) for each soil type respectively and Pearson's square correlations were used to test for linearity between substrate-use and the magnitude of priming. This approach enabled to directly compare mechanisms in both countries, irrespective their variable soil and microbial properties. In both countries, substrate-use was significantly higher in organic soils than in mineral soils, with an approximately two-fold increase in organic soils of one land cover type, compared to their mineral counterparts (Appendix V2-C2-A4). Particularly for the soils with predominantly negative PE, namely the Peruvian forest soils and Swedish organic soils (Fig. 2.4), a strong correlation between the magnitude of priming and substrate-use was evident (Fig. 2.5).

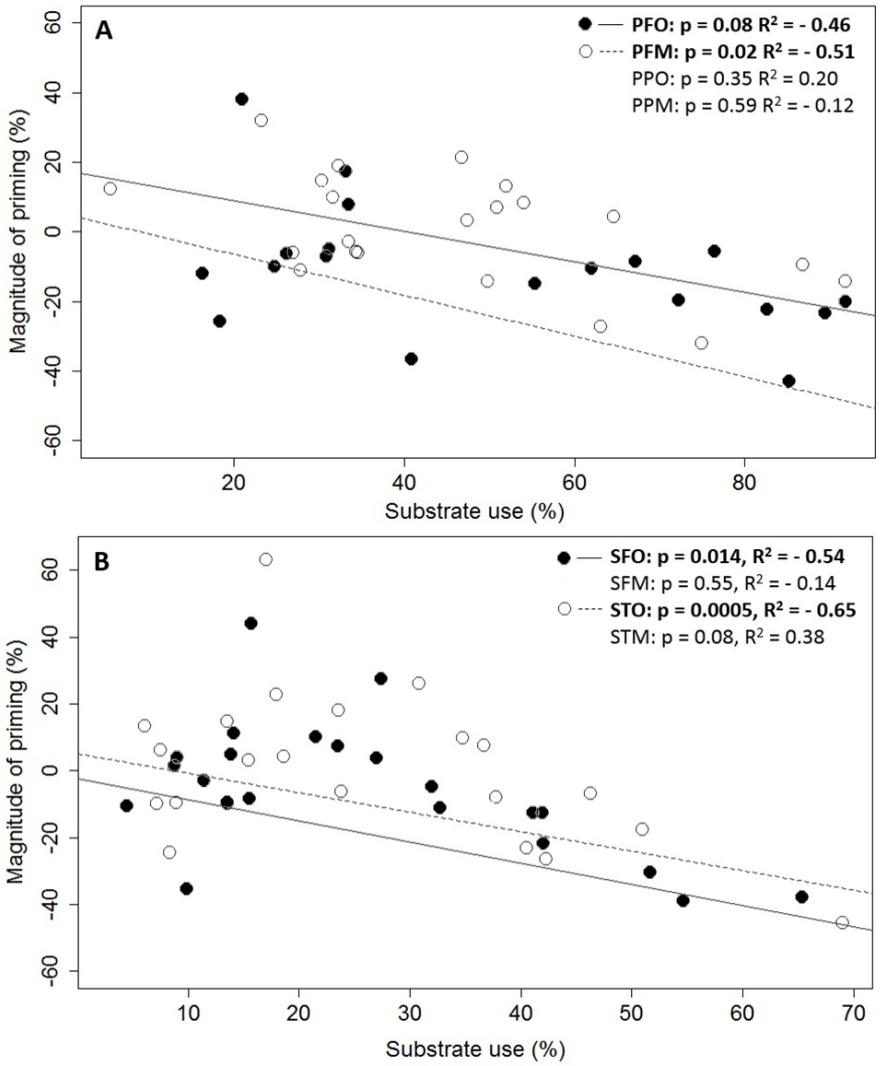


Figure 2.5: Correlation between substrate-use (%) and magnitude of priming (relative PE (%)). Data and regression lines shown for interactions in A: Peruvian forest soils: organic (PFO) and mineral (PFM) and (B) Swedish organic soil: forest (SFO) and tundra (STO). P-values and Pearson's R^2 of all soils provided in figure.

Beta regression was performed to disentangle microbial substrate utilisation respective of contrasting soil C and N contents and the different size and C and N characteristics of the microbial communities in the different soils. Amongst the factors included, models provided consistent results of significant correlation between substrate-use and microbial biomass C and N, as well as the imbalance between the carbon contents of soil, microbial biomass and added substrates coherently for both Peruvian (Table 2.5 A) and Swedish (Table 2.5 B) data sets.

*Table 2.7: Modelled factors potentially determining substrate-use ((%), given different soil and microbial C and N properties in soils from Peru (A) and Sweden (B). Abbreviations: minN: soil mineral nitrogen, Std.Error: Standard error, Pr(>|t|): p-value in reference to t-statistic, Sig.code: significance level at α = 0.05 with codes for p < 0.001***, 0.01**, 0.05*.*

A: Peru	**Substrate use (%)**				
Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**	**Sig.code**
(Intercept)	-1.24	4.58	-0.27	0.79	
soilC	9.67	9.92	0.98	0.33	
soilN	-65.30	158.00	-0.41	0.68	
minN	0.01	0.01	0.74	0.46	
soilCN	-0.13	0.24	-0.54	0.59	
mbC	-0.01	0.005	-2.96	0.003	**
mbN	0.02	0.01	1.98	0.05	*
mbCN	0.41	0.18	2.33	0.02	*
subC	-0.04	0.09	-0.45	0.65	
subN	0.46	0.34	1.33	0.18	
subCN	-0.0005	0.003	-0.21	0.84	
soilC:mbC:subC	0.003	0.001	2.40	0.02	*
soilN:mbN:subN	-0.23	0.20	-1.14	0.25	
B: Sweden	**Substrate use (%)**				
Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**	**Sig.code**
(Intercept)	-2.74	0.66	-4.17	$3.07e^{-5}$	***
soilC	-0.68	1.06	-0.64	0.52	
soilN	8.72	29.65	0.30	0.77	
minN	0.04	0.03	1.38	0.17	
soilCN	0.002	0.01	0.18	0.86	
mbC	-0.001	0.0006	-2.14	0.03	*
mbN	0.04	0.01	3.36	0.0008	***
mbCN	0.03	0.02	2.08	0.04	*
subC	0.001	0.001	1.12	0.26	
subN	2.53	3.60	0.70	0.48	
subCN	-0.001	0.003	-0.50	0.62	
soilC:mbC:subC	-2.74	0.66	-4.17	3.07^{e-5}	***
soilN:mbN:subN	-0.68	1.06	-0.64	0.52	

Combining the observations from the two countries enabled the study of a set of eight distinct soil types, analysing substrate induced priming effects in organic and mineral soils from high Andean tropical forests and Puna grasslands and sub-arctic boreal birch forests and Tundra heath. To test whether consistency amongst models of substrate-use extends to drivers of priming on a trans-national scale, data sets of Peru and Sweden were combined and analysis of covariance was performed with a similar structure as for the individual data sets (Table 2.4). Three-way ANCOVA testing for the interaction of the C:N ratios of soil, microbial biomass and substrate and the response variable primed C indicated that microbial biomass C:N may execute a significant control on priming effects on a transnational scale ($p = 0.0002$, Table 2.8).

*Table 2.8: Results of three-way ANCOVA for combined data of Peruvian and Swedish soils testing for differences of sample means of observed priming effects under four different substrate treatments in soils with different soil and microbial C and N peculiarities. p-values to significance levels: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' where no numbers are given, variables were non-significant (ns) with p > 0.05.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig.code
mbCN	**1**	**17829**	**17829**	**14.97**	**0.0002**	*******
subCN	3	3935	1312	1.10	0.35	
soilCN	1	909	909	0.76	0.38	
mbCN:subCN	3	3284	1095	0.92	0.43	
mbCN:soilCN	1	703	703	0.59	0.44	
subCN:soilCN	3	2218	739	0.62	0.60	
mbCN:subCN:soilCN	3	3855	1285	1.08	0.36	
Residuals	159	189361	1191			

2.5 Discussion

2.5.1 Revision of hypothesis

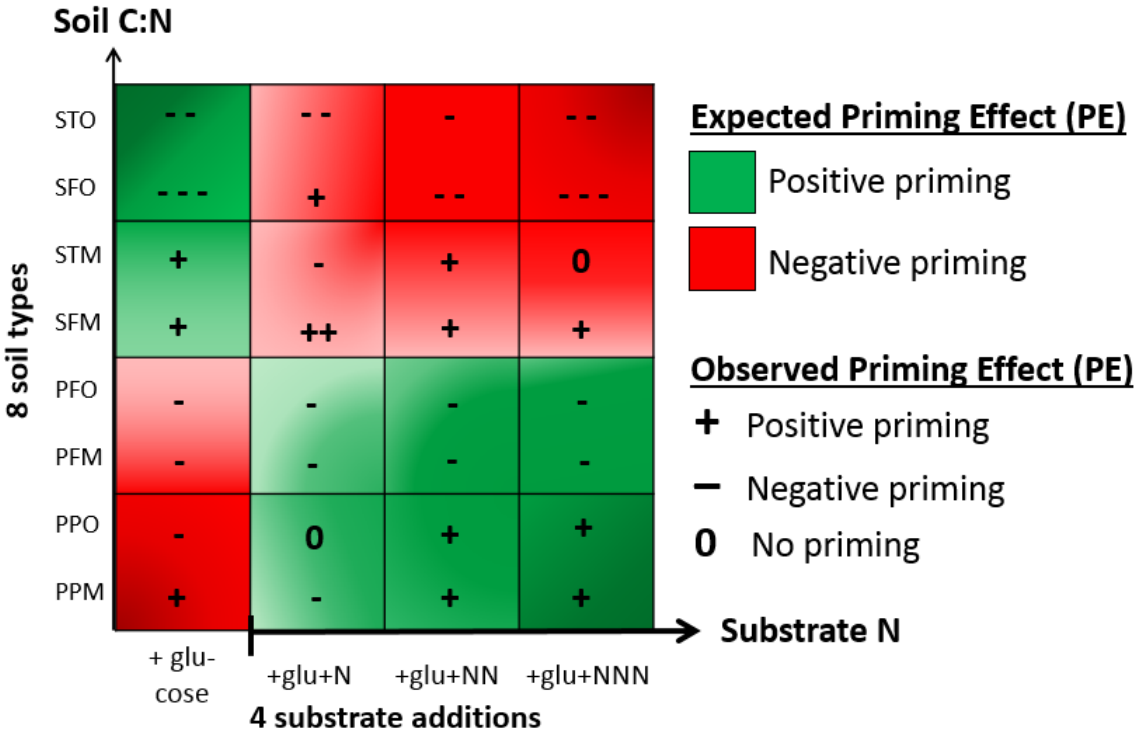


Figure 2.6: Revision of hypothesis: Expected and observed priming effects: Coloured squares represent direction (green: positive, red: negative) and magnitude (brightness) of priming according to hypothesis; plus and minus signs indicate actually observed priming effects with "+" positive and "-" negative priming and number of signs indicating magnitude of PE; Abbreviations: STO: Sweden Tundra Organic, SFO: Sweden Forest Organic, STM: Sweden Tundra Mineral SFM: Sweden Forest mineral PFO: Peru Forest Organic, PFM: Peru Forest Mineral, PPO: Peru Puna Organic, PPM: Peru Puna Mineral, C: carbon, N: nitrogen, PE: priming effect, x-axis: 4 treatments glu: glucose addition, increasing substrate-N additions (at C:N 71, 17, 7), y-axis: soils ranked from low to high C:N ratios with further consideration of extractable Nitrogen and total C and N contents (Table 2.3)

Under hypothesis one (H1) glucose (C) only addition should result in positive priming in soils with high C:N, following the N-mining hypothesis (Chen et al., 2014). This was supported for the Swedish mineral soils (STM, SFM + glu) only, while rejected for the Swedish organic soils (STO, SFO + glu) which showed distinct negative priming under C-only instead. The major difference between the two soil horizons is, that although they both have high C:N ratios, the total contents of C and N are at least twice as high in the organic soils compared to the mineral soils. Hence, the N-mining theory may only apply to soils with limited N availability, but without microbial nutrient limitation. The fact that

substrate C:N was rarely correlated with the observed PE in the here studied soils (Table 2.4, Fig. 2.4) further challenges the N-mining theory, which is in line with other recent studied (Wild et al., 2019; Mason-Jones et al., 2017).

According to the second hypothesis (H2) C+N additions should have cause negative priming in soils with high C:N, as microbes switch their nutrient acquisition to the labile compounds (Wang et al., 2015). This was supported for the Swedish organic soils (STO, SFO + glu+NN + glu+NNN), but rejected for the Swedish mineral soils (SFM + all additions, STM inconsistent). For the organic soils, the significant correlation of the strong negative priming and substrate-use (Fig. 2.5) provides support for preferential substrate-use in these soils. The negative priming in organic forest soils was also accompanied by reduced overall soil respiration (Fig. 2.2). This has been previously observed (Murphy et al., 2015), and also in organic soils from Abisko (Rousk et al., 2016), where the authors suggested that reduced microbial respiration could be due to labile C inputs shifting SOM degradation to more N-rich compounds, and hence reducing total SOM use.

Under hypothesis three (H3) glucose (C) only addition would induce negative PEs in soils with low C:N, as microbes prefer the readily available labile C and nutrients from substrate over taxing SOM-degradation (Cheng et al., 1999). This was supported for most of the Peruvian soils, supporting the preferential substrate use theory for soils with abundant N and no other nutrient limitations (PFO, PFM, PPO + glu). Only for the Peruvian mineral Puna soil (PPM) C-only addition caused positive priming instead (see also Appendix Figures V2-C2.4 & 5).

The fourth hypothesis (H4) predicted positive priming in low C:N soils following combined C+N additions (Zhu et al., 2018). This was observed for the higher N-additions on the Puna soils (PPO, PPM + glu+NN + glu+NNN), but is rejected for the Peruvian forest soils (PFO, PFM + all treatments), where all substrate amendments caused negative priming. Given the large contribution of substrate to soil respiration (Fig. 2.3), the preferential substrate-use theory seems to be applicable to at least three soils under all treatments in this study (Fig. 2.5).

2.5.2 General mechanisms of priming effects

Taken together, some clear conclusions could be drawn regarding the general mechanisms of soil carbon priming in the study soils. The presented experiment was designed to study the mechanisms of priming effects under constant C and varying N + nutrient additions in eight soil types. Confounding substrate-induced pH shifts (Rousk et al., 2010), apparent priming (Blagodatskaya & Kuzyakov, 2008) and bias from inherent contrasting soil properties were excluded by using a buffered nutrient solution, maintaining a constant mbC:subC ratio (30%) and normalisation of the observed priming effects for soil carbon content. Thus, this study was able to relate the observed PEs to the interaction of the C:N ratios of the three compartments of soil, microbes and substrate, and thus represents a multifactorial test of the different mechanisms proposed to describe and explain the changes in microbial activity in soil following substrate C and N additions.

The classical N-mining hypothesis predicts positive priming effects in N-limited systems, when additional energy (C) is invested to mobilise N from SOM (Jingguo & Bakken, 1997). This mechanism could explain the PEs observed for the mineral Peruvian Puna soils (PPM), where the addition of glucose only caused positive priming. But as C + N-additions at higher N-rates also caused positive priming, N-mining does not seem to be the only possible mechanism. However, N-mining powered by glucose could also be the underlying process of the positive priming effects observed for the Swedish mineral soils (both forest and Tundra). However, these soils differ from the Peruvian Puna soils, as regardless their high soil C:N ratios, the overall C and N contents are low (Table 2.3) and the soils have a sandy texture. Hence, soils in their natural state could be limited both in C- and N, which is consistent with the finding that for Swedish mineral soils positive priming prevailed under most treatments (Fig. 2.3). A further characteristic distinguishing Swedish from Peruvian mineral soils are the microbial C:N ratios of Peruvian mineral soils (2.9, 3.6), which are significantly lower compared to the Swedish (5.9, 8.2). This indicates that other factors might be driving soil carbon priming in these soils, such as microbial traits like carbon use efficiency (Mooshammer et al., 2014a) or activity of different functional groups (Fontaine et al., 2003).

Moreover, it could be proposed that soil carbon in OM of the Peruvian soils is not necessarily readily available to microbes, for which clay minerals could be responsible (Keiluveit et al., 2015; Merino et al., 2016). This framework of **physical SOM protection** (Cotrufo et al., 2013) and enhancement of microbial activity through provision of energy (C) and essential nutrients (N et al.) (Sollins et al., 1996), which then triggers positive priming, could be the mechanism behind the PEs reported for the PPM soil in this study. The mineral associated fraction of OM might be more prone to priming, because the addition of labile energy and nutrients might increase microbial ability to produce extra-cellular enzymes, in turn increasing the bioavailability and accessibility of this OM fraction (von Lützow et al., 2006; Keiluveit et al, 2015). This is in agreement with other studies suggesting that deep soil C might be more vulnerable to priming than top soils (Bernal et al., 2016; Heitkötter et al., 2017). Although NMR-analysis of soil aggregate fractions was not within the frame of this study, Zimmermann et al. (2010b) performed such analysis on top soils in the same area in Peru, including a high elevation forest (3030 m), for which they report that 61 % - 98 % of C is present in the form of mineral-bound organic matter (MOM), while 2 % - 39 % of C is in particulate organic matter (POM). The impact of the presence of a resistant pool of SOM, mainly built of recalcitrant carbon physio-chemically bound to the soil matrix, on microbe-governed SOM-mineralisation has been demonstrated for different soils and ecosystems (Averill & Waring, 2017; Cotrufo et al., 2013; Craine et al., 2007; Mikutta et al., 2006).

With the priming effects observed for the Swedish organic forest soils (SFO), namely negative priming under all C+N additions, together with reduced soil respiration compared to control soils, data is provided to support a possible mechanism of **selective targeting of N-rich compounds** (Murphy et al., 2015, Rousk et al., 2016), possibly related to **dynamic adjustment of microbial carbon / nitrogen use efficiency** (Soares & Rousk, 2019). For the organic forest soils from both countries (PFO, SFO) predominantly negative priming under additions of C-only and the different C: N ratios was reported (Fig. 2.4) and a strong negative correlating with substrate-use (Fig. 2.5). Hence, **preferential substrate use** (Cheng & Kuzyakov, 2005) could be an important mechanisms in soils that are naturally neither C- nor N-limited, but was also demonstrated for Peruvian mineral forest soils (Fig. 2.5).

2.5.3 Potential implications for carbon cycling in treeline ecosystems

Soils from two contrasting treelines of a high altitudinal ecosystem in Peru and a high latitudinal ecosystem in Sweden were incubated with different substrates in a short term lab incubation. Consistently for all soils, the observed priming effects changed direction from negative priming below the treeline (within the forest ecotone) to positive priming in upland soils (Puna grassland in Peru and tundra heath in Sweden). If climate change facilitates the expansion of plant species into the uplands above the current treelines, this could also modifying microbial SOM-mineralisation in these regions (Hartley et al., 2012; Parker et al., 2015). The here presented results indicate that modified carbon and nutrient inputs to upland soils can enhance microbial mineralisation of SOM.
However, the carbon source provided to soils in this experiment was glucose only, added at a single time. While this is indeed a naturally highly abundant component of low molecular weight carbon in soils and exudates (Jones et al., 2009; Gunina & Kuzyakov, 2015), and a major determinant of heterotrophic soil respiration (Hoegeberg & Rerad, 2006), it was metabolised quickly and the observed PE ebbed away after 7 days of incubation. Single-time substrate addition also cannot account for other changes in nutrient inputs under treeline advance, such as organic and amino acids and enzymes exudated by roots or varying litter inputs. It can nonetheless still be an important component of long-term soil carbon priming in natural environments, where root exudation continuously and repeatedly provides microbes with labile C and determines rhizosphere priming effects (Shahzad et al., 2015).

Moreover, this study focused to explicitly link priming effects to soil, substrate and microbial C:N ratios. Though in natural environments, further nutrient limitations can impact soil C cycling and PE, for example, phosphorous in tropical soils (Nottingham et al., 2015; Hicks et al., 2019). The observed priming effects under varying C:N inputs therefore not necessarily represent rhizosphere priming in the real ecosystems of soil origin. Further studies could address how the availability of micronutrients differentially affects priming in such contrasting ecosystems as the arctic and the tropics, which may be strongly linked to mineralogy and factors like clay content.

Lastly, estimating the impact of PEs on soil C stocks to capture the potential for soil C sequestration or loss requires careful evaluation of many other factors, such as microbial turnover (Kyker-Snowman et al., 2019) and long-term OM stabilisation processes (Schmidt, Torn et al., 2011). It would be ambitious to extrapolate findings and mechanisms derived in a controlled laboratory soil-with-substrate incubation to ecosystem C-cycling in complex large-scale biomes. Inclusion of PEs in earth system models cannot be claimed based purely on laboratory incubations, mainly because lab incubations do not account for a plant sink or dynamic environmental parameters of plant-soil-microbe interactions.

2.6 Conclusion

This study provides evidence that both positive and negative priming can occur in soil of high latitudes and high altitudes. Negative priming prevailed and these effects are likely also a continuous feature of root-microbe interactions in plant-soil systems, where root exudates provide a continuous source of LMW-sugars to microbial communities.

Support is provided for preferential substrate utilisation in Peruvian forest soils and Swedish organic soils, where increasingly negative priming was significantly correlated with increased substrate use. For the soils whose priming effects have been studied here, the results provided indicate possibly two rate-limiting steps for soil carbon priming: initially, microbial stoichiometry could determine the degree of substrate utilisation, which then consecutively determines the magnitude of priming.

Chapter 3:

Plant induced rhizosphere priming and microbial mediators

(Michel J, Revaillot S, Whitaker J, Hartley IP, Fontaine S)

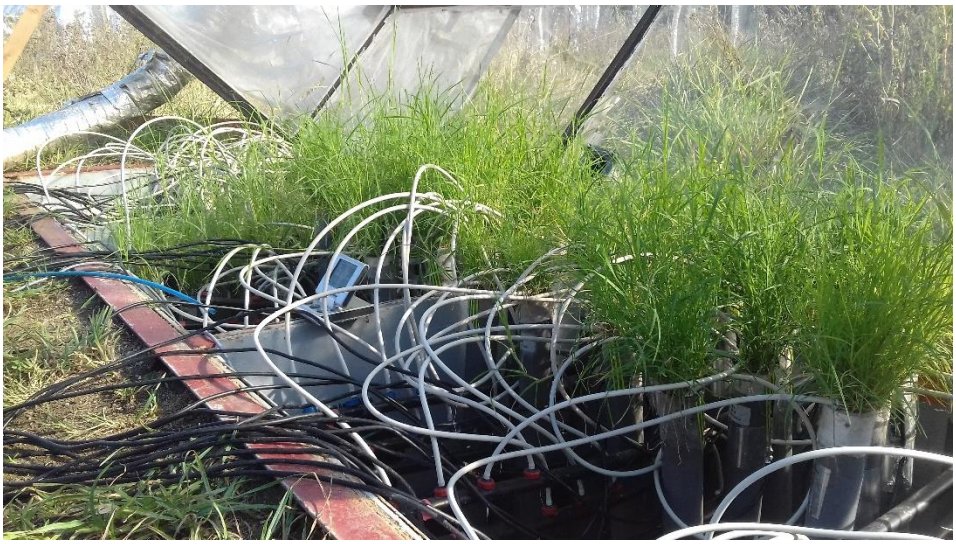


Figure 3.0: Plant-soil mesocosms in the greenhouse

Chapter 3: Plant induced rhizosphere priming and microbial mediators in contrasting ecosystems 105

3.1 Abstract 107
3.2 Introduction 108
3.3 Material and methods
3.3.1 Experimental set-up 113
3.3.2 RPE measurements 114
3.3.3 Post-harvest laboratory analysis 115
3.3.4 Calculation of RPE 118
3.3.5 Data uncertainty 119
3.3.6 Statistical analysis 121
3.4 Results
3.4.1 Data uncertainty 123
3.4.2 RPE amongst different soil types 125
3.4.3 Soil and microbial characteristics 128
3.4.4 Environmental and plant parameter 130
3.4.5 Microbial parameters 132
3.4.6 RPE and key soil and microbial parameter 136
3.4.7 Potential drivers of RPE 138
3.5 Discussion
3.5.1 Seasonal RPE *in vivo* (H1) 140
3.5.2 Distinct priming in organic and mineral soils (H2) 144
3.5.3 Microbial community composition (H3) 146
3.5.4 Plant-soil-microbe interactions 148
3.5.5 Data uncertainty 149
3.6 Conclusion 150

3.1 Abstract

It has been demonstrated that climate change and its effects will be most pronounced in high altitudes and high latitudes, with the arctic and montane ecosystems most severely affected. The treeline ecotone can be an early indicator of climate change induced species shifts. Modifications of aboveground plant communities in these vulnerable regions is likely also impacting the processes by which carbon (C) and essential nutrients (like nitrogen (N)) are transformed or recycled within these ecosystems, in particular within their soils. Understanding the mechanisms that regulate carbon - climate feedbacks is crucial to quantify and predict possible effects of climate change induced species shifts on ecosystem C cycling.

This study provides an assessment of potential rhizosphere priming effects (RPE) in soils from above and below treelines in the Peruvian Andes and sub-arctic Sweden, by introducing a live plant (C_4-grass). Capturing priming effects in presence of a vivid rhizosphere also accounts for the temporal and chemical variation of nutrient flows through root exudates and carbon cycling from plant CO_2-fixation, root exudation and root nutrient uptake, to microbial activity in the rhizosphere. Two soil profiles were differentiated (organic and mineral) for each land cover type (forest and upland: tundra heath / Puna grassland) and RPE estimated at three timepoints during the late growing season (September – November).

Most rhizosphere priming effects reported were negative (reduced mineralisation of soil organic matter (SOM) following the introduction of a plant) and of a similar order of magnitude. Only the Swedish organic tundra soils showed initially positive priming. The observed RPE were combined with the analysis of microbial community structure in respective soils (PLFAs and microbial biomass $^{12/13}C$ and N) to provide a comprehensive approach to the dynamics underlying complex plant-soil-microbe interactions, which could be the pivot-point for large scale ecosystem C-cycling.

3.2 Introduction

Ecosystems in high altitudes and high latitudes share two features, which are of particular importance in the context of climate change: Their soils contain large carbon (C) stocks (chapter 1; Yang et al. 2018; Rolando et al. 2017; Saatchi et al. 2011; Zimmermann et al., 2010) and these biomes are predicted to experience greater than average increases in temperature (Wang et al. 2016; Classen et al. 2015; Wookey et al. 2009; Cramer et al. 2004). Hence, the current carbon sinks of the undisturbed ecosystems in high altitudes and high latitudes are highly vulnerable to climate change with the risk of becoming future carbon sources.

The direct effects of climate change on plant productivity and soil microbial activity, such as increased temperature and elevated carbon dioxide (CO_2), are well studied (Raich et al., 2006; Davidson & Janssens, 2006; Heimann & Reichstein, 2008; Powers et al., 2009; Wood et al., 2012; Hopkins et al., 2012), but less is known about indirect effects, such as changes in plant species distributions. Altered plant community composition will modify plant-soil interactions and how nutrients, particularly C and nitrogen (N), are allocated within the plant-soil-atmosphere-continuum (Pancotto et al., 2005; Raich et al., 2006; van de Weg et al., 2014; Hobbie 2015). Their availability in soils depends on abiotic factors, like physical protection of soil organic matter (SOM) (Cotrufo et al., 2013; Keiluveit et al., 2015), but is centrally regulated by the degradative activity of the soil microbiome. Microbes lead the processing of labile C inputs and their functional capacity determines the extent of C (energy) investment to nutrient acquisition from SOM (Fontaine et al., 2003; Nottingham et al., 2009; Rousk et al., 2010; Mooshammer et al., 2014a,b; Silva-Sánchez et al., 2019).

The degree of SOM-mineralisation by soil microbes can be significantly altered, depending on the aboveground plant species and their interactions with soils and microbes. Changes to aboveground species distribution, as a result of climate change, could therefore have contrasting effects on the carbon cycle and soil organic carbon (SOC) stocks. Increased above and belowground biomass and different recalcitrance of litter from different species can increase the potential for new SOM formation (Rolando et al., 2017; Lange et al., 2015). But counterintuitively greater nutrient inputs to soil do not always result in greater C and N storage (Fontaine et al., 2004), as plant litter and root

exudation can enhance soil organic matter mineralisation by microbes and significantly increase the amount of CO_2 released to the atmosphere (Hartley et al., 2012). This phenomenon of altered degradation of recalcitrant SOM through modified microbial activity following the addition of a labile energy source is known as the priming effect (PE) (Bingemann et al. 1953, but see Kuzyakov, 2000).

In addition to early pioneer work studying priming effects *in vivo* (Cheng & Kuzyakov, 2001, Dijkstra et al., 2006, Shahzad et al., 2015) PE have been mostly studied in controlled laboratory experiments. These generally comprise restricted amounts of soil, which are amended with isotopically labelled substrates, and priming is quantified using the isotopic signature of the stable carbon isotopes ^{13}C:^{12}C, reported in parts per thousand relative to a standard ($\delta^{13}C$), of soil and substrate-derived CO_2 (chapter 2). While there are numerous advantages of such controlled incubations for studying fine scale mechanisms on a molecular level, their suitability to represent and understand priming effects on an ecosystem level is limited by the lack of realistic abiotic and biotic interactions in the common laboratory incubation unit (jar) (Crawford et al., 2017). One of the key processes determining priming effects in nature are plant-soil-microbe interactions in the rhizosphere. Here, the input of rhizodeposits, including root exudates, to the soils alters microbial degradation of SOM, resulting in rhizosphere priming effects (RPE). A positive RPE describes a situation, where plant inputs enhance microbial SOM-degradation and therewith the amount of available nutrients. Negative rhizosphere priming refers to the opposite situation, where microbial degradation of SOM is reduced under plant growth. This can be attributed to decreased demand of nutrients by plants and/or microbes, preferential substrate-use when microbes primarily feed on plant exudates/rhizo-decomposites, an already sufficient supply of available nutrients in the rhizosphere or molecular and/or physical limitations to SOM-degradation (e.g. missing N or phosphorous (P) for enzyme synthesis or aggregate complexation of minerals in the soil matrix). Furthermore, RPEs are subject to diurnal and seasonal fluctuations and their continuous study requires continuous labelling. The easiest way to achieve such labelling is to use the natural difference of fractionation between heavy and light carbon isotopes ($\delta^{13}C$) in C_3 and C_4-plants (Balesdent et al., 1987). Most commonly, C_4 plants

($\delta^{13}C$ approx. -15) are introduced to C_3 soils ($\delta^{13}C$ approx. -25) and the isotopic ratio of their carbon inputs is used to partition old (C_3-soil) and new (C_4-plant) C in soil respiration.

In this chapter, the C_3-C_4 approach was applied to soils collected across treelines in the high altitudes of the Andes and the high latitudes of the Northern hemisphere. Although priming effects have been studied in a range of laboratory experiments and on different soils (reviewed in Blagodatskaya & Kuzyakov, 2008), there is little information on priming effects in tropical environments, particularly in tropical montane soils. This study therefore addresses the potential effects of climate change induced species shifts at the treeline and the possible implications for SOM mobilisation. It is of great interest to extend this research to investigate priming effects induced by live plants, and to quantify potential rates of new SOM formation in response to plant inputs. Introducing live plants to these soils allows to capture the dynamics of continuous C-inputs through rhizodeposition at the root-soil interface and the biomechanical impact of root growth into soil and their relation to SOM decomposition and priming.

Therefore, a mesocosm experiment was conducted using soils from the Peruvian and Swedish field sites introduced in chapter 1 and planting the perennial C_4-grass *Cynodon dactylon* (Bermuda grass) separately on organic and mineral soils from boreal and tropical forests, tundra heath and Andean Puna grasslands. This provided the unique opportunity to distinguish continuously between plant, root and soil derived carbon, while also monitoring carbon uptake within the microbial community. This provided an insight to the dynamics of atmospheric carbon sequestration during plant growth simultaneously with potential carbon release from soil and rhizosphere by microbial activity. Moreover, possible links to the mechanisms underlying carbon mobilization when plants colonise certain ecosystems are indicated, as well as the role different functional groups of microbes could play in carbon cycling in plant-soil-interactions.

A close link has been suggested between resource C:N and priming effects in soils, but no consensus has been reached regarding the effect on magnitude and direction of priming (Cheng et al., 1999; Qiao et al., 2016; Zhu et al., 2018; Hicks et al., 2019). Therefore, additional factors seem to strongly determine occurrence of priming effects, particularly in the rhizosphere of live plants. These may include differences in soil physiochemistry, nutrient limitations and microbial communities, which are inherent to different soil types depending on soil origin and soil history (Gunapala et al., 1998; Fierer & Schimel, 2002; Garbeva et al., 2008; Schulp & Verburg; 2009; Keiser et al., 2011). This study will address these factors by analysing RPE in soils from two countries representing a high altitudinal ecosystem (Peruvian Andes) and a high latitudinal ecosystem (Swedish sub-arctic), four land cover types (Andean tropical forest, Puna grassland, boreal forest and tundra heath) and two soil horizons each (organic and mineral). Moreover, during the natural course of seasonal plant growth, plant nutrient uptake and organic inputs to soils change. Therefore, investigating how priming effects change over time offers an opportunity to identify mechanisms promoting positive and negative priming.

In the previous soil-only incubation (chapter 2), priming effects were determined by soil C:N across all soil types (Tables 2.5, 2.6). Most positive priming was observed in soils of lowest soil C:N ratios (Peruvian Puna) and in mineral soils from Sweden. Most negative priming was observed from Peruvian forest soils and in organic soils from Sweden, which have high C:N ratios. In contrast to substrate addition experiments, under conditions where plants are competing with microbes for nutrients, we may observe different priming effects. On the one hand, if microbes preferentially use labile C inputs from plants exudates, less SOM may be metabolised, resulting in a reduction of RPE (Kuzyakov, 2002; Blagodatskaya et al., 2011). On the other hand, if microbial growth is stimulated and plants deplete the rhizosphere of nutrients, the increased demand for nutrients may be met with SOM mining, causing positive RPE (Dijkstra et al., 2013; Meier et al., 2016; Lynch et al., 2018). We therefore expected magnitude and direction of RPE in the different ecosystems to depend on soil C:N and to potentially vary with time of year as plant-activity affects the relative availability of labile C versus nutrients (H1).

In the organic top soils, the introduction of plants will result in increased competition between plants and microbes (Zak et al., 1990; Ke & Wan, 2019). As these soils provide ample nutrient supply, it is less likely that microbes will become nutrient limited and there may be the potential for microbes to shift to using new plant inputs. On the other hand, we expected an enhancement of SOM degradation under plant growth in the C and nutrient poorer mineral soils. This supports the idea that in the lower soil profiles microbes are limited by the supply of fresh C (Fontaine et al., 2007), which will be provided when we introduce plants. We therefore hypothesised (H2) stronger (more positive) RPE in mineral soils compared to organic soils, as the introduction of plants can facilitate microbial access to soil-bound nutrients (Keiluveit et al., 2015), which can induce positive RPE (Dijkstra et al., 2013) and plants can also enhance microbial activity through provision of labile energy (Jones et al., 2009), with stronger effects in deep soils than top soils (Bernal et al., 2016; Shahzad et al., 2018).

The soils studied here comprise a wide range of microbial C:N ratios and are thus very suitable to study the governance of soil nutrients and microbial communities over plant-microbial competition and possible links to RPEs (Fontaine et al., 2003; Eilers et al., 2010; Männistö et al., 2016). While fungi have been shown to induce positive priming through enhanced enzyme synthesis (Fontaine et al., 2011; Bastida et al., 2019), they also sequester considerable amounts of C into their biomass (Kramer et al., 2012; Treseder & Holden, 2013; Kallenbach et al., 2016). We therefore expected greater (positive) RPE in soils with higher fungal than bacteria abundance (higher F:B), but lower magnitude of RPE relative to the amount of C sequestered in microbial biomass (H3).

Given the variability of plant inputs and microbial community dynamics, consequent RPEs are difficult to predict. Measuring the seasonal change of RPE in contrasting soils and investigating the potential roles of microbial C:N ratios, fungal to bacteria ratios (F:B) and microbial community composition the following three hypotheses were tested:

H1: The magnitude and direction of RPEs will depend on the soil type studied (soil C:N), plant productivity (C inputs~photosynthesis) and the availability of nutrients (N et al.) for microbes.

H2: RPEs under live plants will be lower in organic soils, while mineral soils will respond with stronger positive RPE.

H3: Microbial community composition will change following plant growth, resulting in positive or negative RPE, depending on microbial biomass C:N and fungal: bacteria ratios (F:B).

3.3. Material and methods

3.3.1 Experimental set-up

In this study plant-induced RPEs were investigated in organic and mineral soils from above and below treelines in Andean Peru and sub-arctic Sweden. Details of the sampling campaigns conducted 2016 can be found in chapter 1, which also describes how composite samples were generated to differentiate eight characteristic soil types by country of origin, land cover type and soil horizon: PFM: Peru Forest Mineral, PFO: Peru Forest Organic, PPM: Peru Puna Mineral, PPO: Peru Puna Organic, SFM: Sweden Forest mineral, SFO: Sweden Forest Organic, STM: Sweden Tundra Mineral and STO: Sweden Tundra Organic.

Before the experimental phase in the greenhouse in summer 2018, samples were homogenised again, remaining large roots and rocks removed, clay and other conglomerates loosened and bags of soil carefully mixed manually. A sub-sample of each soil type was analysed for micronutrients and classified following standard protocols (LUFA Nord-West, PKS: CAT-method) to complement existing data sets (Table 3.3). For each of the eight soil types, eight pots (d = 5.5 cm, V = 1.02 l) were filled with equal amounts of soil, with final weights at field moisture as follows: Peru mineral: 200 g, Peru organic: 150 g, Sweden mineral: 400 g, Sweden organic: 150 g). Fertiliser containing mineral nitrogen was added to all pots to ensure good plant establishment and soils

were allowed to settle and pre-incubate one month before planting the C_4-plants. Additions were proportional to soil PKS (1:1) on a dry weight basis (Table 3.3), with reduced rates for the Swedish mineral soils, to account for their heavy, sandy texture at low C and N contents and low microbial abundance (Appendix V2-C3-A3). Subsequentially, four pots were randomly selected from each soil type and planted with the C_4 species *Cynodon dactylon,* at a density of 20 seeds / pot (following germination tests) and plants were grown for three months. Throughout the greenhouse phase, soil moisture was continuously monitored and maintained within the defined range above permanent wilting point (PWP) and below field capacity (FC) for each soil type (Appendices V1-P2 and V2-C3-A1).

3.3.2 RPE measurements

Over the course of three months, three plant-soil-respiration measurements were performed to estimate RPE *in vivo*: RPE 1: September (35 days after sowing), RPE 2: October (80 days after sowing) and RPE 3: November (95 days after sowing). For each measurement, pots were removed from the greenhouse, placed in dark respiration chambers under controlled temperature conditions (21.05 ± 1.15 °C, Appendix V2-C3-A1, Fig. 3.1.F) and flushed with CO_2-free air (Fig. 3.1.G) before chambers were sealed. Plant-soil systems were incubated 24 hrs and unplanted soils for 48 h. Incubation times were chosen after trial incubations, to obtain reasonable ratios of soil:plant-soil respiration for planted soils and comparable concentrations from unplanted soils. All values were corrected for 24 hrs incubation in RPE calculations. 120 ml gas samples for $^{12/13}CO_2$-analysis were taken (Fig. 3.1.M). Accompanying the first and second plant-soil respiration measurements (RPE 1 and 2) a range of plant growth parameters were measured. Photosynthesis (PS) was measured at leaf level with three replicates per plant (i.e. 12 per soil type) using an infrared gas analyser (LICOR, LI-6200, Fig. 3.1.E). Corresponding leaf area was measured using a scanner (LI-3100C) and photosynthetic active radiation (PAR) and leaf area index (LAI) of whole pots were measured using a ceptometre (Accupar LP-80), with one segment adapted to fit the size of the pots (Fig. 3.1.D).
As the estimates of priming obtained by the in vivo method (RPE 1- 3) were attached with considerable uncertainty (Table 3.1), the upper 15 cm of root-soil-

column were incubated under controlled laboratory conditions (RPE 0) to improve accuracy of RPE estimates. Firstly, above-ground plant material was removed, and set aside to be dried, weighed and ground. Pots were cut to size as described in Shahzad et al., 2018 (Fig. 3.1.J) and top 15 cm of columns of root-soil and root-free control soils were incubated separately for 48 hrs / 60 hrs in 6 litre glass flasks (Fig. 3.1.K). Flasks were fitted with rubber stoppers to enable direct measurement of $^{12/13}CO_2$ in the headspace by connecting the flasks to the analysers (gas chromatograph (Clarus 480, Perkin Elmer) and Picarro (G2101i)).

3.3.3 Post-harvest laboratory analysis

After the root-soil incubations, plant and soil material from each replicate pot was separated and several parameters were measured. These comprised root biomass, $^{12/13}C$ and N of plant tissues and bulk soil and mineral N, pH and moisture content of each soil (four planted and four unplanted controls per soil type). For $^{12/13}C$ and N analysis, plant and soil material was oven-dried (60 °C / 105 °C), ground and analysed following standard protocols (INRA, Nancy). Mineral N was extracted with 20 ml KCl from subsamples of 5 g soil for each replicate. Extracts were analysed using a TOC/TN analyser (Shimadzu TNM-L) and values were blank corrected. Soil pH was measured on 5 g subsamples of soil in 12.5 ml deionised water (Schott instruments Lab870). Soil water content was determined by weight loss of 10 g fresh soil oven-dried at 105 °C for 48 h. Microbial biomass $^{12/13}C$ and N were extracted using the vapour-phase chloroform fumigation technique modified after Vance et al, 1987. Soils were sub-set into two sets of 5 g soil each. In the first set (F = fumigated), microbial cells were lysed by exposing the soil samples to vaporous chloroform (EtOH - free) for 24 hrs in an evacuated desiccator. After ventilation, this set and the second reference set of non-fumigated soils (NF = non-fumigated) were shaken with 20 ml of 30 mM K_2SO_4 to extract C and N. Though, it must be mentioned that the efficiency of the fumigation method is possibly affected by soil texture, particularly clay minerals and WHC. Therefore, no single correction factor was applied, to reduce the risk of amplifying unfair comparison amongst the here studied contrasting soil types (see Appendix V2-C3-A3). Extracts were freeze-dried and elemental C and N contents and isotopic composition of the carbon

compound were analysed following standard protocols (INRA, Nancy). Microbial C and N were calculated by subtracting the respective amounts of non-fumigated extracts from the fumigated ones. Microbial biomass (mb) $\delta^{13}C$ was calculated using mass balance:

$$\delta^{13}C\ mb = (\delta^{13}C_F * C_F - \delta^{13}C_{NF} * C_{NF})/(C_F - C_{NF}) \qquad (Eq.\ 1)$$

Moreover, broad microbial groups of fungi, actinomycetes, gram+ and gram- bacteria were determined by analysing phospholipid fatty acids (PLFAs, Appendix V1-P3). Subsamples of approximately 10 g soil were frozen at -80°C and stored until analysis. For that purpose, samples were then freeze-dried and ground. Extraction was performed following a modified Bligh-Dyer protocol (after White et al., 1979) and FAMEs were analysed on an Agilent 6890 gas chromatograph coupled to a mass spectrometre detector (Agilent 5973). Concentrations were blank corrected, normalised against an internal standard (methyl nonadecanoate C13:0 and C21:0, Sigma Aldrich, UK), classified by standard nomenclature (Frostegard et al., 1993) and reported on a soil mass basis as PLFA µg g^{-1} dwt soil. Biomarkers were assigned after Rinnan & Baath (2009), adding 18:1(n-9) for fungi (against Zelles,1997, but see Treonis et al., 2004; Chung et al., 2007; Denef et al., 2009; DeDeyn et al., 2011) and with the methyl-branched saturated fatty acids 10Me-16:0, 10Me-17:0 and 10Me-18:0 for actinomycetes after Zelles (1997,1999). The two fungal biomarkers were tested for correlation (R^2 between 0.88 and 0.96 in all batches) to avoid mis-ID-ing gram- bacteria through the 18:1(n-9) biomarker (Zelles, 1997). Final assignments were: saprophytic fungi (18:2, 18:1(n-9)), actinomycetes (10Me-16:0, 10Me-17:0, 10Me-18:0), other gram positive bacteria (15:0i, 15:0a, 16:0i, 17:0i, 17:0a), gram negative bacteria (16:1(n-7), cy17:0, 18:1(n-7), cy-19:0) and remaining unspecified PLFAs (14:0i, 14:0, 15:1i, 15:1a, 16:1(n-9), 16:1(n-5), 16:0, 17:1i(n-8), 17:0, 18:0i, 18:1(n-5), 18:0, 19:1). In this conservative assignment, the group of unspecified PLFAs also comprises, potentially, AMF as identified by 16:1(n-5). It was not separated to avoid mis-ID-ing bacterial lipoproteins (Nakayama et al., 2012), a possible error, which would differentially affect soils from different land cover types (potentially more AMF in grasslands) and overshadow resolution of respective community structures and comparison amongst soil types.

Figure 3.1: Timeline: A: Sowing of Cynodon dactylon at 20 seeds / pot (02.08.2018) B: Greenhouse with irrigation system connected, pots with four replicates of each planted and unplanted soils for each soil type (n=4x2x8=64) C: Plants in September, 35 days after sowing (RPE 1: 05.09-07.09.2018) D: Leaf area index (LAI) measurement E: Photosynthesis (PS1) measurement F: Plants in October, 80 days after sowing, in respiration chambers (RPE 2: 25.10-28.10.2018) G: Set-up and flushing of respiration chambers H: LAI, PAR and PS2 measurements I: Plants in November, 95 days after sowing, leaves starting to senescent (RPE 3: 05.11-11.11.2018) J: Harvesting of plant-soil-systems, set-up for root-soil-incubations K: Root-soil-incubations (RPE 0: 19.11 + 20.11.2018, conducted in dark chamber) L: Roots (washed) and leaves set to dry M: 120 ml gas samples for GC and CRDS N: soil samples prepared for oven-drying O: Stage 2 of PLFA extractions

3.3.4 Calculation of RPE

Rhizosphere priming was calculated after measuring total soil respiration (CO_2-total) of planted and unplanted soils in dark respiration chambers and partitioning between soil and plant-derived carbon, based on the ^{13}C abundance of the CO_2-C in gas samples. The amount of primed carbon (CO_2-primed) was specified as the organic matter-derived CO_2-SOM respired from planted soils (CO_2-total) relative to the amount of C mineralised and CO_2 respired from organic matter of unplanted control soils (CO_2-control).

Firstly, the amount of plant-derived CO_2 was calculated following

$$CO_2\text{-plant} = CO_2\text{-total} \times \frac{d^{13}\text{C}-\text{total} - d^{13}\text{C}-\text{SOM}}{d^{13}\text{C}-\text{plant} - d^{13}\text{C}-\text{SOM}} \qquad (\textit{Eq. 2})$$

and then the amount of SOM-derived carbon in the control soils was subtracted from the amount of SOM-derived carbon of the planted soils to quantify the fraction of C mineralised through rhizosphere priming

$$CO_2\text{-SOM} = CO_2\text{-total} - CO_2\text{-plant} \qquad (\textit{Eq. 3})$$

$$CO_2\text{-primed} = CO_2\text{-SOM} - CO_2\text{-control} \qquad (\textit{Eq. 4})$$

Rhizosphere priming was calculated accordingly following three short-term plant-soil incubations at three time points during the first phase of the experiment (RPE 1-3) when plants were growing in the greenhouse (when not incubated in dark chambers). The same mass balance approach with the terminology as above, where “plant” corresponds to roots only, was also used to calculate RPE of the final incubation of root-soil-columns after aboveground plant removal in the laboratory (RPE 0). Here, partitioning between soil and root-derived carbon was based on the ^{13}C abundance of the CO_2 measured from the jars. For all calculations, the amount of primed C was expressed as (µg) CO_2-C per (g) soil C, in order to normalise for the differences in soil type and carbon contents and increase comparability amongst the soils and their priming potential.

3.3.5 Data uncertainty

Labelling intensity is defined as Δ $\delta^{13}C$ plant-soil, i.e. the difference of $\delta^{13}C$ between the natural soil-C and the $\delta^{13}C$ of plant inputs. There likely was deviation in isotopic composition of the soil released CO_2, because soil respiration is composed of carbon originating from the process of microbial mineralisation of materials with different isotopic composition. This includes different fractions of SOM, as well as different plant materials (labile LMW-C and complex carbon such as cellulose), which do not have the same isotopic composition (Collister et al. 1994). The $\delta^{13}C$ of the soil end-member is hence best represented by the $\delta^{13}C$ of bulk SOM, as well as the $\delta^{13}C$ of control soil respiration to account for the deviation of $\delta^{13}C$ in different soil fractions. The $\delta^{13}C$ of the plant endmember can be inferred from $\delta^{13}C$ of plant tissues and together with $\delta^{13}C$ of leaf and root respiration, to obtain a proxy of the isotopic composition of carbon compounds generated from the plant-endmember, as exudates can hardly be measured in vivo, especially not continuously. For this experiment, using the C_3-C_4-approach with *Cynodon dactylon* ($\delta^{13}C$ approx. –15) and a variety of soils, the labelling intensity was between 9-11‰ and variation of endmember isotopic signature was up to ± 0.78 (plant) and ± 2.3 (soil), both depending on soil type (see Appendix V2-C3-A4 for details on $\delta^{13}C$'s of the different compartments). For each individual measurement of RPE, formula 1 can therefore be completed with various values for the $\delta^{13}C$ of the different end-members, which results in various different values of RPE. Uncertainty in RPE estimate was defined after Cros et al. (2019)

$$\text{RPE uncertainty} = \frac{(\text{RPEav} - \text{RPE}_{av} \pm \text{stdv})}{\text{RPE}_{av}} x\ 100 \qquad (\textit{Eq. 5})$$

where RPE was first calculated using all the different $\delta^{13}C$s of soil and plant endmembers and their combinations to then determine the average RPE (av) and its standard deviation (stdv). For the here presented consideration of RPE uncertainty, two possible $\delta^{13}C$'s were included for the soil end-member (bulk and respiration) at natural abundance labelling.

Given the large uncertainty attached to each RPE value (Table 3.1), exceeding 100% in some cases, conclusions regarding the magnitude of priming must be taken with care. Thus, an approach was developed to validate the informative quality of each RPE estimate regarding the direction of priming. The measured

$\delta^{13}C$'s from all plant tissues and their respiration were integrated, to define the possible range of plant $\delta^{13}C$ values (hatched area in Fig. 3.2b). This assumes, that any carbon compound released from the plant through rhizosphere processes can only have a $\delta^{13}C$ which ranges between the $\delta^{13}C$ of structural components (tissue) and the $\delta^{13}C$ of metabolic processes (carbon respired from leaves and roots). Following this, hypothetical $\delta^{13}C$ values were calculated (formula 6: $\delta^{13}C_{NO\text{-}PRIME}$, dark triangles in Fig. 3.2b, c) for null priming. This is a theoretical $\delta^{13}C$ value, that would have been necessary for a plant input to cause no priming effect at all, for each given respiration measurement. Furthermore, a hypothetical $\delta^{13}C$ value was calculated, which would have been necessary in order to cause a priming effect similar to the average RPE calculated (i.e. same magnitude), but with opposite direction (formula 7: $\delta^{13}C_{RPE++}$, light triangles in Fig. 3.2b, c). These were compared to the measured values and as they fell outside the range of possible $\delta^{13}C$'s, uncertainty about the direction of the observed rhizosphere priming effects could be resolved (Fig. 3.2). Some uncertainty none the less persists, because of fluctuations of $\delta^{13}C$ of atmospheric CO_2 and the variation of $\delta^{13}C$ from soil derived carbon. The latter could, theoretically, be narrowed down using $\delta^{13}C$ from rhizosphere soil respiration (Appendix V2-C3-A4), but as this was only measured at the end of the experiment, this value does not chronologically correspond to RPE measurements and would introduce yet another uncertainty.

Verification of the direction of priming was hence performed following

$$\delta^{13}C_{NO\text{-}PRIME} = \frac{(CO_2{-}PS\ x\ d^{13}C{-}PS){-}(CO_2{-}control\ x\ d^{13}C{-}SOM)}{(CO_2{-}PS - CO_2{-}control)} \qquad (Eq.\ 6)$$

$$\delta^{13}C_{RPE++} = \delta^{13}C_{NO\text{-}PRIME} + (\delta^{13}C_{NO\text{-}PRIME} - \delta^{13}C_{INITIAL}) \times 2 \qquad (Eq.\ 7)$$

where PS is the value measured from plant-soil incubation, CO2-control is the value measured from plant- and root-free control soil. The values obtained using equation 7 were not linear, so they were manually adjusted to provide RPE values corresponding to the original ones, but in opposite direction. All theoretical values were tested by using them in the original calculations of average RPE and comparing the thus calculated values (actual and theoretical $\delta^{13}C$'s, Fig. 3.2c). Uncertainty analysis and theoretical $\delta^{13}C$ tests were

performed for each RPE measurement (Table 3.1, RPE 1-3 of plant-soil incubations and RPE 0 of root-soil incubations) with detailed data exemplary presented for RPE 0 in Fig. 3.2 and all further analysis in Appendix V2-C3-A2.

It must be noted, that the values for RPE 1 (September) were calculated differently, because $\delta^{13}C$ values (Appendix V2-C3-A4) indicated bias from either chamber leaks, atmospheric CO_2 fluctuation or the isotopic analyser. To account for this, only $\delta^{13}C$ of corresponding soil respiration was used for the soil endmember at this time-point.

3.3.6 Statistical analysis

All analysis was conducted using R version 3.2.2 (2015) and additional packages as stated. Differences in RPE amongst soil types were tested with ANOVA followed by Tukey's tests. Significant differences in microbial C and N and of microbial functional groups were determined using Student's T-tests ($\alpha = 0.05$), with pairing for unplanted*planted replicates within each soil type. Correlations between RPE and soil and microbial C:N ratios and F:B ratios were tested by Pearson's product-moment correlation with alternative hypothesis: true correlation is not equal to 0.

More complex plant-soil-microbe interactions were modelled in generalised linear models. Because in this experiment the ideal data set can comprise only a maximum of 2 x 32 observations for respiration measurements (CO_2) and 32 observations for priming (RPE), no complex Bayesian statistics or ordination could be applied. After evaluation of replicate, soil pH, moisture and temperature as random effects in various linear mixed models (package lme4, Bates et al., 2015), data was analysed using generalised linear models, including multiple continuous predictors and multiple interactions as described below. Validity of model assumptions were evaluated and most parsimonious models kept and further fitted using step-wise deletion (Chi^2) were applicable. Some models include main effects, which also appear in interaction terms. They were not removed to increase comparability between the four models.
Response: For full plant-soil-microbe interaction, average RPE (RPE_av, part 1 in Table 3.7) from the three plant-soil incubations was used as response variable, as for each set of the individual measurements, RPE estimates had

varying accuracy amongst the different soils and individual reps. From root-soil-incubations, both calculated RPE (RPE_0, part 2, Table 3.7) and pure CO_2-concentrations (CO2_RS and CO2_control, log-transformed to meet normality criteria, parts 3a and 3b, Table 3.7) were used to determine drivers of soil respiration without bias from the uncertainty of RPE-values.

Fixed effects: For **soil and microbial** compartments, total C and N were used, together with further functional parameters: mineral nitrogen, micronutrients (PKS), texture (humus and silt & clay) and pH for soils and PLFA functional groups (relative abundance of fungi, actinomycetes, gram positive and negative bacteria, total PLFAs and F:B ratios) for microbes. For **plant** parameters leaf N and biomass were used, as well as root C, N and biomass, as measured at the end of the experiment. In part 1, where the average RPE of plant-soil-incubations is the response variable, the additional parameters photosynthesis (PS) and leaf area index (LAI) were included.

Interactive effects were restricted to two-way interactions to avoid over-parameterisation of the models and included correspondingly for each interaction: carbon/dwt, carbon/carbon, nitrogen/nitrogen, nitrogen/mineral nitrogen, C:N/dwt, C:N/total PLFAs, C:N/F:B, functional groups of PLFAs individually linked with each C and N of both plant and soil. Other soft parameters (PKS, LAI etc.) were not considered for interactions. Interactive terms were grouped into 1. plant-soil, 2. soil-microbe and 3. plant-microbe interactions.

Final models were then compared and factors assigned to four groups ranked by overall significance and abundance amongst models. Group I comprised factor significant ($p < 0.05$) in all models. Group II included factors with overall high significance (present in at least three models, in at least two models with $p < 0.05$ and others $p < 0.1$). Group III comprised factors present in all models, but without consistent significance and group IV included factors present in all models, that account for plant-soil-microbe interactions (parts 1 – 3a), but without consistent significance.

3.4 Results

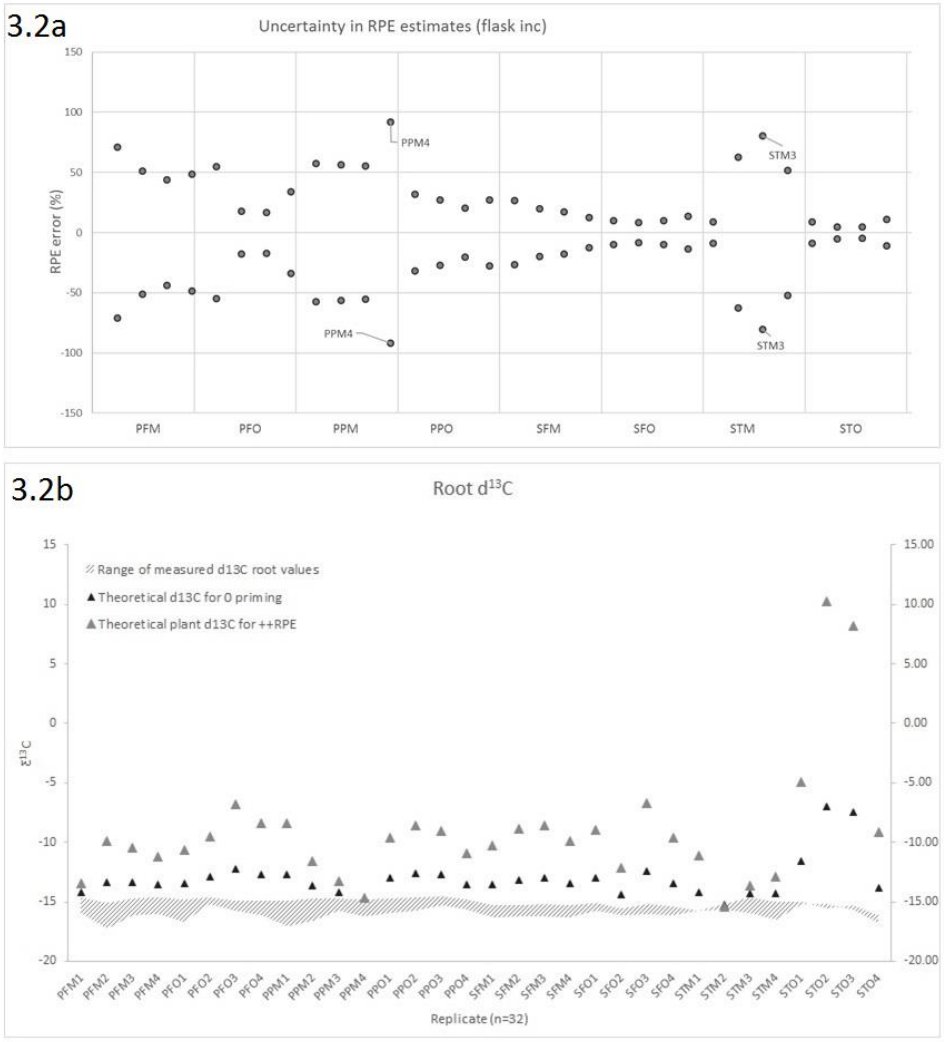
3.4.1 Data uncertainty

The uncertainty attached to each individual RPE estimate ranged between 0.86 % and 254.08 %, with the lowest overall uncertainty calculated for the final root-soil incubations (Table 3.1).

Table 3.1: Uncertainty and data accuracy of RPE estimates for the different incubations

	Uncertainty of RPE estimates				
	Plant-soil				Root-soil
	RPE 1: Sept	RPE 2: Oct	RPE 3: Nov	RPE average	RPE 0
n incubations, accuracy of data	n=29, adjustment of soil moisture, respiration chamber leaks	n=31	n=32	fluctuation of atmospheric CO_2 in greenhouse between measurements	n=32, controlled laboratory conditions
Calculated uncertainty (%)	3.88 – 246.98	15.38 - 254.08	34.71 – 201.59	0.86 – 254.08	4.80 - 91.74
n (corrected)	27	27	30	30	28

To ensure conclusions about the direction of priming are accurate, theoretical $\delta^{13}C$ values were calculated for each RPE value at each measurement (Fig. 3.2 for RPE 0, please see Appendix V2-C3-A2 for RPE 1- 3). These represent hypothetical isotopic compositions, that would have been necessary for a plant input to soil in order to cause no priming (dark triangles in Fig. 3.2 b, c), or priming in the opposite direction (light triangles in Fig. 3.2 b, c). These were compared to the values actually measured from plants (hatched area) to test, if they fall within the range of experimentally accessed $\delta^{13}C$'s from plants. Analysis was conducted for each replicate individually (Appendix V2-C3-A4), as plants grew differently on different soils (Table 3.4, Fig. 3.1 C,H). Albeit attached with considerable uncertainty, our analysis shows that the majority of here presented estimates of RPE are possible, with confidence about their direction (negative RPE) and seasonal trend.



Uncertainty (a), theoretical d13Cs (b) and their test in original calculations of RPE (c) for final root-soil-incubations (RPE0). ***3.2a*** *shows the possible ± error (%) of each replicate of each soil type.* ***3.2b*** *shows the range of $\delta^{13}C$ values measured from roots for each replicate of each soil type (hatched area) and the theoretical $\delta^{13}C$ values, which would have been necessary for no priming (dark triangles) or priming of the same magnitude, but in the opposite direction (light triangles).* ***3.2c*** *shows the corresponding values for primed C, which are calculated based on the theoretical $\delta^{13}C$s. The area between the two dashed lines corresponds to the hatched area in figure 3.2b, but for all replicates of all soil types. To confidently identify a priming effect, ideally all theoretical values would be outside of this range. Note that analysis was performed before normalisation of primed C for SOC, hence y-axis in 3.2c: primed C µg g^{-1}.*

Figure 3.2: Example of how the uncertainty was calculated, showing data for the final root-soil incubation (RPE 0). The same procedure was applied to RPE values from plant-soil incubations using $\delta^{13}C$s from all plant compartments (Appendix V2-C3-A4).

3.4.2 RPE amongst different soil types

In this experiment plants overall decelerated SOM mineralisation, that is, induced a negative priming effect. RPE became more differentiated between different soil types during the course of the experiment (Table 3.2; Fig. 3.3). All organic soils showed a tendency for progressively greater negative priming during the course of the experiment (Fig. 3.3). No consistent trend was observed for the mineral soils. For the Peruvian soils, the organic soils initially (RPE I) showed more negative priming than their mineral counterparts, while this was reversed later in the experiment (RPE II, III, 0). For the Swedish soils, the contrast between organic and mineral soils was most pronounced during the final root-soil incubations (RPE 0, Fig. 3.4). In sum, flourishing plants did not significantly stimulate additional microbial mineralisation of SOM in most of the soils, only for the organic tundra soils initially positive priming was reported (Fig. 3.3).

Table 3.2: RPE amongst soil types. Letters indicate similarity of sample means between soil types following ANOVA and post-hoc Tukey tests for each RPE respectively. Soil type has eight levels, derived from combining Country (two levels: Peru and Sweden, as dummy codes for a tropical and an arctic ecosystem), land cover type (four levels: high Andean forest, boreal forest, Puna grassland, tundra heath) and soil horizon (two levels: organic and mineral). ANOVA performed for each and p-values and significance code given.

	RPE				
	Plant-soil				Root-soil
	I: Sept	II: Oct	III: Nov	average	0
PFO		a	bc	a	abcd
PFM		ab	ab	ab	abc
PPO		a	c	a	ad
PPM		ab	ab	a	bc
SFO		a	ab	ab	d
SFM		a	ab	ab	abcd
STO		b	a	b	abd
STM		a	bc	a	c
Soil type	0.0754 .	**0.00494** *	**4.66e-05** ***	**0.00203** **	**0.000159** ***
Country	0.144	0.289	**0.0216** *	**0.0386** *	0.373
Land cover	0.453	0.112	0.063 .	0.122	0.208
Soil horizon	**0.00243** **	0.364	0.235	0.183	**1.32e-05** ***

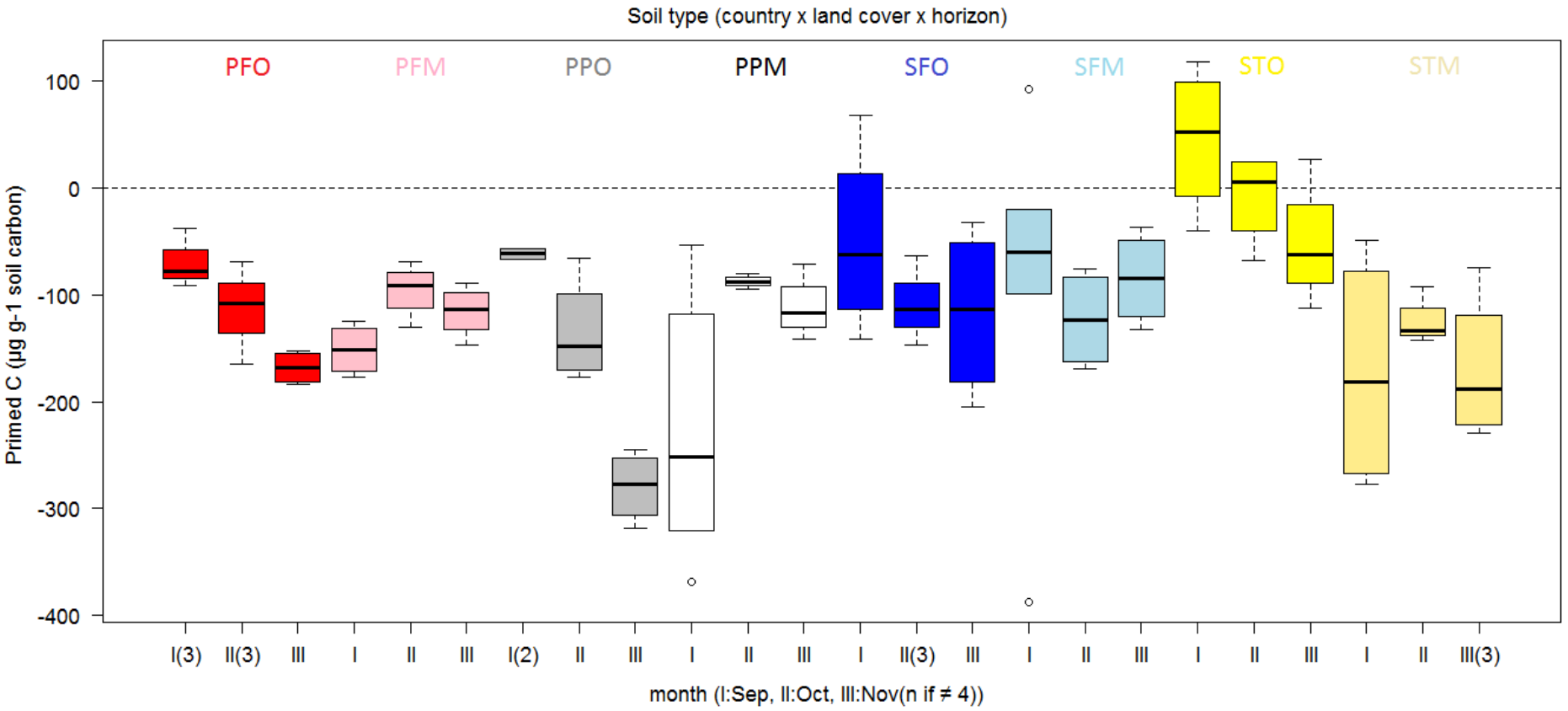


Figure 3.3: Changes in rhizosphere priming effect (RPE) quantified as µg primed C g^{-1} soil carbon in plant-soil incubations for the eight soil types studies at three timepoints: I: September: 35 days after sowing, II: October: 80 days after sowing and III: November: 95 days after sowing. Boxes show median lines and interquartile ranges for n = 4 if not indicated otherwise.

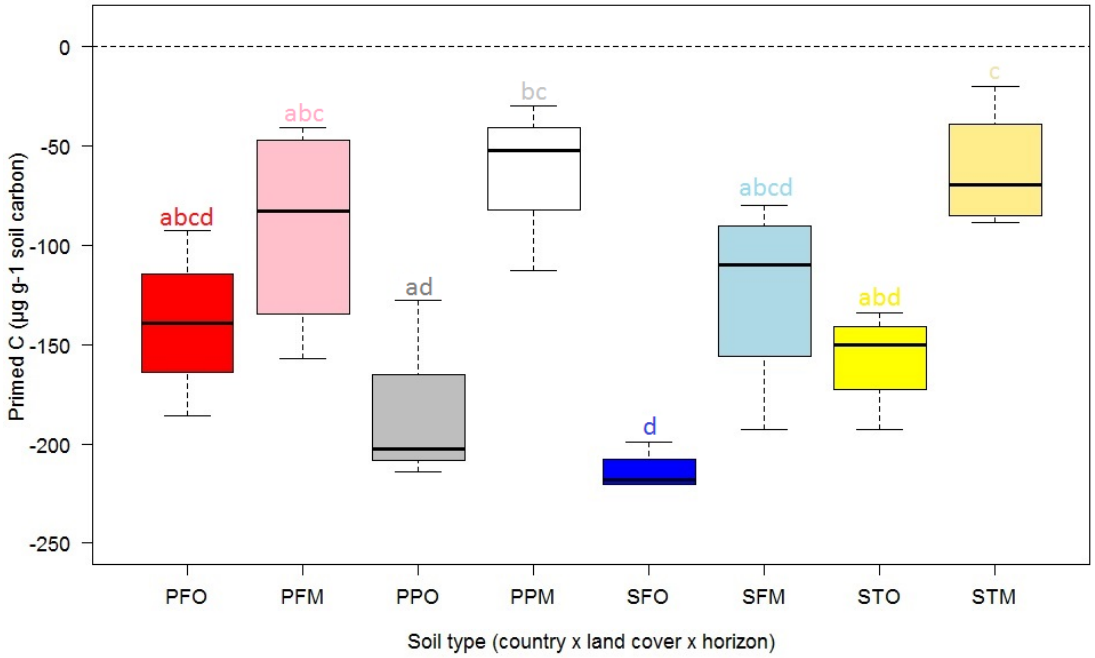


Figure 3.4: Rhizosphere priming effect (RPE 0) quantified as µg primed C g^{-1} soil carbon in root-soil incubations of upper 15 cm of soil columns, where aboveground plant material has been removed instantly before the incubation. Letters indicate similarity of sample means following ANOVA and post-hoc Tukey's test. Significant differences between soil horizons (p = 1.32e-05***, f = 27.8). Boxes shows median lines and interquartile ranges for n = 4.

3.4.3 Soil and microbial characteristics

Priming effects were studied on a set of eight diverse soils, comprising samples from organic and mineral soil horizons from boreal and tropical forests, tundra heath and Andean Puna grasslands sampled 2016 in Sweden and Peru. Most analysis of soil and microbial parameters was performed on planted and un-planted soils at the end of the experiment. Calculations based on their comparison therefore account not only for the effect of plant growth in the planted soils, which induced e.g. shifts in microbial community composition and soil mineral N and ultimately RPE. Such comparison of planted and unplanted soils therefore hold bias from changes of soil and microbial parameters in the unplanted soils, which cannot be attributed to the growth of plants. Still some parameters can be assumed to be relatively stable, such as soil texture and bulk soil carbon and nitrogen contents. In this regard, the studied soils comprised a set of very different samples, spanning a gradient of soil C:N from 14 – 30 and comprising sandy mineral soils, as well as humic organic and clayey soils (Table 3.3). Microbial biomass C:N ratios in the unplanted soils at the end of the experiment ranged from 2.85 – 17.45 and fungi:bacteria ratios (F:B) between 0.18 and 0.63 (Fig. 3.6).

Table 3.3: Key soil and microbial parameters of unplanted soils measured at the end of the experiment. Abbreviations: org: organic soil horizon, min: mineral soil horizon. Soil texture and PKS analysed on composite samples for each soil type prior to the experiment, all further properties analysed on unplanted soils at the end of the experiment, n=4 and ± standard error given, but n ≠ 4 for microbial C and N, please see Table 4 for details

Sample ID				Soil						Microbes			
Country	Land cover	Horizon	Soil ID	Texture	PKS (µg g^{-1})	pH	C (%)	N (%)	C:N	C (mg g^{-1})	N (mg g^{-1})	C:N	F:B
PERU	High Andean tropical forest	org	PFO	humus (8.1-15%), silty clay (silt>50%)	244.6	5.18 ± 0.11	20.52 ± 1.12	1.39 ± 0.06	14.81	0.60 ± 0.21	0.03 ± 0.01	15.70	0.19 ± 0.02
		min	PFM	humus (4.1-8%), silty clay (silt>50%)	84	5.07 ± 0.12	9.07 ± 0.59	0.65 ± 0.03	14.01	1.65 ± 0.17	0.17 ± 0.04	10.75	0.18 ± 0.02
	Puna grassland	org	PPO	humus (4.1-8%), silty clay (silt>50%)	141.7	5.61 ± 0.21	14.36 ± 0.35	1.16 ± 0.02	12.37	4.82 ± 1.1	0.34 ± 0.15	17.45	0.23 ± 0.02
		min	PPM	humus (0-4%), silty clay (silt>50%)	64	5.12 ± 0.15	7.36 ± 0.17	0.59 ± 0.01	12.38	4.01 ± 0.22	0.32 ± 0.05	12.63	0.26 ± 0.02
SWEDEN	Sub-arctic birch forest	org	SFO	humus (> 30%), bog like	227.8	5.67 ± 0.15	38.40 ± 0.57	2.03 ± 0.03	18.93	1.47 ± 1.5	0.10 ± 0.02	13.94	0.24 ± 0.05
		min	SFM	humus (0-4%), sandy, very silty clay (silt>50%)	55.1	5.66 ± 0.15	2.17 ± 0.11	0.13 ± 0.02	16.92	0.48 ± 0.05	0.17 ± 0.04	3.32	0.21 ± 0.06
	Tundra heath	org	STO	humus (> 30%), bog like	146	5.01 ± 0.06	46.05 ± 0.39	1.53 ± 0.03	30.19	1.76 ± 0.93	0.17 ± 0.03	10.01	0.63 ± 0.15
		min	STM	humus (0-4%), sandy, very silty clay (silt>50%)	55	5.33 ± 0.16	1.98 ± 0.62	0.098 ± 0.03	20.18	0.88 ± 0.51	0.32 ± 0.03	2.85	0.20 ± 0.01

3.4.4 Environmental and plant parameters

Ambient temperature in the greenhouse varied considerably during the course of the experiment (August – November), with both diurnal and seasonal fluctuation (Appendix V2-C3-A1).When not placed in incubation chambers under controlled temperature conditions, the continuum of plants, soils and microbes was exposed to this variation, as well as to oscillation of solar radiation and atmospheric CO_2. All these factors likely had direct and/or indirect effects on the plants and microbes. The effects of these variations on plants were made through measurements of photosynthesis (PS), leaf area index (LAI) and photosynthetic active radiation (PAR) accompanying the first and second plant-soil incubations (RPE 1 & 2). Plants showed symptoms of senescence towards the end of the greenhouse phase (Fig. 3.1.I), which was manifest in strongly reduced rates of photosynthesis (Table 3.4). Finally, soil moisture was continuously measured and adjusted to suit plant demand without over-watering soils to reduce bias of extreme soil water status on SOM-mineralisation processes and the physical structure of the soil matrix (Appendices V1-P1 and V2-C3-A1).

Cynodon dactylon was planted under uniform conditions on eight different soil types. Plant establishment and phenotypes strongly varied amongst the different soil types, which is reflected in the parameters measured at time of incubation such as PS and those measured post-harvest, including biomass and root:shoot ratios (Table 3.4). Plants had in common, that root nitrogen was below 1 %, while leaf nitrogen was above 1.5 % and leaves had lower $\delta^{13}C$ than roots. Hence leaf tissue was enriched in N compared to root tissue, resulting in distinct C:N ratios (C:N leaves ≈ 25, C:N roots ≈ 60). Roots were enriched in $\delta^{13}C$ compared to leaves, while $\delta^{13}C$ from root respiration was closer to leaf bulk material than to root bulk material (Appendix V2-C3-A4). Plant biomass and total C in plant biomass was greater for the plants growing in organic soils, compared to those grown in mineral soils. Root:shoot ratios were higher in mineral soils, with the highest ratio for Swedish mineral forest soils.

Table 3.4: Plant parameter determined on live plants: leaf area index (LAI) and photosynthesis (PS: µmol m^{-2} s^{-1}) as measured corresponding to first and second plant-soil incubations to estimate RPE (1: September, 2: October), n =12 per soil type and analysis of leaf and root $^{12/13}C$ and N and biomass (dry weight (dwt)) and biomass carbon (C) as determined post- harvest, n = 4 per soil type. Standard error (±SE) below each value.

		Live plant				Post - harvest						
Soil type	Plant material	LAI_PS1	LAI_PS2	PS1	PS2	C mg g^{-1}	N mg g^{-1}	C:N	$d^{13}C$	Gram dwt	root:shoot	total C (mg) in biomass
PFO	leaves	2.16 ± 0.33	1.835 ± 0.24	24.445 ± 5.54	8.945 ± 3.90	0.451 ± 0.009	0.019 ± 0.004	23.840	-15.588 ± 0.51	5.841	0.313	3.415
	roots					0.473 ± 0.006	0.008 ± 0.001	56.733	-14.804 ± 0.15	1.645		
PFM	leaves	2.1775 ± 0.21	1.94 ± 0.16	24.26 ± 8.76	3.6111 ± 1.42	0.450 ± 0.007	0.017 ± 0.002	26.936	-15.801 ± 0.56	3.450	0.357	2.129
	roots					0.480 ± 0.003	0.007 ± 0.001	69.939	-14.763 ± 0.22	1.200		
PPO	leaves	1.7925 ± 0.37	1.7825 ± 0.16	20.91 ± 7.69	6.512 ± 1.38	0.454 ± 0.003	0.018 ± 0.003	25.681	-15.606 ± 0.44	5.678	0.217	3.149
	roots					0.475 ± 0.008	0.007 ± 0.001	69.844	-14.673 ± 0.11	1.203		
PPM	leaves	2.9175 ± 0.05	1.58 ± 0.11	16.0875 ± 5.37	4.807 ± 1.66	0.450 ± 0.005	0.013 ± 0.005	41.152	-15.868 ± 0.61	3.133	0.247	1.767
	roots					0.478 ± 0.002	0.007 ± 0.0005	65.064	-14.770 ± 0.08	0.745		
SFO	leaves	3.0775 ± 0.31	2.0525 ± 0.12	15.835 ± 4.83	6.339 ± 2.70	0.447 ± 0.003	0.026 ± 0.004	17.928	-15.916 ± 0.1	5.153	0.284	2.980
	roots					0.475 ± 0.009	0.011 ± 0.002	43.232	-15.306 ± 0.21	1.423		
SFM	leaves	1.62 ± 0.29	1.68 ± 0.09	14.27 ± 6.45	4.819 ± 0.95	0.433 ± 0.007	0.020 ± 0.004	21.842	-16.083 ± 0.36	1.771	0.634	1.198
	roots					0.440 ± 0.037	0.007 ± 0.0005	65.507	-15.256 ± 0.04	0.981		
STO	leaves	1.7725 ± 0.47	1.9975 ± 0.13	19.2025 ± 6.33	5.786 ± 1.26	0.446 ± 0.006	0.019 ± 0.004	23.812	-15.709 ± 0.53	4.408	0.167	2.304
	roots					0.481 ± 0.004	0.007 ± 0.0003	64.595	-15.487 ± 0.51	0.705		
STM	leaves	1.72 ± 0.45	1.7125 ± 0.17	14.095 ± 7.85	5.55 ± 1.77	0.440 ± 0.005	0.017 ± 0.004	26.487	-15.909 ± 0.75	1.875	0.219	0.986
	roots					0.442 ± 0.036	0.006 ± 0.0005	71.339	-15.133 ± 0.48	0.363		

3.4.5 Microbial parameters

Microbial biomass C and N were distinct for the different soil types, with significant changes occurring following the introduction of live plants (quantified as the difference between unplanted and planted soils, Fig. 3.5, Table 3.5). Microbial biomass N increase was significantly greater in planted compared to unplanted soils, except for the Peruvian Puna organic soils (PPO). This was accompanied by varying degrees of changing microbial biomass carbon, with significant increases for organic forest soils from Peru (PFO) and mineral forest soils from Sweden (SFM). For the Peruvian soils, the nitrogen gain exceeded carbon gains for all soils, resulting in lower microbial biomass C:N ratios of microbes in the planted soils. This was also observed for the Swedish organic soils, while in the mineral soils increase in microbial biomass carbon exceeding nitrogen gains, while increasing C:N ratios. The strongest correlation of microbial C:N and RPE was observed for the final root-soil incubations (RPE 0: $p = 0.014$, $R^2 = - 0.44$), while no coherent correlation was found for plant-soil incubations (RPE 1-3), as microbial biomass C:N was significantly correlated only with the RPE estimates of November (RPE 3: $p = 0.02$, $R^2 = - 0.41$).

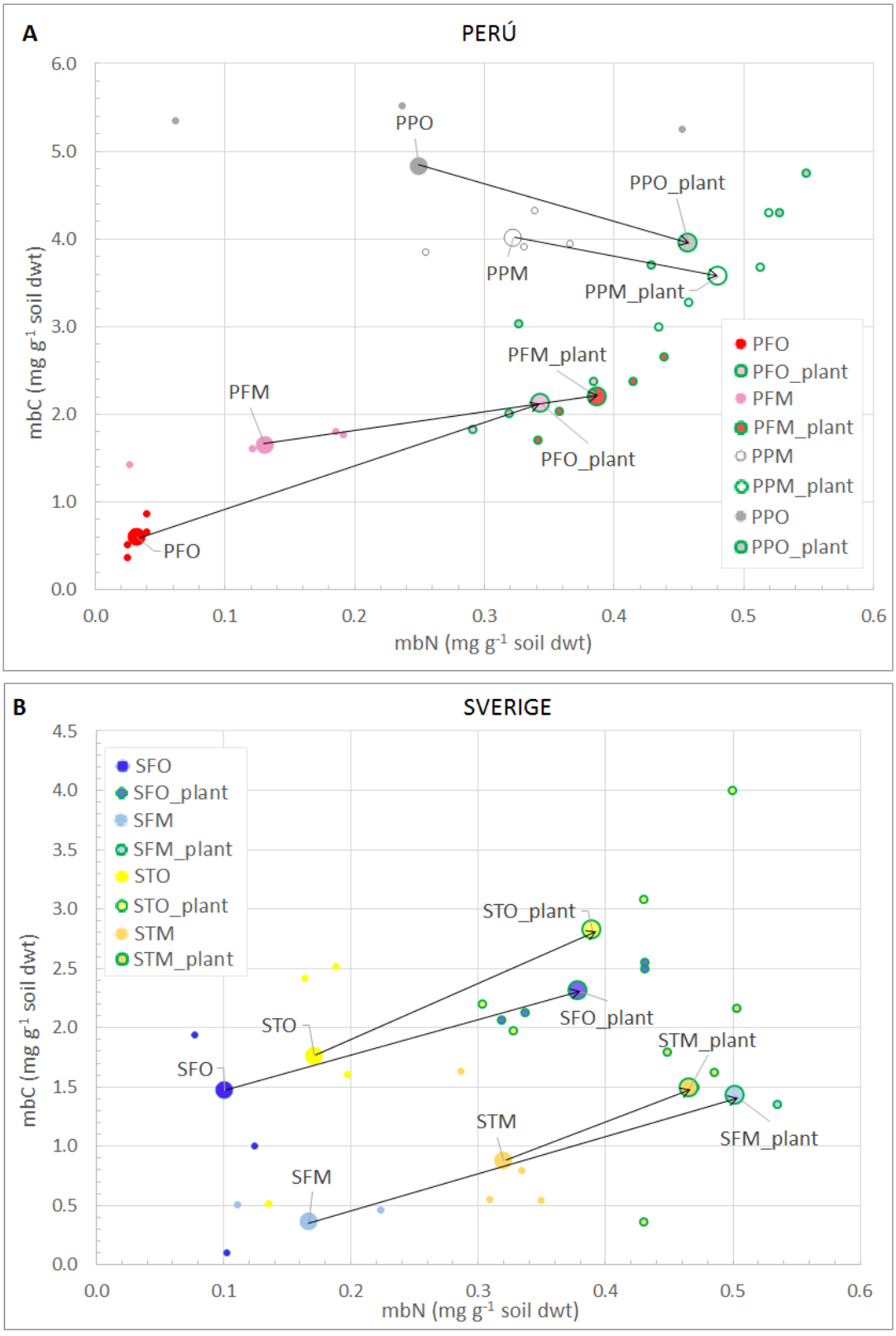


Figure 3.5: Differences in microbial biomass carbon (mbC) and nitrogen (mbN, mg g^{-1} soil dwt.) between planted (_plant) and unplanted soils of each soil type in (A) Peru and (B) Sweden. Small dots represent the individual replicate values, larger dots represent average of n = 4, arrows indicate the change in mbC or mbN between unplanted to planted soils. Statistical analysis is detailed in Table 4 (*n < 4 for PFM, PFO, PPO, SFM, SFO)*

Microbial community composition was characterised as identified by phospholipid fatty acids (PLFAs). Total PLFAs were significantly higher in organic soils, compared to their mineral counterparts for each land cover type, with mineral soils having between 1:3 (Peruvian forest) to less than 1:10 (Swedish forest) of total biomarkers (Appendix V2-C3-A3). Overall microbial community composition as determined through PLFAs was mostly unaffected by the introduction of live plants for all soil types. Full community PLFA profile did not change significantly between planted and unplanted soils, however, significant changes were observed for specific biomarkers. The functional group most affected were gram+ bacteria, which significantly decreased in Swedish organic soils (SFO & STO). Community shifts significantly decreased F:B ratios in Peruvian mineral Puna soils (PPM), while simultaneously the ratio of gram + to gram – bacteria increased. These parameters also changed in the Swedish tundra soils, where the F:B ratio significantly increased in the mineral soil, while the gram + to gram – bacteria ratio significantly decreased in the organic soils (Fig. 3.6, Table 3.5).

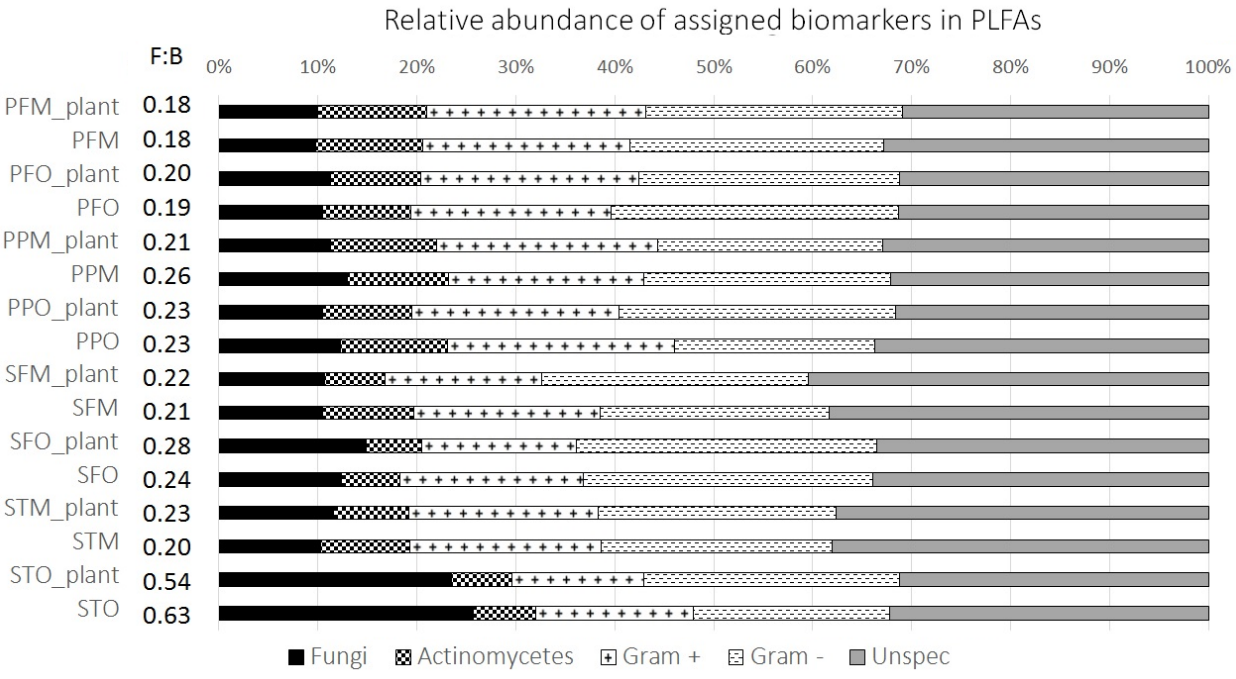


Figure 3.6: PLFAs indicating relative proportions of key functional groups of soil microbes as assigned to biomarkers for fungi, actinomycetes, other gram positive and gram negative bacteria and remaining unspecified PLFAs. Fungi to bacteria ratio (F:B) displayed in column between sample label and PLFA bars. Data presented for planted and unplanted soils for each soil type respectively, n = 4.

*Table 3.5: Microbial parameters: Left hand side (columns 2 – 4): Microbial C:N and $\delta^{13}C$ shifts (Δ i.e. the change in value attributed to plant action) and % change in microbial biomass (mb) carbon (C) and nitrogen (N). Values were excluded when element contents of non-fumigated samples > fumigated and / or $\delta^{13}C$ > 0 (see Appendix V2-C3-A3 for comment on extraction method). Absolute values for C:N ratio and $\delta^{13}C$, percentage for mbC and N. Right hand side (columns 6 – 13): Change (Δ) to individual groups of assigned biomarkers in PLFAs (μg g^{-1} dwt soil). Final column compares community composition between unplanted and planted soils (two-sided paired Student's T-test, α = 0.05). All values calculated using individual measurements from planted soils compared to average of unplanted soils for each soil type correspondingly. ± Standard error (SE) given below each value. n=4 for each set of planted/unplanted, if different, numbers are given in brackets next to value of SE. *significant differences (in bold) of parameter between planted and unplanted soils.*

Soil type	Δ C:N	Δ $d^{13}C$	mb C gain (%)	mb N gain (%)	Δ total PLFAs	Δ Fungi	Δ Actino-mycetes	Δ Gram +	Δ Gram -	Δ F:B	Δ Plus: Minus	t.test PLFAs (p)
PFO	**-9.51*** ± 0.22(4/2)	**3.09*** ± 0.77	**251.76*** ± 40.18	**959.85*** ± 144.43(4/2)	15.80 ± 11.24	2.48 ± 2.46	1.58 ± 1.16	5.04 ± 2.92	1.84 ± 2.87	0.01 ± 0.03	0.14 ± 0.09	0.80
PFM	-5.11 ± 0.45(4/3)	**2.53*** ± 0.61	33.17 ± 25.09	**195.94*** ± 35.27(4/3)	3.04 ± 1.28	0.31 ± 0.02	0.34 ± 0.09	0.90 ± 0.26	0.76 ± 0.42	-0.01 ± 0.001	0.03 ± 0.02	0.89
PPO	-8.75 ± 0.46(4/2)	**3.29*** ± 0.73	-18.03 ± 15.5	82.82 ± 40.71(4/2)	-27.54 ± 10.76	-4.96 ± 1.04	-3.05 ± 1.18	-5.42 ± 2.64	-5.33 ± 2.89	-0.0004 ± 0.05	-0.13 ± 0.08	0.63
PPM	**-5.22*** ± 0.62	**3.07*** ± 0.20	-10.91 ± 14.16	**49.01*** ± 12.83	2.46 ± 3.05	-0.26 ± 0.47	0.46 ± 0.25	1.32 ± 0.64	-0.13 ± 0.76	**-0.05*** ± 0.01	**0.20*** ± 0.03	0.90
SFO	-7.79 ± 0.33(4/3)	3.43 ± 0.45	56.94 ± 16.95	**275.08*** ± 59.45(4/3)	-42.91 ± 21.59	-2.69 ± 3.81	-2.92 ± 1.24	**-10.88*** ± 3.25	-11.48 ± 6.83	0.04 ± 0.03	-0.10 ± 0.04	0.61
SFM	1.24 ± 3.56(2/4)	-1.23 ± 0.27(4/3)	**349.77*** ± 32.16(2/4)	**51.68*** ± 13.74(2/4)	0.51 ± 1.46	0.08 ± 0.20	-0.33 ± 0.13	-0.27 ± 0.40	0.58 ± 0.24	0.01 ± 0.02	-0.26 ± 0.11	0.96
STO	-2.88 ± 0.81	1.42 ± 1.16	59.98 ± 52.31	**127.71*** ± 58.22	-9.57 ± 10.94	-5.22 ± 3.27	-0.87 ± 1.12	**-4.83*** ± 1.06	5.73 ± 3.19	-0.10 ± 0.03	**-0.31*** ± 0.03	0.91
STM	0.66 ± 0.87	1.36 ± 2.58(3/4)	111.91 ± 31.66	**45.78*** ± 10.43	1.14 ± 0.52	**0.31*** ± 0.05	-0.10 ± 0.04	0.19 ± 0.15	0.38 ± 0.16	**0.03*** ± 0.004	-0.04 ± 0.01	0.91

3.4.6 RPE and key soil and microbial parameter

Relationships between soil and microbial stoichiometry, microbial community composition and priming effects are summarised in Table 3.6, providing Pearson's product moment correlation of soil and microbial biomass C, N, and C:N ratios, as well as soil mineral N and fungi to bacteria ratios (F:B), with the different RPE values. The measured values of RPE are mostly negative. Therefore, a positive correlation indicates that as one variable increases, RPE gets less negative. Similarly, a negative correlation indicates that as one variable increases, the more negative RPE gets.

Soil C:N was significantly and positively correlated with negative rhizosphere priming for all three measurements of RPE in vivo (RPE 1-3), as well as their average (RPE average), but uncorrelated to RPE of final root-soil-incubations (RPE 0). For these, soil C and N individually and mineral N were significantly and negatively correlated.

Microbial biomass C:N was significantly and negatively correlated with the last plant-soil priming estimate (RPE 3) and the post-harvest root-soil RPE 0. No consistent trend was observed for microbial biomass carbon, while microbial biomass nitrogen was significantly correlated with all RPEs apart from RPE 3. F:B ratios were significantly and positively correlated with the first two measurements of RPE (RPE 1 & 2) and the average RPE.

Notably, rhizosphere priming from the final plant-soil incubation (RPE 3) was least correlated with the parameters included here, with significant correlations only with soil and microbial C:N ratios. Furthermore, mineral N was significantly correlated only with rhizosphere priming measured in the final root-soil incubations (RPE 0).

Table 3.6: Pearson's product moment correlation of soil and microbial carbon, nitrogen and C:N and fungi to bacteria ratios (F:B) with each estimate of rhizosphere priming as measured during three consecutive plant-soil incubations (RPE 1 - 3), the average rhizosphere priming effect from that period (RPE average) and rhizosphere priming quantified in laboratory root-soil incubations (RPE 0) after removal of above-ground plant material. Significant ($p < 0.05$) correlations highlighted. cc = correlation coefficient (R^2), df = degrees of freedom

Pearson's product-moment correlation		RPE 1 (Sep)	RPE 2 (Oct)	RPE 3 (Nov)	RPE average	RPE 0
soil C:N	t	**2.75**	**3.80**	**2.90**	**4.23**	-0.93
	df	**27**	**27**	**30**	**30**	28
	p-value	**0.01**	**0.0008**	**0.007**	**0.0002**	0.36
	cc	**0.47**	**0.59**	**0.47**	**0.61**	-0.17
soil C	t	**3.43**	**3.25**	1.46	**3.79**	**-4.07**
	df	**27**	**27**	30	**30**	**28**
	p-value	**0.002**	**0.003**	0.15	**0.0007**	**0.0003**
	cc	**0.55**	**0.53**	0.26	**0.57**	**-0.61**
soil N	t	**2.65**	1.47	-0.03	1.92	**-4.98**
	df	**27**	27	30	30	**30**
	p-value	**0.013**	0.15	0.97	0.07	**2.935e-05**
	cc	**0.46**	0.27	-0.006	0.33	**-0.69**
mineral N	t	1.82	0.41	-0.21	1.10	**-4.89**
	df	27	27	30	30	**28**
	p-value	0.08	0.68	0.84	0.28	**3.752e-05***
	cc	0.33	0.08	-0.04	0.20	**-0.68**
microbial C:N	t	0.74	-0.20	**-2.47**	-0.61	**-2.62**
	df	27	27	**30**	30	**28**
	p-value	0.47	0.84	**0.02***	0.55	**0.01**
	cc	0.14	-0.04	**-0.41**	-0.11	**-0.44**
microbial C*	t	**2.69**	1.68	-0.42	1.71	**-3.16**
	df	**27**	27	30	30	**28**
	p-value	**0.01**	0.11	0.68*	0.10	**0.004**
	cc	**0.46**	0.31	-0.077	0.30	**-0.51**
microbial N*	t	**3.09**	**2.12**	-1.16	**2.52**	**-3.71**
	df	**27**	**27**	30	**30**	**28**
	p-value	**0.005**	**0.04**	0.26	**0.02**	**0.0009**
	cc	**0.51**	**0.38**	-0.21	**0.42**	**-0.57**
F:B	t	**2.91**	**3.22**	t=1.87	**3.75**	-1.54
	df	**27**	**27**	30	**30**	28
	p-value	**0.007**	**0.003**	0.07	**0.0008**	0.14
	cc	**0.49**	**0.53**	0.32	**0.57**	-0.28

** p-values of correlations differ significantly between unplanted, planted and Δ of variables*

3.4.7 Potential drivers of RPE

Individual Pearson's square correlations of soil and microbial C:N and F:B with RPE (Table 3.6) indicated that soil and microbial parameters equally contribute to microbial SOM-degradation and hence determine priming effects. As this doesn't directly account for plant-induced changes and interactions between the three compartments of plants, soils and microbes, generalised linear models were built to statistically include some of these factors. Four models (parts 1, 2, 3a, 3b) were fitted and significant parameters ranked by their relevance amongst models (Table 3.7).

Group I comprised factors with significance ($p < 0.05$) in all models (where applicable), which were leaf nitrogen content, soil silt & clay content and microbial groups of actinomycetes and bacteria (combined gram+ and gram-). Furthermore, the interactions between leaf and soil mineral N, root and soil N and between actinomycetes and soil (min)N and leaf N were significant in all models. In all models, root nitrogen was also well correlated with either actinomycetes or fungi.

Group II included factors with overall high significance (present in at least three models, in at least two models with ($p < 0.05$) and others with ($p < 0.1$) and comprised root N, soil mineral N and soil C contents.

Group III included factors which remained present in all fitted models, but showed no consistent significance amongst them. Those were root biomass (dry weight, dwt), soil N and fungi as well as the interactions between soil C and gram+ bacteria and soil C and actinomycetes.

Finally, group IV comprised factors consistently present in parts 1 - 3a (models accounting for plant-soil-microbe interactions), but without coherent significance. These were soil micronutrients (PKS) and the interactions between soil C:N and total PLFAs.

Table 3.7: Potential drivers of RPE (part 1, 2) and soil respiration with (3a) and without (3b) plants, as determined by generalised linear models. For each part, p-values presented for terms present in final best-fit models. (+) (-) indicate positive or negative effect of fixed term on response variable (note: RPE is negative). When direction consistent amongst significant terms in all models, (+/-) indicated behind fixed effect. Factors highlighted according to their abundance and significance amongst models. sig.code: significance codes for *p < 0.001***, 0.01**,* 0.05*, 0.1., df = degrees of freedom, AIC: Akaike Information Criterion. Detailed individual model outputs can be found in Appendix V2-C3-A5.

		part 1	part2	part 3a	part 3b
	Response variable →	**RPE_av**	**RPE_0**	**CO2_RS**	**CO2_control**
Class	**↓ Fixed term**	microbes + plant-soil	microbes + root-soil	microbes + root-soil	microbes + soil
Plant	LAI	0.368 (+)	n.a.		
	leaf_N	**0.002** (-)**			
	root_N (-)	0.087. (-)	**0.023* (-)**	**0.012* (-)**	n.a.
	root_C			0.034* (+)	
	root_g_dwt	0.026* (-)	0.063. (-)	0.256 (+)	
Plant-soil	**leaf_N:soil_minN**	**0.002** (+)**	n.a.		
	root_g_dwt:soil_C	0.003** (+)	0.028* (-)		n.a.
	root_N:soil_N	**0.017* (-)**	**0.014* (+)**	**0.019* (+)**	
Soil	**soil_siltclay**	**0.007** (+)**	**0.018* (-)**	**0.040* (-)**	**0.047* (+)**
	soil_PKS	0.406 (-)	0.034* (-)	0.172 (-)	
	soil_minN (-)	**0.003** (-)**	**0.014* (-)**	**0.034* (-)**	
	soil_C (+)	**0.002** (+)**	**0.021* (-)**	0.089. (-)	**2.55e^{-05}*** (+)**
	soil_N	0.002** (-)	0.058. (+)	0.369 (-)	0.460 (-)
Soil-microbes	soil_CN:PLFA_total	0.477 (+)		0.161 (+)	
	soil_CN:F_B	0.002** (-)			
	soil_C:Gram_plus	0.222 (-)	0.077. (+)	0.097. (+)	0.0003*** (-)
	soil_C:Gram_neg		0.022* (+)	0.132 (+)	
	soil_C:Actinomycetes	0.002** (-)	0.173 (+)	0.068. (-)	0.161 (-)
	soil_C:PLFA_total			0.167 (-)	
	soil_minN:Actinomycetes		**0.015 * (+)**		
	soil_N:Gram_plus		0.106 (-)		
	soil_N:Fungi	0.006** (-)		0.079. (+)	
	soil_N:Actinomycetes (+)	**0.002** (+)**		**0.255 (+)**	**0.097. (+)**
Microbes	**Actinomycetes**	**0.002** (-)**	**0.0427 * (-)**	**0.036* (-)**	**9.16e^{-05}*** (-)**
	Fungi	0.240 (+)	0.463 (-)	0.210 (-)	0.199 (-)
	Gram_plus	**0.004** (-)**	0.446 (+)	**0.045* (+)**	0.292 (+)
	Gram_neg (-)		**0.047* (-)**	**0.045* (-)**	**3.04e^{-06}*** (-)**
	PLFA_total			0.400 (-)	
	mb_C			0.007** (-)	0.035* (-)
Plant-microbes	**leaf_N:Actinomycetes**	**0.003** (+)**	n.a.		
	root_N:Actinomycetes		**0.031* (-)**	**0.018* (+)**	
	root_N:Fungi	**0.037* (+)**	0.155 (+)		
	root_N:Gram_plus		0.021* (+)		
	root_C_Gram_plus		0.030* (-)	0.040* (-)	n.a.
	root_C_Gram_neg		0.049* (+)		
	root_C_Actinomycetes		0.029* (+)		
	root_g_dwt:Gram_plus		0.076. (+)		
	root_g_dwt:PLFA_total	0.019* (-)			
AIC		275.05	244.28	506.66	437.87
df residuals		6	3	6	18

3.5 Discussion

3.5.1 Seasonal RPE *in vivo* (H1)

Hypothesis 1 stated that the magnitude and direction of RPE would depend on soil carbon and nitrogen availability. RPE from plant-soil incubations (RPE 1-3 and average RPE) were indeed consistently positively correlated with soil C:N, while RPE from the final root-soil incubations (RPE 0) was negatively correlated with mineral nitrogen (Table 3.6). This is a surprising result, as one would expect mineral N to be more important in the presence of a live plant, which has an active uptake of mineral N. Even though the initial phase of negative priming *in vivo* could be attributed to the addition of mineral nitrogen, this is not supported statistically, as plant induced rhizosphere priming (RPE 1-3) was not correlated with mineral N (Table 3.6).

Although significant correlation for soil C:N and mineral N with RPE estimates were found, no consistent trend was identified for magnitude and direction of rhizosphere priming. Predominantly negative priming was observed for all soils, apart from the organic Tundra heath soil. The greatest magnitude of negative priming was observed for organic soils from above the treeline in both Peru and Sweden: the organic Puna soil showed strong negative priming in November (PPO, RPE III, Fig. 3.3), while the organic Tundra soil showed strong positive priming in September (STO, RPE I, Fig. 3.3). The changes of magnitude of rhizosphere priming observed during the course of the experiment are likely the result of several interacting factors: the initially higher availability of mineral nitrogen facilitated plant growth and microbial activity, different stages of plant growth and productivity can result in different amounts of nutrient uptake from soil to plant and differential rates of root exudation to soil. The microbial community differentially adapted to this situation, while microbial demand for nutrients, primarily of C and N, could be met by labile sources at all times (negative priming) for the majority of soils.

The different patterns of rhizosphere priming during the experiment showed contrasting directional progression for organic and mineral soils. In the Peruvian soils, rhizosphere priming became more negative over time in the organic soils, but less negative over time in the mineral soils. If SOM contains large quantities of undecomposed organic matter (plant litter), plants can also have a negative

effect on SOM mineralisation by absorbing mineral nutrients, which would otherwise be taken up by microbes that decompose the C-rich plant compounds (Chaparro et al., 2014). The Swedish mineral soils showed no temporal change of priming intensity, while for the organic soils more negative rhizosphere priming was observed. Notably for the organic tundra soils, this reversed the initial positive priming to negative priming. Generally, the soil microbiome in organic soils is considered to be more nutrient limited (Dijkstra et al., 2013), while microbes in mineral soil are rather energy (C) –limited (Fontaine et al., 2007). Hence the introduction of plants has different effects in the two soil horizons and their C and N status interacts with plant growth during the growing season. At the time of the first RPE measurement, plants were flourishing, reflected in high rates of photosynthesis and large LAI (Table 3.5). This likely has a two-fold effect in the rhizosphere: on the one hand, increased plant productivity would also increase root exudation and hence increase the supply of fresh C to microbes (Kuzyakov & Cheng, 2001; Jones et al., 2009). On the other hand, increased plant productivity is likely also increasing the nutrient demand of the plant (Cheng & Gershenson, 2007), detracting this resource from microbes. At the beginning of the experiment, more negative priming was observed from the mineral soils. This suggests that even though these soils have less than half the amount of C and N compared to their organic counterparts (Table 3.3), which should intensify competition when a plant is introduced, this led to a large reduction in SOM-mineralisation, potentially due to microbes in these mineral soils being energy limited.

One possible mechanism to regulate plant and microbial supply and demand, is the establishment of a mutualistic relationship, where microbes utilise the labile C provided through root exudation to degrade SOM rich in nutrients, which are then also available to plants via root uptake. Similar effects of reduced SOM - mineralisation have been observed in laboratory incubations (Murphy et al., 2015; Rousk et al., 2016; chapter 2). For plant-soil experiments, negative priming can occur when the overall supply of nutrients in the rhizosphere exceeds plant and microbial demand (Dijkstra et al., 2013). This can be further enhanced when plant productivity is reduced, switching the nutrient sink from plant to soil. Near the end of the experiment, reduced plant productivity (low PS and senescent leaves, (Table 3.4) was observed. Different plant traits, such as

specific leaf area and root biomass have been shown to determine rates of plant exudation (Guyonnet et al., 2018). This again can have a two-fold effect on rhizosphere processes: on the one hand, root exudation could be reduced as a result of lower plant productivity, reducing the energy supply to microbes. On the other hand, root decomposition of aging plants could be increased, which could form a new source of nutrients (Cheng & Gershenson, 2007). The effect of senescing plants on SOM-mineralisation is therewith unclear, but it could be a change in the type of soil inputs from simple and labile sources of exudates (LMW sugars) to a greater range of complex compounds in senescing roots, and / or a change in the C:N of these compounds. According to this proposition, direction and magnitude of rhizosphere priming effects could then depend on the microbial capacity to utilise these different sources. For the early senescence phase, more negative priming was observed in organic soils compared to mineral soils (Fig. 3.3). Given that the introduction of a plant likely enhanced biological (microbial) activity compared to unplanted soils, particularly in the rhizosphere (Dijkstra et al., 2013), this would result in increased microbial demand even towards the end of the growing season, when plant productivity declined. In organic soils, enhanced microbial demand could be met easier, through either higher availability of C and N from the labile fraction of SOM (Kolář et al., 2009) or more labile C from rhizodeposition, as higher root biomass and higher overall C in plant biomass in organic soils was observed (Table 3.4). The mechanisms at play could not be unravelled in detail for this study, but likely include both plant-microbe-interactions (mutualistic, commensalistic or parasitic) as well as microbe-microbe interactions (competition and cooperation).

Shahzad et al. (2018), also reported strong seasonality in plant-induced RPE, but in contrast to their study, where exclusively positive priming occurred, in this experiment predominantly negative priming effects were observed (Fig. 3.3, 3.4). This could potentially indicate differences in plant-soil interactions between C_3 and C_4-grasses, which have also been shown to be differentially associate with fungi (C_3) or bacteria (C_4) (Cheng et al., 2016). Extrapolating prevailing negative priming to ecosystem C cycling would presume a mechanism of C sequestration over time, as plant growth – in addition to the C stored in biomass – also reduced the amount of C mineralised from SOM and released to the

atmosphere via soil respiration. The soils studied here originate from undisturbed ecosystems. The presented results suggest that, in a balanced ecosystem, plant-soil-microbe interactions are finely tuned to dynamically adjust soil nutrient supply to plant and microbial demands. This continuous adjustment, which is manifested in more or less intense priming effects, could be based on the principle of soils functioning as a bank (Fontaine & Barot, 2005; Perveen et al., 2014): plants and microbes dynamically debit and deposit into a continuous store of nutrients in the soil matrix. On a larger scale, in undisturbed ecosystems, priming effects are hence not necessarily altering the overall ecosystem C balance, as they fluctuate according to plant and microbial requirements. Though, it has been shown that in agricultural and highly managed soils, priming effects can lead to significant losses of carbon from SOM (Bell et al., 2003).

In this study, positive priming (additional mineralisation of SOM) following the introduction of a live plant was observed only for organic soils from the Swedish tundra. These soils stand out with various parameters lying at the far end of the scale: highest soil C (> 45 %), highest soil C:N (> 30), highest F:B (> 0.5), highest total PLFAs, greatest fungal abundance and distinct vegetation, which comprises Ericaceae (e.g. *Vaccinium myrtillus*). Tundra soils might therefore be prone to positive priming for different reasons: Firstly, they are rich in C and N (Table 3.3) and hence unlikely to limit microbial activity in these key elements, even when plant and microbial demand are increased. Secondly, they host a large microbial community, which could be functionally broad (Fanin et al., 2015), as the tundra biome naturally undergoes seasonal patterns (temperature and vegetation) where the presence of recalcitrant litter with a high C:N or lignin:N ratio can enhance immobilization of inorganic N and thus reduce net N-mineralisation (DeLuca et al., 2002; Keeney 1980; Scott and Binkley, 1997). Finally, C cycling in tundra soils can be slowed down under ericaceous plants, as they take up organic N and release complex OM and phenolics (Kielland, 1994; Wardle, 1997), while the experimentally introduced grass likely provided more labile C and acted as a weaker N-sink. In contrast, the high Andean Puna vegetation is composed of relatively uniform grasses and does not undergo summer-winter seasonality (chapter 1), which could lead to a restricted

microbial community with lower rates of SOM-mineralisation, especially in the presence of labile C-inputs from root exudates.

3.5.2 Distinct priming in organic and mineral soils (H2)

Under hypothesis 2 rhizosphere priming was predicted to be marginal in organic soils and more pronounced in mineral soils. This is not supported by the data gathered in this study, as all soils showed predominantly negative priming of a similar magnitude, with only organic tundra soils demonstrating positive or marginally negative priming (Fig. 3.3). While no differences in microbial SOM-degradation between organic and mineral soils were observed (apart from tundra), higher rates of photosynthesis, plant biomass and total C in plant and microbial biomass for organic soils were detected, compared to the corresponding mineral soils (Table 3.4). Although all predominantly negative, characteristic patterns of rhizosphere priming over time were observed for the two soil horizons. Two possible contributory factors are the soils' contrasting soil texture and C and N contents (Chen et al., 2019). These differences are more pronounced in the Swedish soils, where the mineral soils are sandy loam and the organic soils are composed of primarily pure organic matter (Table 3.3), and indeed the final root-soil incubation revealed a proportional distinction of RPE (Fig. 3.4). This observation of stronger rhizosphere priming effects (less negative) in mineral soils, compared to the organic soils, supports the proposition that mineralisation of deep soil C can be enhanced through labile organic inputs at greater rates as is the case of top soil C (Fontaine et al., 2007; Bernal et al., 2016).

Interestingly, the here observed differences in RPE between organic and mineral soils were less pronounces in the Swedish soils regarding the seasonal development of priming effects. A potential seasonal decrease of RPE was observed amongst the organic soils, particularly the Peruvian Puna grassland and Andean forest. These samples have comparable soil texture and nutrient status of organic and mineral horizons. In contrast to Chen et al. (2019), for the here observed priming effects it could therefore be assumed, that soil mineral structure and C and N are not primarily causing the observed seasonal trends of RPE. If this was the case, one would expect an even stronger differentiation

between organic and mineral soils amongst the Swedish samples, but particularly the mineral soils showed no temporal pattern (Fig. 3.3). One possible reason for the absence of seasonality in these sandy soils could be that the low total soil C and N contents (Table 3.3) limited the magnitude of microbial SOM-mineralisation as neither enough energy nor nutrients were available (Gilbert et al., 2008).

For the Swedish forest mineral soil, several parameters stood out: plants developed particularly high root:shoot ratios (0.6), while aboveground plant biomass was comparable to plant biomass on mineral tundra soil, there was up to three-times more root biomass in organic soils compared to mineral soils (Table 3.4). This could indicate, that nutrient acquisition in the mineral forest soil was impeded for the plant and hence more roots were needed to acquire sufficient nutrients to meet plant demand (de la Riva et al., 2017). On a spatial scale, the strength of the plant sink was thus enhanced and increased the competition between the plant and soil microbes. This is in accordance with Poirier et al. (2018), who showed that root traits can determine C stabilisation in soil. This idea is further supported by the finding that the large increase in microbial biomass carbon (+ 349.77 %) in this soils was accompanied by a decrease of δ^{13}C in microbial biomass carbon (Table 3.5). This suggests, that the additional carbon in microbial biomass originates from a soil source, rather than plant source. Mechanistically, this could link to the idea that the plant was already struggling itself to obtain sufficient nutrients from SOM or that N-immobilisation in microbial biomass was a remedy for the plant to store the excess of mineral nutrients. This is supported by Chaparro et al. (2014), who demonstrated that the assembly of microbes in the rhizosphere follows the seasonal growth of plants and could be one of the mechanisms of carbon and nutrient trading in the rhizosphere (Jones et al., 2009). Interestingly, in this study the large increase of microbial biomass C in the mineral soils from Swedish forests did not induce significant positive rhizosphere priming (Fig. 3.3). This is only possible, if a) unlikely: microbial metabolic processes of growth and maintenance respiration can be selectively assigned to labile carbon from plants for respiration and soil-C for biomass b) Rhizosphere priming was determined only three times in 100 days of plant growth. Maybe, some positive priming happened (see outlier in September, Fig. 3.3) c) note that the change of

$\delta^{13}C$ in microbial biomass is, statistically, non-significant. For the scope of this experiment microbial utilisation of plant-provided carbon therefore goes beyond further interpretation. However, seasonal phyto-mediation of microbial SOM-mineralisation could be further tested in studies explicitly designed to address them.

3.5.3 Microbial community composition (H3)

Hypothesis 3 stated fungal to bacteria ratios would be related to priming effects. Indeed, F:B ratios were correlated with the RPE estimates of the first two plant-soil incubations (RPE 1 and 2) and microbial biomass C:N was correlated with RPE of the last plant-soil incubation and the final root-soil incubation (RPE 3 and 0, Table 3.7). F:B ratios were non-significant in generalised linear models, while distinct dependence was instead identified for individual functional groups of biomarkers assigned to actinomycetes, fungi and bacteria (Table 3.5).
The most notable increase in microbial biomass nitrogen was observed for microbes from the Peruvian forest soils (organic: + 959.85 %, mineral: + 195.94 %), while the increase in microbial biomass N in the Puna soils was much lower. A significant increase in microbial biomass carbon was only observed for the organic forest soils (Fig. 3.5, Table 3.6). Nitrogen limitation has been shown to be higher at higher elevations at this altitudinal gradient in Peru (Hicks et al., 2019; Whitaker et al., 2014a,b; Nottingham et al., 2015, 2009), where in the latter study the authors also showed a decrease in N-associated enzymes and N-mineralisation at higher elevations. The here reported increases of microbial biomass nitrogen between +195.94 % (mineral) and + 959.85 % (organic, Table 3.6) support the idea of high microbial N-demand for these high Andean forest soils. Together with the previously reported reduced rates of N-mineralisation (chapter 1), the large increase in microbial biomass N suggests high nitrogen use efficiency (NUE) of these forest microbes (NUE = N in biomass / N mineralisation, Mooshammer et al., 2014b). Our results do not support this for the Puna soils above the treeline and also don’t support the idea of microbial homeostasis, as significant decreases of microbial C:N ratios were observed for the two soils (PFO, PPM).

Differences in microbial C and N between planted and unplanted Swedish soils followed characteristic patterns in each soil horizon. In the mineral soils, the

increase in microbial biomass carbon in planted soils was at least double the increase of microbial biomass nitrogen, while in the organic soils, the increase in microbial biomass nitrogen was at least twice as high as the increase of microbial biomass carbon (Fig. 3.5, Table 3.5). This supports the idea that microbial activity in mineral soils could be energy (carbon) limited, while in the organic soils carbon is more abundant and nutrient limitation can restrict microbial activity. For the Swedish organic soils, significant decreases of gram+ bacteria were concurrently observed (Table 3.5). If the increase in microbial biomass nitrogen in the organic soils is mechanistically linked with the simultaneously decreased abundance of gram + bacteria, this could indicate that this functional group is not predominantly involved in microbial N-cycling. Supplementary, their role in carbon cycling is supported in the modelling approach, where the interaction of gram+ bacteria and soil carbon was present in all models and highly significant for control soil respiration ($p = 0.0003$, Table 3.7). For all Swedish soils, the magnitude of increase of the element of lower gain in microbial biomass (organic soils: carbon, mineral soils: nitrogen) was approximately 50% and no significant changes of microbial biomass C:N were observed anywhere (Table 3.5).

Interestingly, overall microbial community composition (as determined by PLFAs only at the end of the experiment) did not change significantly during three months of plant growth (Fig. 3.6, Table 3.5). Despite that, a steady state of microbial communities cannot be concluded, given the significant changes of microbial carbon and nitrogen (Fig. 3.5, Table 3.5). Unfortunately, these were not consistently correlated with the changes of individual functional groups of microbes. Apart from gram+ bacteria in Swedish organic soils, no consistency between stoichiometric microbial parameters (C and N) and biomarkers (PLFAs) was reported. However, total PLFAs and microbial biomass carbon were significantly correlated ($p = 0.0003$, $R^2 = 0.39$, Appendix V2-C3-A3). The modelling approach further indicates, that actinomycetes and fungi are more closely involved with nitrogen cycling than bacteria (Table 3.7). The former group of actinomycetes could play a central role in microbial SOM-degradation and priming, as they could link high CUE (as generally assigned to bacteria) with the capacity to facilitate access to recalcitrant SOM through their growth form of filamentous and branching mycelia, which could breach soil minerals.

But to better understand the role of individual groups of microbes and their community dynamics, more continuous measurements would have been necessary, especially of soil from the rhizosphere directly. But then, one must account for hot spots of microbes along the root. Ideally, this would also include parameters such as enzymes, to understand the link between microbial diversity and functionality.

In summary, these results suggest that different functional groups could be associated with C and N cycling at different extent. For example, actinomycetes could play a part in carbon and nitrogen acquisition - as well as fungi, but they were outcompeted by actinomycetes in the model - while gram+ bacteria were not decisive for microbial biomass nitrogen gains, but instead interact with soil carbon to determine RPE. Interestingly, there is indication that actinomycetes are also quite competitive in nature, antagonising other bacteria and fungi (Broadbent et al.,1971; Oskay et al., 2004). Their study therefore merits further attention, not only because of their potentially dominant role in shaping microbial community composition. Also, they have been shown to promote plant growth and health (Bhatti et al., 2017) and there is indication that actinomycetes and AMF could be more affected by temperature than, for example, gram+ and gram- bacteria (Xue et al., 2018). This would strengthen their importance in plant-soil interactions on a warmer planet in the future.

3.5.4 Plant-soil-microbe interactions

The modelling approach indicates that fungal to bacteria ratios do not necessarily determine priming effects (H1), but rather individual groups of microbes and their individual metabolic capacity. This links them to plant and soil nutrients, why a more comprehensive modelling approach was developed including the three different compartments of plants, soils and microbes. The mechanisms behind priming effects in nature go beyond simple correlations such as between F:B ratios and RPE estimates. While restricted reductive studies (excluding aboveground plant-material, for instance, or soil-substrate incubations) do have their value, they are not suitable to understand plant-soil-microbe interactions on an ecosystem scale. However, with increasing data and model complexity increases the data uncertainty, but also the eagerness for ecological understanding. For this reason the modelling presented in Table 8

was conducted. Nonetheless, caution must be applied to the linear models, based on a small sample size and uncertain RPE estimates. Independent of model AIC or other statistic measures to validate goodness of fit, the different response variables are *a priori* attached with different degrees of uncertainty given the methodology of their measurements. Moreover, the initial number of fixed effects logically varied between the four approaches (Table 3.7, part 1 included aboveground and belowground plant parameters, parts 2 and 3a belowground plant parameters and part 3b included no plant parameter at all), hence model complexity and additivity increases accordingly, reducing the statistical power of each part. As with many modelling approaches, the presented framework is dependent on the range of variables that are inputted and caution should be used when implying causal relationships. The model represents one possible interpretation of the measurements taken. Adding to this the quality of the raw data (uncertainty of RPE estimates, effectiveness of microbial biomass extraction), the modelling approach should be understood as hypothesis about possible, and complex, plant-soil-microbe interactions, which could be more specifically tested in future experiments explicitly designed for testing these hypotheses.

3.5.5 Data uncertainty

In this study the natural abundance of ^{13}C and its fractionation by a C_4-plant were used to determine rhizosphere priming effects in soils. This is why the resulting uncertainty is higher than reported in other studies using artificial CO_2-labelling (Cros et al., 2019, Djkstra et al, 2010). The presented uncertainty clearly shows that better approaches of isotopic labelling *in vivo* are needed, if the aim is to realistically and reliably estimate and predict the effect of plant-soil-microbe interactions on the ecosystem carbon balance. However, extrinsic labelling cannot override the plants' radical freedom to continuously produce a variety of labile carbon compounds and decompose at varying degrees. A source of uncertainty that should not be underestimated is the unknown amount and isotopic composition of root exudates. Collister et al. (1994) reported a general order for plant $\delta^{13}C$ as alkanes < lipids < bulk plant tissue with 8 – 10‰ depletion between compartments. Moreover, rates of root and microbial

turnover continuously change on temporal and spatial scales. Several proxies have been suggested, including plant productivity (Dijkstra et al., 2006) or biomass (Huo et al., 2017)) and a positive correlation of (negative) priming with leaf area index (LAI) was reported. But to fully understand and predict priming *in vivo*, these aboveground parameters need to be mechanistically linked to root-microbe interactions in the rhizosphere. Moreover, soils in this experiment were incubated *ex situ* and after a period of storage (2 years). Thereafter exposing them to increased summer temperatures could have modified both the soil microbiome, as well as extracellular enzyme activity, which could have increased the baseline of net mineralisation in the soils during the course of the experiment.

3.6 Conclusion

Results demonstrate the existence of negative priming effects beyond experimental artefacts and highlight the importance to consider both positive and negative priming effects as important mechanisms of plant-soil synchronisation. By showing that negative priming can occur in microbially mediated plant-soil system over several months, we suggest bilateral RPE (dynamic alteration of positive and negative RPE) as a potential mechanism to regulate nutrient availability in different soils with different CN availability under plant growth. Although several factors limit the transferability of these findings to ecosystem scale (size of pots, planting of one single species in monoculture, *ex situ* set-up), they highlight, that negative RPE can occur in soils under *in vivo* conditions. In an extrapolation of the plant-soil synchronization hypothesis (Swift, 1984; Myers et al., 1994; Perveen et al., 2014), this could have a positive effect on the ecosystem C-balance, which has received little scientific attention so far. Results also indicate that further factors of importance for determining RPE are soil texture and microbial functional capacity and community dynamics, as well as the dynamic and seasonal variation of plant-inputs to and nutrient uptake from soils.

III Synthesis and conclusions 151

III.1 Carbon cycling in the Andes and the Arctic

III.1.1 Résumé 152

III.1.2 Patterns and potential mechanisms of carbon turnover 153

III.1.3 Microbial mediators of organic matter mineralisation 158

III.2 Challenges studying C-cycling across ecosystems

III.2.1 Comparing contrasting soils 159

III.2.2 Substrate vs plant experiments 161

III.2.3 Error propagation and uncertainties 165

III.3 Could positive and negative priming synchronise plant-soil-microbe interactions? 166

III.1 Carbon cycling in the Andes and the Arctic

III.1.1 Résumé

In the experiments conducted most soils from Andean and sub-Arctic forests and the adjacent uplands (Puna grassland and tundra heath) from Peru or Sweden showed reduced SOM-mineralisation both under substrate additions of varying C:N ratios and when hosting a plant. The predominantly negative priming observed contrasts previous studies (Huo et al., 2017; Bastida et al., 2019) and provides empirical evidence that reduced SOM-mineralisation (negative priming) can occur both in short-term laboratory incubations and *in vivo.* This could be further studied to unravel the role of microbial mediation of plant-soil processes in ecosystem functioning and predict the effects of climate change induced species shits and therewith altered plant-soil systems on the overall long-term ecosystem C-balance.

Mechanistically, the presented results support the preferential substrate use theory (Cheng et al., 1999), particularly under substrate-additions. The relative contribution of substrate-C to respiration was measured and negatively correlated with the magnitude of priming. However, in the lab incubation, this was only a short-term effect, as most PE ebbed away after seven days of incubation. Chapter 2 provides further empirical evidence, that negative priming can occur in soils independent of the addition of carbon only, or in combination with nitrogen or other nutrients, disentangling PE from available N and questioning the N-mining hypothesis (Mason-Jones et al., 2017; Wild et al., 2019).

Chapter 3 indicates that negative priming can also be a longer term mechanism in different soils when a plant is introduced. Although several factors limit the transferability of these findings to ecosystem scale (size of pots, planting of one single species in monoculture), they highlight that negative RPE can occur under *in vivo* conditions. In an extrapolation of the plant-soil synchronization hypothesis (Barot & Fontaine, 2005), this could have a positive effect on the ecosystem C-balance, i.e. carbon sequestration, which has received little scientific attention so far. Results from all chapters indicate that further factors of importance for determining (R)PE are soil texture and microbial functional capacity and community dynamics, as well as the dynamic, seasonal and

spatial dynamics of above- and below-ground biodiversity. Microbial N-mining is likely subject to different N-limitations in different soils at different times and potentially follows completely different laws when a live plant is introduced as an active nutrient sink / source.

The main challenges identified in these chapters include i) the need to better understand the (un)coupling of effects of C and N availability from different sources (labile addition and different soil fractions) on microbial activity, ii) the limited suitability to extrapolate findings from controlled laboratory soil incubations, and even pot cultures, to whole ecosystems comprising highly complex plant-soil-microbe interactions and iii) the need to resolve uncertainty of RPE estimates measured in *in vivo* experiments.

III.1.2 Patterns and potential mechanisms of carbon turnover

Even though the observed priming effects differed in their magnitude, some distinct patterns emerged consistently and were valid for both substrate and plant-induced (R)PEs and *in situ* N-mineralisation across the treelines.
In Peruvian soils, despite the pronounced contrast of aboveground plant species between forest and Puna grasslands within a short distance, belowground parameters did not significantly differ between land cover types or soil horizons.

N-mineralisation in the organic horizons in the field was at the same rate, substrate induced PE were all of the same magnitude, irrespective of land cover type. In the substrate experiment (chapter 2), the magnitude of priming was higher in the mineral soils (PFM, PPM) than in organic soils (PFO, PPO) of their land cover counterpart. In chapter 3, in the plant-soil incubations RPE in mineral soils increased (became less negative) over time, while priming decreased in organic soils (became more negative), while in the root-soil incubations, mineral soils primed less negatively than respective organic soils.

For the Swedish soils, PE were distinctive for organic and mineral soils in all experiments. Under substrates of varying C:N ratios, priming was negative for organic soils and positive, but at a low magnitude, for mineral soils. The Swedish organic tundra soils (STO) showed the highest positive priming with

plants, however this reversed to negative priming later in the season when plants started to senescent. The organic forest soils (SFO) showed the largest magnitude of (negative) priming under substrate addition, while all other soils showed significantly lower rates of priming in the controlled laboratory incubation, compared to plant-soil incubations (Figures III.3 and III.4). As true for all soils studied, both positive and negative priming effects have been reported, where negative priming prevailed under live plants. The pattern of RPE in root-soil incubations was very similar for Swedish and Peruvian soils, where priming in mineral soils was less negative than in respective organic soils in all cases. No consistent pattern was identified for RPE in the presence of live plants in mineral soils during the season.

Overall, most priming effects observed were negative, that is: the provision of (labile) substrate reduced native SOM-mineralisation. For both countries, substrate C:N ratio had no significant effect on PE and only one soil from each country showed consistent correlation of substrate-N content and primed C (V2-C2-A3). The amount of carbon primed from soils was at least 10-fold lower in the lab incubation, compared to root or plant-induced RPE, even when normalised per gram of soil carbon. In the plant soil incubation, RPE in all organic soils decreased (became more negative) over time. This highlights that care must be taken when observations and mechanisms determined in laboratory experiments are extrapolated to natural ecosystems. Future studies could specifically address discrepancies between controlled laboratory soil incubations and plant-soil mesocosms, both *in situ* and *ex situ, and* include the effect of seasonality, micro-site conditions and microbial community dynamics on estimates of ecosystem C-cycling.

Table III.1: Summary of findings and their possible interpretations

OBSERVATION	INTERPRETATION	EMPIRICAL EVIDENCE SUPPORTING INTERPRETATION
PERU		
Plant composition changed abruptly across the treeline, soil parameters changed gradually	Above and below-ground biodiversity can be uncoupled	Chapter 1 (in arctic ecosystems: McLaren & Buckeridge, 2019; Fanin et al., 2019)
Substrate C:N treatment had no significant effect on PE	Reject N-mining	Chapter 2, Mason-Jones et al., 2017 ; Wild et al., 2019
Negative priming (both short term response with substrates and long-term response with plants)	preferential substrate-use	Magasanik et al., 1961; Cheng et al., 1999; Hamer and Marschner, 2005; Guenet et al., 2010; Wang et al., 2015
The magnitude of RPE was twice as high in the Puna (Fig. III.3B, D) compared to forest soils (Fig.III.3A, C; please note different y-axis)	Different microbial capacity and / or soil C and nutrient limitations	Chapter 3, Nottingham et al., 2015 (Hicks et al., 2019: higher respiration in Puna soils)
Root induced RPE lower (more negative) than plant-induced RPE in organic soils (Fig.III.3A, B)	Removal of active plant sink -> more C for microbes, nutrients abundant from soil -> preferential substrate use, change of plant inputs	Chapter 3
opposite case in mineral soils (Fig.III.3C, D), where roots induce less negative RPE than measured with live plants	Removal of active plant sink -> more C for microbes -> nutrient limitation from soil: suggests activation of microbes through suspension of energy - limitation	Fontaine et al., 2007
Seasonally decreasing RPE in organic soils (Fig.III.3 (A),B)	Change in identity of plant inputs (simple to complex compounds and/or different C:N), change of microbial community composition	Kuzyakov 2002; Classen et al., 2007; Shazhad et al., 2012
Trend for seasonally increasing RPE in mineral soils (Fig.III.3C, D)	Lower amount of labile plant inputs from senescing plants and/or larger microbial community-> higher demand	(hypothesis)

SWEDEN		
significant differences in ecotone between tundra and forest and edaphic parameters for both horizons	Microbial mineralisation of SOM is governed by different parameters in organic and mineral soils, could be related to rooting depth and PFT, summer-winter-seasonality, mycorrhizal symbiosis type	Hobbie et al., 2002 ; Schimel et al., 2004 ; DeDeyn et al., 2008 ; Rumpel & Kögel-Knaber, 2011; Parker et al., 2016
Substrate-induced PE were negative in the organic soils (Fig.III.4A, B)	Suggests preferential substrate use	Magasanik et al., 1961; Cheng et al., 1999;
and positive in the mineral soils (Fig.III.4C, D).	Suggests suspended energy / nutrient- limitation via substrate addition	Fontaine et al., 2007
Substrate C:N treatment had no significant effect on PE	Reject N-mining	Chapter 2 ; Mason-Jones et al. 2017; Wild et al., 2019
Plant-induced RPE was negative in all measurements for all soils apart from the organic tundra (Fig.III.4C).	Potentially C loss from organic tundra soils from the Abisko National Park	Hartley et al., 2010/12 ; Parker et al., 2018; (but see Liang et al., 2018)
Root-induced RPE was significantly lower than plant-induced RPE in organic soils (Fig.III.4A, B)	Removal of plant sink, change of identity of plant inputs	(hypothesis)
Root and plant-induced RPE were similar in mineral forest soil (Fig.III.4C)	The low C and N contents of the soil prevent dynamic adjustments, other limitation to microbial SOM-degradation (physical protection)	Cotrufo et al., 2013 (but see Keiluweit et al., 2015)
Root-induced RPE was higher (less negative) than plant-induced RPE in mineral tundra soil (Fig.III.4D).	Plant cutting reduced exudation → less labile C available for microbes?	(hypothesis)
Seasonally decreasing RPE in organic soils (Fig.III.4(A),B)	Change in identity of plant inputs (simple to complex compounds and/or different C:N), change of microbial community composition	(hypothesis)
No seasonal change in RPE in mineral soils (Fig.III.4C, D)	low C and N contents of the soil prevent dynamic adjustments(i.e. limited in functional capacity to oxidise significantly more SOM, potentially because not enough “building blocks” for synthesis of enzymes)	(hypothesis)
All other Swedish soils showed opposing direction and contrasting magnitude of priming comparing substrate vs plant induced priming (!!!) (Fig.III.4)	Scale-dependency of priming, thus there are limitations of restricted full factorial lab-experiments to represent natural conditions	Crawford et al., 2017

PERU-SWEDEN		
Magnitude of substrate-induced PE not comparable to plant-induced RPE (*SFO)	Plant-soil-microbe interactions in nature are different from laboratory substrate-soil-(stressed)-microbe interactions	Chapters 2, 3
Large variability (and uncertainty) of plant-induced priming	Limitations of natural abundance isotopic labelling; *in vivo* experiments subject to natural fluctuation of ^{13}C of all compartments	Dijkstra et al., 2010; Cros et al., 2019
Root-only RPE different from live plant	Removal of the active sink of live plant, change in identity of plant-inputs / microbes	Shazhad et al., 2012
Negative priming	suggests preferential substrate-use, could support adjustment of ecosystem C-balance in the long term plant-soil synchronisation processes	(hypothesis)
CONCLUSIONS		
Different (R)PE depending on land cover type and soil horizon	Soil origin determines initial edaphic and microbial parameters (e.g. C and nutrient limitations / use efficiencies) which correlate with priming	Chapters 1, 2, 3
Priming effects vary seasonally	Change in identity of plant inputs (simple to complex compounds and/or different C:N), change of microbial community composition	(hypothesis)
Inconsistency between substrate (PE) and plant induced priming (RPE)	Results from laboratory experiments not necessarily valid in ecosystems (ESMs)	Chapters 2, 3; Hicks et al., 2016 ; Crawford et al., 2017
Positive and negative priming effects can occur	Mechanism to economically adjust SOM-mineralisation to meet plant and microbial demand	(hypothesis)
Considerable uncertainty of RPE estimates	Methodological and experimental limitations, variability of $^{12/13}C$ and turnover rates of all end-members	Chapter 3

III.1.3 Microbial mediators of organic matter mineralisation

Microbial C:N was a good indicator of microbial substrate-use efficiency in the laboratory soil incubations (Tables 2.5, 2.7, 2.8) and several significant interactions were modelled between microbial stoichiometry and RPE in the plant-soil mesocosm study (Tables 3.6, 3.7). The predictive power of these structural parameter could however be improved by establishing a reliable link to microbial functionality, e.g. microbial carbon and nitrogen use efficiencies (CUE / NUE) (Manzoni et al., 2012; Mooshammer et al., 2014a, b), enzymatic capacity (Baldrian, 2009), functional groups (Fontaine et al.,2003), or a combination of several of these parameters (Soares & Rousk, 2019; Malik et al., 2020) and by extending microbial stoichiometry to include phosphorous as a key determinant as well (C:N:P) in both Arctic and tropic ecosystems (Buckeridge & McLaren, 2020; Nottingham et al., 2012).

Six et al. (2006) suggested that bacteria and fungi could have divergent functioning in soil C cycling. While fungi have been shown to induce positive priming effects through their capability to release SOM-degrading enzymes (Fontaine et al., 2011), fungi also are a considerable sink of C into the soil themselves, due to their large biomass (Kramer et al., 2012; Treseder & Holden, 2013). Therefore, the impact of fungi induced priming effects on the net ecosystem C-balance needs to be evaluated carefully, potentially under consideration of different functioning amongst fungal taxa (Fernandez & Kennedy, 2015). Similarly, the precise role of gram positive and gram negative bacteria could be further distinguishing, as they have characteristic responses to seasonality (Buckeridge et al., 2013) and their biomass has different residence times in bulk soil (Schmidt et al., 2011). Results of analysis of RPE in combination with microbial functional groups (Table 3.7) suggested that different groups could have specific functioning in C or N cycling, with actinomycetes and fungi determining N cycling and gram positive and gram negative bacteria monopolising C cycling.

Future studies of RPE could address dynamics of microbial community successions in relation to enzymatic activity and biomass turnover (Fig. III.5) and aim to identify taxa promoting plant growth while reducing C-loss from soil.

III.2 Challenges studying C-cycling across ecosystems

III.2.1 Comparing contrasting soils

Directly comparing soils, which contrast strongly in their properties (edaphic, microbial and life history) in one study enables to address the functional effect of these properties on soil C mineralisation and to better understand how the ecosystems of their origin might respond to climate change. But, it requires careful selection and practice of analysis in order to avoid methodological bias in direct comparison of contrasting study systems.

Example 1: Measuring soil pH

Different soil textures not only cause different water holding capacities (WHC), they can also affect basic measurements such as pH. Soil pH measured in water or KCl can provide values that differ up to 1 unit (own observations, Edmeades et al., 1983, Kome et al., 2018). But this difference is not equal for soils of different texture. The medium of measurement can therefore lead to over- or underestimation of the difference (ΔpH) between the soils (Fig.III.1).

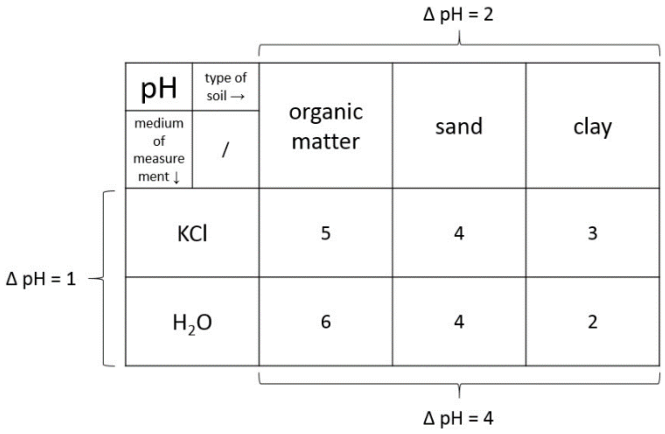


Figure III.1: When measuring soil pH, the medium matters: The absolute values of pH measured in soil solution differ depending on soil texture (organic matter content, clay, sand), with greater variance observed in aqueous solution (deionised water (H_2O)) than potassium chloride (KCl).

Example 2: Microbial biomass extraction from soil

A combination of different soil textures, different soil WHCs and different soil C:N ratios is likely to cause a different extraction efficiency for microbial biomass from differing soils. Although this was not addressed in an experiment specifically designed to directly compare the efficiency of liquid or gaseous chloroform fumigation for different soil types, both methods were independently applied to all eight soils studied here and provided differing results (chapters 2 and 3, Appendix V2-C3-A3), which could be partially assigned to the addition of mineral nitrogen to soils in chapter 3. However, it can be assumed that in soils

with a higher clay and silt content (e.g. those studied here from Peru), not only more organic matter is needed to form stable aggregates, also a differing aggregate stability and soil porosity would differentially affect the surface interaction of chloroform with the soil matrix. The presence of this problem is supported by the finding that in some replicates the amount of microbial biomass C and N determined by gaseous chloroform fumigation in chapter 3 is higher in non-fumigated, than in fumigated soils (values excluded in analysis, Table 3.7). Different correction factors would be needed for soils of different physical properties. Though studies have compared extraction efficiencies between methods of gaseous or liquid chloroform for microbial phosphorous (Bergkemper et al., 2016; Bilyera et al., 2018), more studies could address different conversion factors depending on soil type for gaseous or liquid fumigation techniques (Tate et al., 1988; Dictor et al, 1998; Fierer & Schimel, 2003).

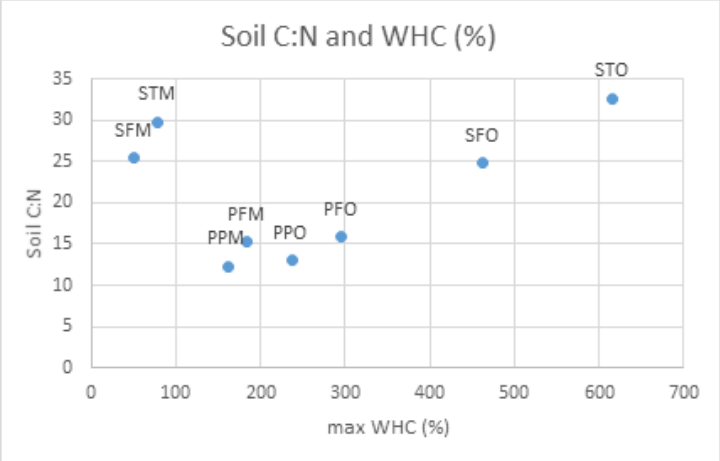


Figure III.2: Correlation of soil C:N and WHC, which is hypothesised to be reflected in the correlation of proportion of microbial biomass C and N extracted and soil physical properties.

Example 3: Irrigation

Different WHCs determine rates and amounts of water additions needed to healthily sustain plants in pot culture. Dense soils (sand) and soils high in clay are more prone to water logging and distribution of water to the lower soil layers in the pots used in chapter 3 (h = 43 cm) was limited by the length of irrigation pins (15 cm) in all soils, and also high WHC in organic soils and compaction in mineral soils. The problem was addressed by continuously measuring soil moisture and administering repeated, low amounts of water (10 ml), tailored for each soil type planted and unplanted (C3-A6). However, it is unlikely that this irrigation system reflects natural ecosystem conditions.

III.2.2 Substrate vs plant experiments

The magnitude of substrate-induced PE was significantly lower than the magnitude of RPE induced by live plants in the majority of soils. The direction of priming was not consistent amongst the substrate treatments and the plant-based measurements (Fig. III.3, 4). The most obvious differences between substrate and pot-experiments are the absence of an active source / sink (plant) in the substrate experiment; the single-time addition and short duration of the lab-incubation vs. continuous root exudation over three months; and the constant ratio of substrate-C to microbial biomass-C vs likely largely varying plant inputs. However, the consequence of the persistent discrepancy between substrate and plant experiments is, that the explanatory power of restricted lab incubations to provide informative values of plant-soil interactions and mimic natural C and N-cycles needs to be specifically tested. Previous studies have shown that significant differences in PE estimated in laboratory incubations can arise already just because of different pre-treatments of soil samples (e.g. sieving (Crawford et al., 2017) and that estimated priming in laboratory incubations is not consistent with priming estimated via field translocation of soil cores (Hicks et al., 2016).

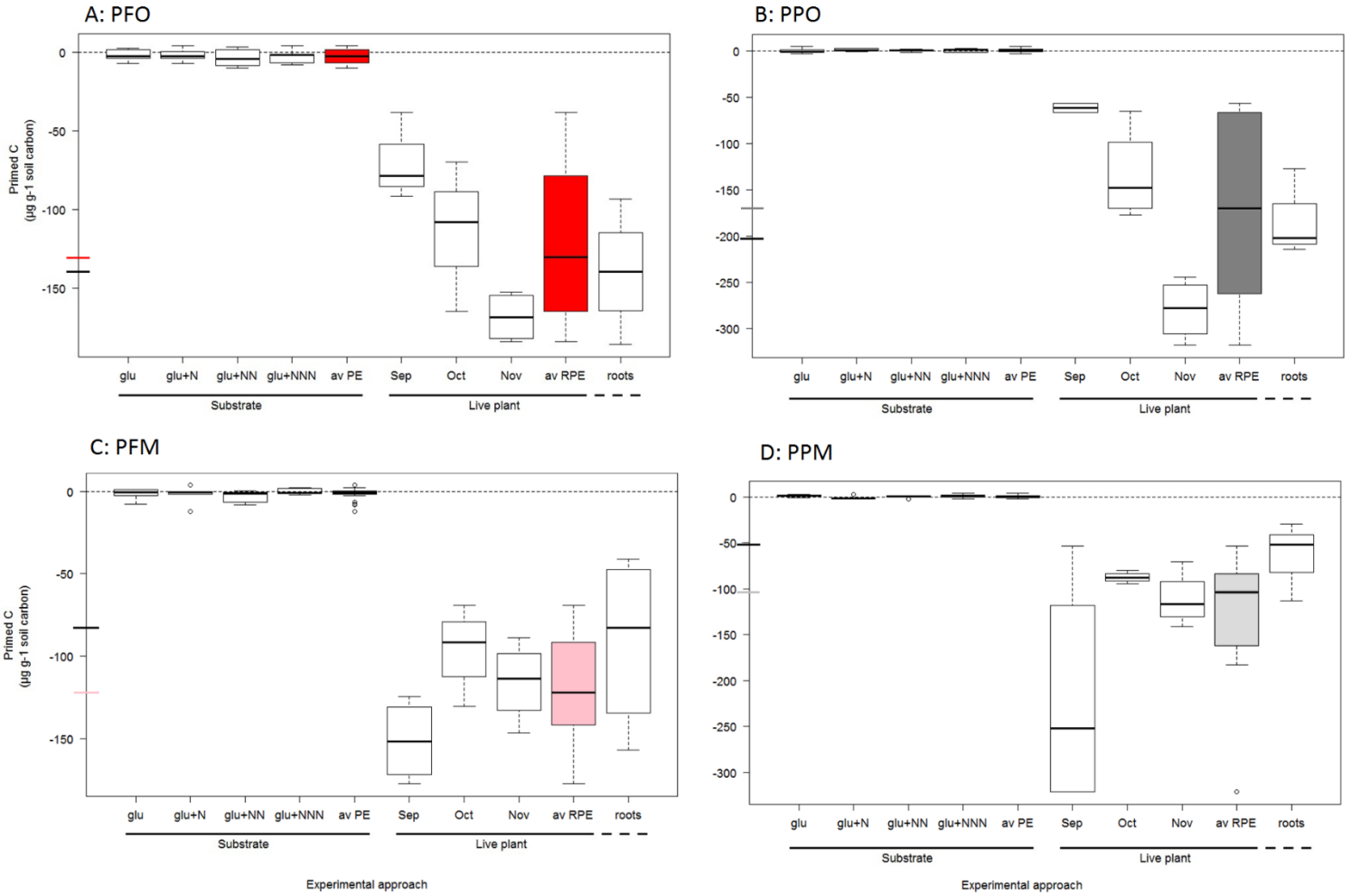
Pot experiments with one single (alien) plant species also only indicate the potential of plant-induced alteration of SOM-mineralisation of different soils and not the possible consequences of increases in plant productivity or vegetation change on SOM-mineralisation in the ecosystems from which the studied soils originate. Many factors act together to determine soil C cycling in nature (Fig. III.5). Lab incubations are therefore likely to serve best to improve the understanding of and unravel mechanisms behind biochemical reactions between soil particles and microbes at a given time. Before conclusions can be extrapolated to natural ecosystems, these mechanisms need to be tested and validated *in situ* in the best case, *ex situ,* but *in vivo,* as a possible compromise.

Plant-soil-microbe interactions are most comprehensively studied when actually involving plants, soils and microbes. However, it must be acknowledged that when studied *ex situ*, the mechanisms at play must not necessarily be the same as those in action *in situ*. In order to address ecosystem responses to climate change, it is necessary to holistically study the dynamic, continuous and

spatially differentiated nutrient and energy flows of living organisms in conjunction with the non-living components of their environment, because they interact as a system. To understand ecosystem functioning, mechanisms are best studied *in situ*, because minor disturbance to the soil samples in transit to the lab can lead to major modification of the processes in subsequent laboratory manipulations, as they may be highly sensitive to initial conditions.

Unlikely, the mechanism of C and N cycling at play in a controlled laboratory soil incubation are not fully representative of large-scale ecosystem C and N processes. Therefore, care must be taken when comprehensive carbon models are based purely on laboratory or greenhouse-based experimental approaches. It would be very ambitious to claim the capture of the complexity of natural ecosystems and their functioning based on such reductionist experiments. It is a scientific challenge to determine the scale-dependency of studying priming effects and the parameters needed in order to build reliable models, incorporating dynamic plant, soil and microbial parameters. This could help to meaningfully address climate change and unravel the potential to improve land management practices for a more careful use of resources.

Figure III.3: (Rhizosphere) priming effects induced by substrate additions and plants / roots for the four Peruvian soil types. Substrate additions were glucose only (glu), with three glucose + nutrient addition of increasing N-content. First colour-filled box represents average of all substrate-induced PEs. Plant-induced rhizosphere priming was measured three times during the growing season, in September, October and November respectively. Second colour-filled box represents average of all plant-induced RPEs. Final box is post-harvest root-induced RPE after removal of above-ground biomass. Boxplots show median lines and interquartile ranges and additional ticks on y-axis indicate mean plant-induced RPE (coloured line) and mean root-induced RPE (black line).

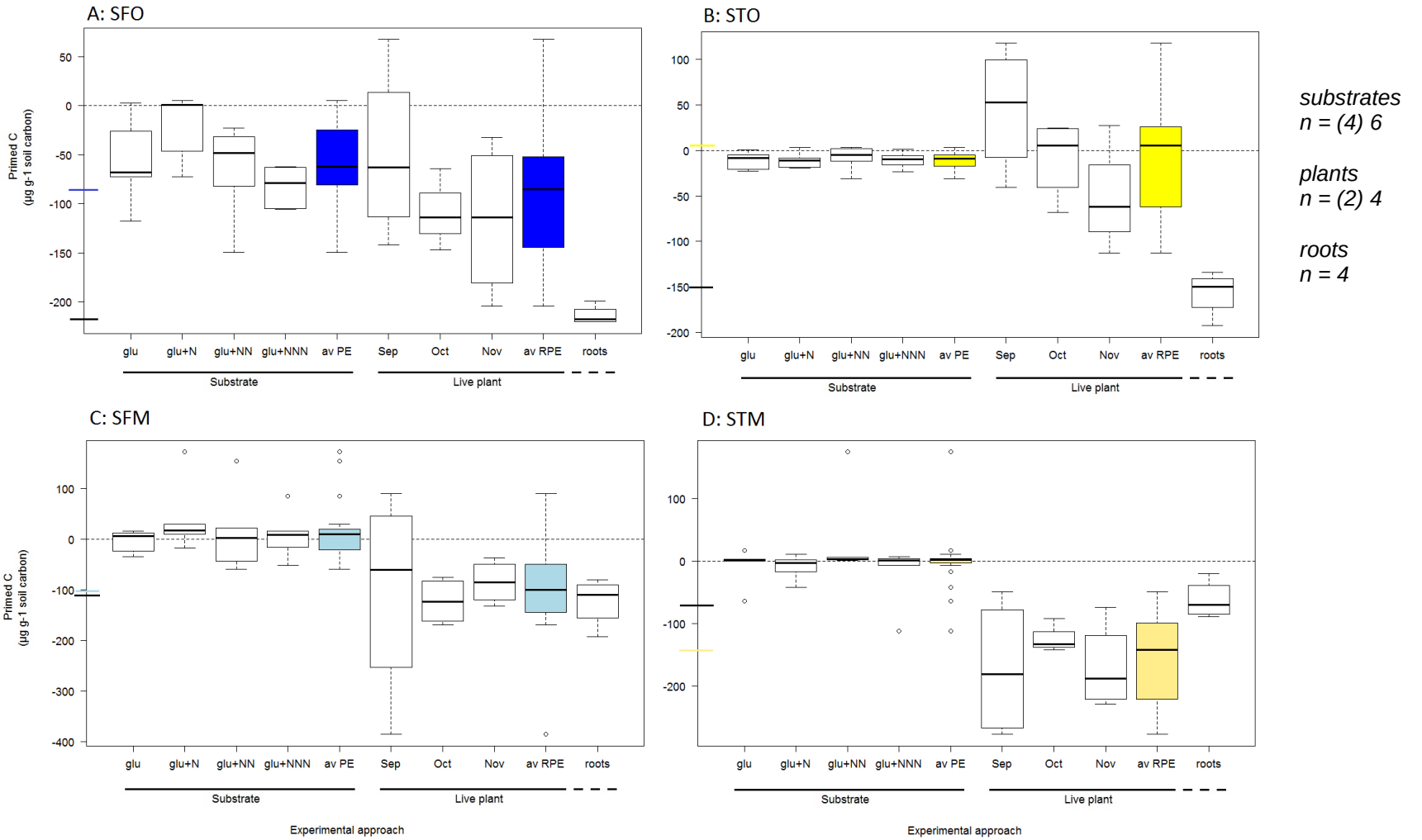


substrates n = (4) 6

plants
n = (2) 4

roots
n = 4

Figure III.4: (Rhizosphere) priming effects induced by substrate and plants / roots for the four Swedish soil types. Substrate additions were glucose only (glu), with three glucose + nutrient addition of increasing N-content. First colour-filled box represents average of all substrate-induced PEs. Plant-induced rhizosphere priming was measured three times during the growing season, in September, October and November respectively. Second colour-filled box represents average of all plant-induced RPEs. Final box is post-harvest root-induced RPE after removal of above-ground biomass. Boxplots show median lines and interquartile ranges and additional ticks on y-axis indicate mean plant-induced RPE (coloured line) and mean root-induced RPE (black line).



substrates
n = (4) 6

plants
n = (2) 4

roots
n = 4

III.2.3 Error propagation and uncertainties

It can be a long way for a soil sample and its microbes from field to laboratory and several factors along the way may introduce errors and uncertainty to the study of its functionality in an experiment. Firstly, the methodology of field sampling can lead to an overestimation of carbon stocks when not accounting for compaction or determining bulk density on small volumes, sampling may yield an artificially selected microbial community and field repetition is essential to obtain representative samples for one soil type or area. Soils in natural environments are not in a steady-state, so it remains to test whether single random samples can account for seasonal fluctuations of temperature, precipitation and plant productivity? Moreover, limited by logistics, sampling can remain bound to a certain spatial range. A spatially nested sampling design can be a further source of random error and data inflation. A factor that may also apply to larger scales, when ecosystems such as “the Arctic” and “the Tropics” are intensively and predominantly studied in certain geographical locations, where logistical support and expert advice are well established, e.g. in proximity of research centres.

In transit to the lab, soil samples may undergo several and / or severe cycles of drying and rewetting, as well as temperature fluctuations exceeding 2 °C. Once in the lab, processing, e.g. sieving, can manipulate soils in a way rendering them unsuitable to represent field conditions (Crawford et al., 2017). Substrate amendments do not account for the variability of plant exudates and they need to be homogenously labelled, but are subject to uncertainty of atomic mass given for elements by IUPAC (Wieser et al., 2013) and added uncertainty of the stoichiometric factor in molecules (Meyer, 2003). But more importantly, lab incubations miss a plant sink / source. When an actual live plant is introduced, isotopic variation between endmembers, particularly under natural abundance labelling, can cause uncertainty of RPE estimates up to 200% (Table 3.1).

III.3 Could positive and negative priming synchronise plant-soil-microbe interactions?

The exchange of carbon and nutrients between plants and soils is highly dynamic. Ecosystem stability and persistence however depends on the establishment of a trading system, which regulates supply of C and N from plants and soils and satisfies plant and microbial demand for these resources (Jones et al., 2009; Perveen et al., 2014). Thus ecosystems would strive towards an equilibrium, which does not connate a static system, but rather a finely tuned exchange and storage of C and N amongst and within the different compartments of the ecosystem (here: plants, soils, microbes) over temporal scales (days, years, centuries). Generally, positive priming can occur when plants and microbes need more C and / or nutrients (e.g. in summer generally, i.e. at peak growing season or diurnally when PS is highest). Negative priming might occur when the present supply is enough to meet plant and microbial nutritional demands (e.g. in autumn, or generally under lower NPP). Negative priming would thus save C in SOM and could be an evolved mechanism of plants to economise soil C resources. This underlying finely tuned balance of mass and energy flows applies to small scales, where plant-soil-microbe interactions determine C-mineralisation from SOM, and nutrient allocation between them (Fig. III.5), and to larger scales, where forests can determine atmospheric CO_2- concentrations. It would be valuable to better understand how positive and negative priming could potentially mediate a stable ecosystem carbon balance in undisturbed and also in managed ecosystems.

Ecosystem C models are being constantly improved by adopting them to newest findings regarding pools and flows of C between compartments (Louis et al., 2016; Woolf & Lehmann, 2019). Recent advances have been made to account for microbial community dynamics (Wieder et al., 2015; Robertson et al., 2019; Kyker-Snowman et al., 2019). However, it remains challenging to include priming effects in model structures (Perveen et al, 2014), because the mechanisms are manifold and highly dependent on temporal and special scales. One approach to account for PE in plant-soil-microbe C-cycling could be to model both stocks and flows of C following a macroeconomic function, with plants and microbes as consumers and depositors of C and nutrients and the soil functioning as a bank (Fontaine & Barot, 2005).

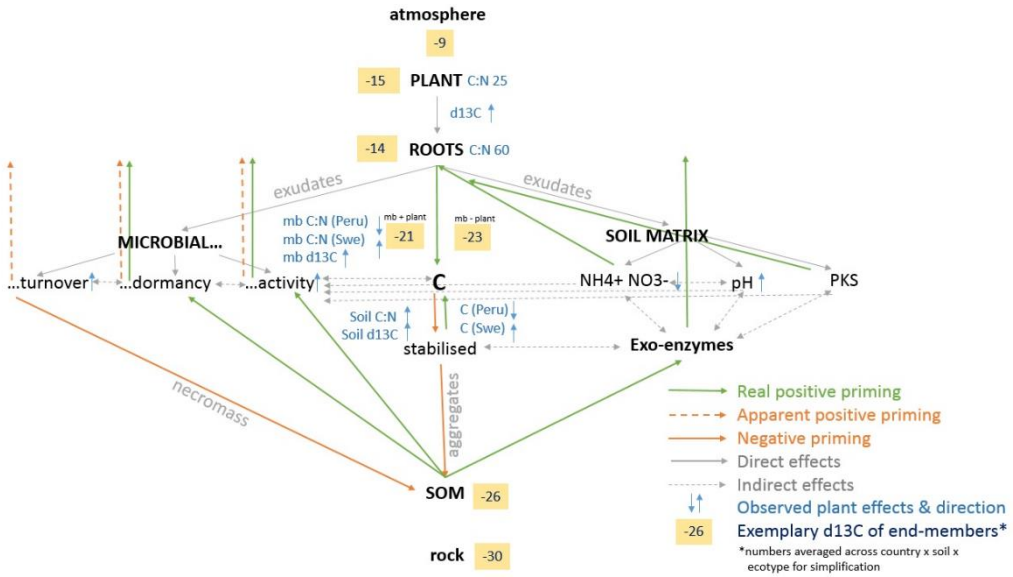


Figure III.5: Exemplary mechanisms and processes in plant-soil-microbe systems and their potential impact on priming effects. Includes data gathered in chapter 3, not all parameters displayed were experimentally accessed.

An alteration of positive and negative priming could function to synchronise supply and demand of C and nutrients between plants and microbes, where microbial SOM-degradation adapts to nutrient availability by switching the nutrient sink from plant to soil when NPP is low (Fig. III.6). Soil nutrient supply can be adjusted to meet microbial and plant demands, with plants and microbes themselves being the actors in that process. The adjustment of soil nutrient allocation could, in addition to (/ as a result of) priming effects, also involve short term storage of both carbon and nutrients in senescing plant tissues and microbial necromass, which could be traded on a daily basis (Chaparro et al., 2014). This would create a potentially nutrient rich source of organic matter in the rhizosphere, potentially important in highly productive plant systems. However, the amount, type and frequency of carbon molecules traded between plants and microbes varies considerably (Jones et al., 2009), which can affect priming effects (Whitaker et al., 2014b; Hicks et al., 2019). Therefore, to develop models incorporating RPE, these different “currencies” need to be specified, as for example in models of marine C-cycling (Christian & Anderson, 2002). Storage of C in soils could be subject to (at least) two rate-limiting steps. Initially, excess C, as well as nutrients, could be sequestered into microbial biomass. This pool is subject to turnover, but remains available until physio-chemical processes stabilise plant and microbial necromass into the soil matrix (Robertson et al., 2019). The more advanced the stabilisation process in the soil matrix is, the higher is the energy investment needed from both plants and microbes into accessing the stored C and nutrients (Skidmore & Powers, 1982;

Newcomb et al., 2017; Jilling et al., 2018). It has for example been shown that plants can recruit distinct groups of microbes depending on environmental conditions (Chaparro et al., 2014; Jones et al.,2009). To confirm these mechanisms, they need to be tested in long-term monitoring of plant-soil-microbe interactions *in vivo*, while determining rates of SOM-mineralisation, microbial turnover and community composition; supply and demand of micronutrients; C stabilisation in the soil matrix and N-mineralisation at high spatial and temporal resolution (Fig. III.5).

The dynamics observed in the experiments of this thesis indicate that it's unlikely that there is a singular mechanism behind priming effects. Likely, all of the many mechanisms proposed to date (preferential substrate use, N-mining, selective N-targeting, stoichiometry) hold part of the truth, as each is valid at some point in natural environments. Ecosystems are complex and dynamic and priming effects constantly occur in plant-soil systems. To address some of the major challenges in climate change mitigation and soil management, it would be very beneficial to study whether plant-soil-microbe interactions could be synchronised to create sustainable plant-soil systems (e.g. cropping systems) and which parameters are necessary, and at what time and spatial scale do they need to be measured, in order to meaningfully inform comprehensive ecosystem C-models and develop reasonable land management practices.

In natural environments, increased plant growth (high NPP) can increase plant nutrient demand. A possible process of phyto-mediation could be increased exudation of compounds that fuel microbial degradation to increase release of nutrients from SOM (positive priming). Contrarily, when NPP is low and plant nutrient demand is reduced, exudation might also be tailored to reduce SOM mineralisation and economise the nutrient stock (negative priming). A particular case could be plant senescence. At reduced rates of plant growth, the plant sink would become weaker and increased inputs of complex compounds from decaying plant tissues become available for microbial degradation (Fig. III.6). The degree to which this affects SOM-mineralisation and stabilisation would depend on microbial community structure, mutualistic, commensalistic or

parasitic interactions between microbes and their functional capacity to degrade the different materials from plants and SOM.

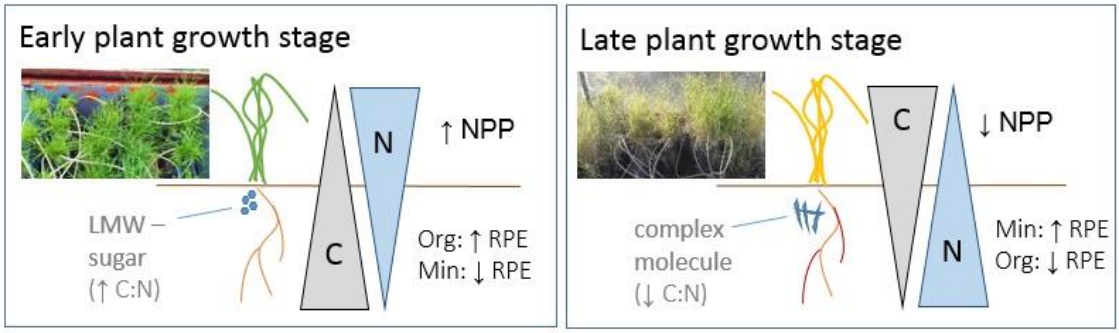


Figure III.6: Conceptual idea of plant-soil-microbe synchronisation during plant growth. Microbial mineralisation of SOM leading to rhizosphere priming effects (RPE) depends on form and frequency of organic input, type of the receiving soil and plant productivity. This framework unifies not only the occurrence of positive and negative priming effects, but also several of the mechanisms proposed (preferential substrate use, microbial N-mining, stoichiometric decomposition). Abbreviations: LMW: low molecular weight, NPP: Net primary production, Org: organic, Min: mineral, C: carbon, N: nitrogen.

This could be tested by addressing the following

Hypothesis: Exudation is adjusted by plants to stimulate different functional groups of microbes. Identity of root exudates changes with plant productivity (nutrient demand) and life cycle. Root decay is higher in senescing plants providing more complex C. Excess nutrients can be stored in – and mobilised from - microbial necromass.

H1: A simple and economic way for a plant to stimulate e.g. bacteria rather than fungi would be to lower rhizosphere pH through exudation of amino acids or other substances that act as “pH-changers”. Or by providing microbes with easily available LMW-sugars. This would be preferred when no additional nutrients from SOM are needed. Theoretically negative priming.

H2: Microbial necromass could function as a buffer, a temporary store, of both carbon and nutrients, which either becomes available to plants and microbes under increased demand, or stabilised into the soil matrix when labile supply is sufficient to sustain plant nutrient uptake and metabolism of active microbes.

H3: When NPP is high and plant nutrient demand is increased and additional nutrients are required from SOM, a possible plant strategies could be increased root growth to break up soil aggregates and / or stimulation of fungi or actinomycetes to mine nutrients from SOM. This could be triggered through release of more complex compounds in exudates.

IV Acknowledgments***

“the whole is something else than the sum of its parts”
(Self-Organization in complex ecosystems, Solé and Bascompte, 2006)

To my supervisors, fellow students, colleagues, friends and family I’d like to express my sincere gratitude. Thank you for all the small, existential and pivotal things I’ll happily remember, and thank you so much for making the big things possible as well. Thank yous muchly also for helping in the field and in the lab and on the screen and for your sympathetic support and sunny smiles in stormy times.

Thanks for your friendly attitude and for being such great role models of dedicated scientists and generously sharing your valuable expertise (and cookies and some field work ☺ thank you!!!). It was a pleasure to work with such knowledgeable people who want to create understanding.

I am truly grateful for having been on this journey of a PhD. It would have been impossible without you. It was an honour to meet such amazing and inspirational people across the globe and see the sun rise at so many wonderful places.

Thank you very much,
muchas gracias,
tusen+1 tack,
merci beaucoup,
vielen Dank,
and cheers.

V Appendices

V.1: Methodological protocols

V.1-P1: N-resin bag protocol

Protocol developed with advice from Kate Buckeridge after DeMarco et al., 2011 and the resin bag protocols of the Templer lab, Boston University, 2011 and the Allison lab, University of California Irvine, 2008.

Similar to the tea bag index approach to measure decay rates of plant material in the field (Keuskamp et al., 2013), commercially available tights can be used to make ion exchange resin bags to measure N-mineralisation in the field.

Material:

- Dowex mr-3 mixed bed ion-exchange resin
- 1.2 M HCl
- nylon tights, nude (some commercially added dye can interact with the ion exchange resin)
- scale
- Milli-Q deionised water
- gloves (HCl is a hazardous chemical and to avoid contamination of membrane and ion exchange resin)

Figure V1-P1: Preparation of resin bags, cores with bags in the field

Procedure:

1. Wash the nylon tights in deionised water, then place in 1.2M HCl solution and allow to soak for 2h.
2. In the meanwhile, determine the size of the cores that will be used to mount bags in the field. Determine appropriate size to cut tights accordingly and uniformly.
3. Remove from HCl (gloves!) and wash thoroughly with deionised water. Equip a suitable clean tray with clean blue roll paper, place the washed tights on the paper and allow to dry. This can be speed up at low temperature in the oven.
4. When dry, wearing gloves, cut the nylon membrane into pieces of suitable size. Bags can be made by making knots at two sides of the cut out nylon pieces. Make one knot first to create a nylon pocket.
5. Weigh out 5 g of Dowex mr-3 mixed bed ion-exchange resin, place inside the nylon pocket and close with a second knot.
6. Keep finished bags in small individual sealable plastic bags. They can be labelled according to field site and core to facilitate deployment in the field. Keep bags to place resin bags into according plastic bags when collecting from the field after the incubation period.
7. Store in the fridge.

V.1-P2: Irrigation protocols

Measurement of soil moisture, field capacity and wilting point

Protocol courtesy of Sandrine Revaillot & Sébastien Fontaine, Grassland ecology group (UREP), INRAE Clermont-Ferrand

1) Definitions

Water in soils is generally present in three states:

i) Gravimetric water: Water that remains in the soil matrix after saturation of the soil. Initially lost quickly from the soil matrix, leaving the largest pore spaces empty to fill with air. This water occupies the macro-porosity of the soil. When the water runoff stops, the wet soil reaches its "moisture level at field capacity (% FC)", a value that reflects its water retention capacity.

ii) Plant-available water: Water usable by the plant, i.e. retained in the soil, either inside fine capillaries, or in the form of thin films around solid soil aggregates. The plant absorbs this water while lowering the water content. When it is deprived, the plant suffers from drought and cannot maintain metabolic capacity, i.e. it reaches its permanent wilting point (PWP). However, at this stage there is still water in the soil matrix.

iii) Water unusable for the plant: It forms around aggregates in a very thin film. This water is retained by the soil with much more energy than the suction force of the plant roots.

The capillary potential measures the suction force of the water by the soil. The suction force of water by the soil can be expressed as pressure in g cm $^{-2}$, or in atmosphere (1 atm = 1033 g cm $^{-2}$). It is more commonly expressed by the logarithm of this pressure called pF. The wetter the soil, the lower the suction, so less water is retained by the soil. The more the soil dries out, the more this suction force increases.

Table V1-P2.1: Values of soil water retention force (*pF) corresponding to the characteristic values of soil moisture*

Suction P (g cm $^{-2}$) 1 atm (1033 g cm $^{-2}$)	pF = log P	Water status
0		Saturation
562	2.75	Field capacity
15849	4.2	Permanent wilting point
Env 100000	5	Soil air dry (hum relative = 92)

The characteristic values of soil moisture, field capacity and permanent wilting point correspond to constant pF's for one soil type. The same pF corresponds to a variable humidity according to the texture of different soils (e.g. comparison between sandy soil /clay soil). The humidity measured for field capacity and permanent wilting point is likely weak for sandy soil, because as the texture is coarse (sand), there is less contact with water films. This water retention capacity of a soil, although theoretical, can be useful in soil water management operations: for the water supply of plants, for irrigation and sewage treatment. It is also useful for the construction of models describing the transport of water and solutes within the soil matrix and in plant-soil systems.

2) Soil water retention applied in centrifugation:

It is possible to determine the pF of a soil by applying centrifugal force to saturated soil. The mechanical energy created by centrifugation makes it possible to apply a force equivalent to the suction exerted by the plant in the soil on the water it contains. A constant centrifugation speed corresponds therefore to a state of equilibrium of water in the soil. During centrifugation, the local acceleration experienced by a point located at a distance R from the axis of the rotor is described by the equation:

$P = 11.18 \times R \times N^2 \times 10^{-6}$

with

P: pressure of suction (g / cm²), 11.18: constant, R: rotor radius (cm), N: number of spins / min.

Transposed to obtain the number of spins for the centrifuge, this provides:

$$\mathbf{N=} \sqrt{\frac{P}{11.18 x R x 10^{-6}}}$$

3) Measurement of field capacity

Material:

- ThermoScientific SL16R Centrifuge with TX200 rotor (90° angle)
- 40 ml tubes with holes of 1cm deducting the thickness of the tube holder
- Filter paper (to prevent soil loss through the holes)
- Cuvette, Water, Soil, Aluminium trays

i) The day before measurements:

Take the tubes and place the filter paper at the bottom, then insert the soil by tapping the tube on the bench slightly to compact, adjust to the line of one centimetre.

Place the tubes in a bowl filled with water, halfway up the ground level (i.e. about 0.5 cm of water), take a drip bottle and slightly wet the surface of the soil. Make 3 repetitions per soil horizon.

ii) The next day: Drain the tube container, cover with parafilm to prevent evaporation during centrifugation. Place in the centrifuge (rotor tx200 with bucket 3659 (reference "bucket") with the 3 microtubes at the bottom to facilitate the flow of water, mop rounds to absorb the rejected water, then put the samples in the green racks of the centrifuge.

Rotate at 1400 rpm (for 2.5 pF) for 10 min

Take out the tubes, note the tare of an aluminium tray and unmold the soil taking care to remove the filter, and note the fresh weight.

Dry in the oven for 24h at 105° C.
iii) Two days later: Weigh (dry weight) the whole tray (previously cooled in a desiccator about 10 min).

4) Measurement of soil water content at permanent wilting point
Material:

- Soil sieved to 2 mm
- 2 types of micro tubes: 2 ml tube (cut cap) and 0.5 ml tube prepared with holes on the sides (4 rows of 4 holes each and a hole in the bottom of the tube).This is an important criterion as the holes will allow the water to escape from the soil matrix according to the centrifugal force.
- Blotting cloth, cut into small squares of 3 x 3 cm placed into the micro tubes of 0.5 ml. Previously, the small piece of fabric is wrapped around a 1ml tip and heated with an alcohol lamp flame to weld the tip. It can then be carefully filled with soil using a 1 ml pipette tip cut at the end to act as a funnel.

i) Mark the height of soil with a permanent marker on all micro tubes, about 1.8 cm of soil, make a preliminary mark on the drilled micro tube. It is important to have the same soil height in each micro tubes, because the calculation of the speed to apply depends on it. Allow to soak the day before.
ii) The day before measurements:
Take the micro tubes with holes and place the blotting cloth, then place the soil by lightly tapping the micro tube on the bench, adjust soil level in tubes to the line of 1.8 cm.
Soak the micro tubes in a rack with micro tubes that are filled with water, and use a pipette to slightly wet the soil surface.
As a precaution, put a rubber band around the rack to prevent flotation of the tubes.

Prepare at least 3 replicates per soil horizon.
iii) The next day:
Drain the micro tubes with holes. Place them in the larger micro tube. Place the micro tubes in the rotor of the centrifuge. Rotate at 13800 rpm for 10 minutes (see Table 2).
In the meanwhile, note the weight of tare of (numbered) aluminium trays.
Remove the micro tubes, retrieve the soil by removing the filter, place in an aluminium tray and note the fresh weight (aluminium weight + weight of the sample).
Dry in the oven for 24h at 105° C.
iii) Two days later: Weigh (dry weight) the whole tray (previously cooled in a desiccator about 5 min).

Table V1-P2.2: Summary table of the speed to be applied, for a given humidity measurement

variation of pF as function of centrifuge rotor			
		rotor étoile	**rotor microtubes**
		1cm soil in tube	1.8 cm soil in **eppendorf 0.5ml**
pF	P	R=14.8 cm	R=7.45 cm
	g/cm²	tr/min	tr/min
2	100	796	1096
2.5	316	1416	1949
2.75	562	1889	2598
3	1000	2519	3465
	1020	2544	3499
3.3	1995	3558	4894
3.7	5012	5639	7757
4.2	15849	10027	13794

Bibliographie

Soltner D., Les bases de la production végétale, le sol – tome 1, p 67-71, 1986
Calvet R., Le sol, propriétés et fonctions – tome 2, p 154-155, 2003

Modification of an industrial irrigation system to suit scientific watering in small soil pots

(Disclaimer: No commercial conflict of interest is declared.)

The RainBird ®ESPme terminal (Rain Bird Corporation) allows repeated and precisely timed release of water through individually called valves. This provides a solid basis for irrigation that matches plant demand at high temporal and spatial resolution. However, it needs to be adjusted in order to meet scientific precision.

Firstly, water is provided through irrigation needles, which reach 15 cm into the soil (used pots were h = 43 cm, d = 5.5 cm). This constantly provides the top soil with more water than the sub soil. This is also the case in nature, however, depending on soil texture and drainage, water can accumulate at the surface of the pots. Initially, this problem was bypassed by adding overflow-holes and later by reducing the water additions to many repeated amounts of 10 ml water during the day.

Secondly, the amount of water provided by the system is defined as a function of

- i) water pressure in the system and
- ii) the time of opening of the valves.

Both cannot be reduced below a certain threshold, beneath which the system doesn't work efficiently anymore, because it needs to be ensured that independent from distance to the central distribution port, equal amounts of water are released through the individual outlets. The lowest possible pressure was experimentally and empirically tested and adjusted, but still water amounts were too large for the pots of the used size.

The Irrigation System distributes water on a timed base to a maximum of 22 independent stations. Each of these stations is operated by a separate solenoid valve. The system-related shortest irrigation time is 1 minute. Only one solenoid valve is opened at a time and a one-minute pause is taken before switching to the next valve. The system uses a common return line for all solenoid valves (line "C") and offers the possibility to control a so-called main valve.

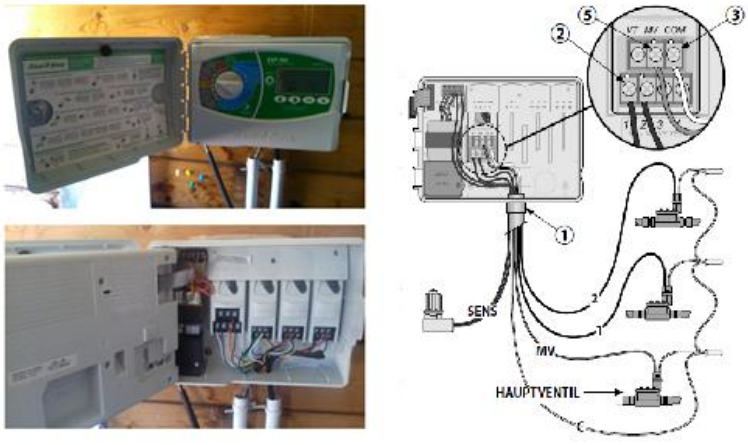


Figure V1-P2.1: ESPMe terminal and circuit diagram

The control of the main valve takes place exactly parallel to the individual solenoid valves of the irrigation. An effective, reproducible reduction of the amount of water added can only be achieved by a well-defined shortening of the minimum irrigation time of 1 minute. The common return line of the solenoid valves offers the chance to intervene in the system and adjust the irrigation times for all solenoid valves synchronous to achieve such reductions. For the experiment presented in chapter 3, a microcontroller relay was mounted to the common return line of the solenoid valves to reduce the irrigation times for all solenoid valves synchronously. The microcontroller was connected to the outlet of the RainBird, which is designed for the main valve. When valves / stations are called individually, this position is programmed “closed”. The relay is mounted in the "normally closed" position so that the irrigation system is not affected in its function. Using a simple keyboard and a four-line LCD display, the irrigation time could be reduced at seconds. Programmed timings were stored non-volatile, so they would be also available after a power failure.

The process of irrigation time reduction in detail:
The system is on and a watering time is entered.
The RainBird system starts a watering cycle, the main valve exit and solenoid valve 1 are activated.
By controlling the main valve, the microcontroller is controlled and relays the sprinkling time as programmed. After expiration of the pre-set time, if the relay opens, the current flow to the active solenoid valve is interrupted. In this case valve 1, and irrigation, stop.
After the set time in the RainBird system, irrigation time will be the output main valve and the corresponding solenoid valve (now 1) is disabled.
The electronics deactivate the interrupted relay and the system is ready for the next cycle.
Due to the pause between the control of the solenoid valves, the trigger time can be accurately detected and the irrigation time reduction work is reliably for all valves.

Figure V1-P2.2: From top to bottom row: Individual valves, water outlets in the greenhouse and irrigation pins to measure water amounts, final set-up with pots

Bibliography:
Rain Bird Cooperation, ESP-Me user manual (6904178-03), 2017

V.1-P3: PLFA protocol & FAME assignment

Adapted from White et al., 1979 by the Plant-Soil laboratory at Lancaster University and the Plant-Soil laboratory at CEH Lancaster

!! Always work in a fume cupboard. Wear lab coat, solvent-protective gloves, and eye protection. Chloroform destroys plastic over time – never invert a pipette with a tip attached and use barrier tips where possible. Tell the lab manager if you think you have aspirated chloroform into a pipette !!

1) Generally

Ensure that you have received appropriate training before using this method by discussing with your line manager. Ensure that you have read through and understood this protocol, risk assessment and MSDS information before proceeding. Store all solvents in sealed solvent bottles in flammable cabinet next to fume cupboard.
Always ensure any open solvents or samples are in the fume cupboard.
Make sample transfers using a Pasteur pipette and handler. Use a clean pipette for each sample.
Rinse out each plastic pipette tip with the relevant solution before use.
Use gloves when using the vortex in case of spillage.
Always replace the right lid on the right tube.
Dispense small quantities of solvents into glass-stoppered bottles for use in fume cupboard.
MINIMISE EXPOSURE OF FATTY ACIDS TO WATER, HEAT, LIGHT AND OXYGEN. These all affect PLFA and reduce the efficiency of recovery.
All containers containing chemicals need to be clearly labelled with the name of the substance, date of opening and initialled. Contact laboratory manager for guidance on chemicals for disposal.
Washing
All glassware, tubes and lids should be washed in phosphate free detergent (Decon 90), rinsed thoroughly with DI water. Glass centrifuge tubes should be dried in an oven at 250°C for two hours or overnight. Plastics lids should be dried in a drying oven (NOT at 250°C!). It is very important that all labware should have no residue and be completely dry and clean.
Reagents
Check N2 cylinder – if low, tell SO or KM.
Ammonia solution (35% ammonium hydroxide) Flammables cabinet B77
Acetic acid (100% glacial) Flammables cabinet B77
Acetone Flammables cabinet B77
Chloroform Flammables cabinet B77
Citric acid PLFA-prep bench
Iso-Hexane Flammables cabinet B77
Methanol Flammables cabinet B77
Potassium hydroxide PLFA-prep bench
Sodium hydroxide pellets PLFA-prep bench
Toluene Flammables cabinet B77
Methyl tridecanoate (C13 standard) Freezer (-20°C) B74
Methyl heneicosanoate (C21 standard) Flammables fridge B77
1,2-dinonadecanoyl-sn-glycero-3-phosphocholine
(C19 standard) Freezer (-20°C) B74

2) STAGE 1 – EXTRACTION

Chloroform and Methanol are DANGEROUS if handled incorrectly. They cause eye and skin irritation and are toxic. Chloroform is potentially carcinogenic and has the potential to cause serious irreversible damage to health if inhaled or swallowed. Inhalation causes nausea, vomiting, dizziness and possible unconsciousness. Chloroform also destroys plastic over time – never invert a pipette with a tip attached and use barrier tips where possible to avoid causing damage. Chloroform and methanol vapours must not be allowed to escape into the laboratory atmosphere.

ALL WORK MUST BE CARRIED OUT IN A FUME CUPBOARD. WEAR GLOVES, LAB COAT AND SAFETY GLASSES. NO LONE WORKING. NEVER INVERT A PIPETTE WITH A TIP ATTACHED AND USE BARRIER TIPS WHERE POSSIBLE TO AVOID CHLOROFORM AND SOLVENT DAMAGE TO PIPETTES

You need to be trained in this procedure and shown to be competent. Seek advice before starting work for the first time.

Reagents

- METHANOL (MeOH)
- CHLOROFORM ($CHCl_3$)
- CITRATE BUFFER 0.15M, pH 4 (+/- 0.02). Fill a 250mL volumetric half-way with Milli-Q water (room C92 3rd floor). Dissolve 7.88 g of citric acid in cylinder and make up to 250mL. Empty solution into clean, labelled glass Citrate Buffer container, adjust to pH4 using NaOH pellets (1 pellett ~ + 0.1, no responsibility is accepted for the accuracy of this information). Replace weekly
- EXTRACTANT (BLIGH AND DYER) ($CHCl_3$:MeOH:citrate buffer, 1:2:0.8v/v/v). Suggested volumes: 70 ml chloroform, 140 ml methanol, 56 ml citrate buffer. Replace weekly
- PC19:0 PHOSPHOCHOLINE WORKING STANDARD (See spreadsheet for preparation). Remember to check and record weigh of stock solution before and after use. Record weight of working solution before and after use. Store in amber glass bottles and, after recording final weight, flush quickly and gently with N_2 before storing in the freezer (-20°C). Replace at beginning of your set of extractions, or every 6 weeks if made up recently.

Procedure

Note: All tubes referred to are round-bottomed, Pyrex glass centrifuge tubes. We have them in large (36mL) or small (9mL). All caps used must have Teflon liner. Make sure you check, as they can fall out. Turn the lights off. Keep the multi-pipette tips used for citrate buffer, chloroform and Bligh and Dyer for later use.

1. Weigh out 1.5 g freeze-dried, ground soil (0.5 g if the soil is very organic) into a large tube (rinsed with chloroform). Remember to include tubes for at least one blank and one appropriate standard soil (i.e. mineral or peat) per batch.
2. Add 1.5 ml citrate buffer
3. To each tube, add 1.9 ml chloroform
4. To each tube, add

- 3.8 ml methanol, then
- 2.0 ml Bligh and Dyer

5. Put on Teflon lined screw top
6. Vortex for one minute

7. Cover with tin foil and leave to separate for 2 hours
8. Remove foil. Vortex for one minute
9. Centrifuge at 650 RCF for 10 minutes
10. Transfer the supernatant to a large tube (rinsed with chloroform) using a Pasteur pipette and red squeezy handler
11. Wash the soil pellet again using 2.5ml of Bligh and Dyer (including blank)
12. Vortex for one minute
13. Centrifuge at 650 RCF for 10 minutes
14. Transfer the supernatant as above
15. Split the phases by adding to each tube

- 3.1ml chloroform
- 3.1ml citrate buffer

16. Cap and vortex for one minute
17. Cover with foil. Leave overnight to separate.
18. Leave the tube containing the soil pellets open to the air in the fume cupboard to evaporate overnight, then rinse down the sink

The following day:

19. Transfer 3ml of the lower phase to a small tube (rinsed with chloroform) using 3 transfers with a filtered tip. Top tips: 1) Use a small beaker of chloroform to rinse each tip before use in the sample to ensure 1 mL is taken in the first aspiration; 2) Don't compress the pipette all the way to the first "stop". Instead, compress it all the way and then raise it a TINY bit before putting it in the sample. Once in the lower phase, gently compress back down to the first stop, so a little bubble of air sneaks out but doesn't mix the suspension, then draw up your sample. This prevents the top phase from entering the bottom of the tip and ending up in your transferred sample. Ask Kelly to show you if you don't understand!
20. Evaporate under a stream of N2 in the sample concentrator (heating block turned OFF). Place the tubes in the block. Place the needles, point upwards, in the B holes – push hard if necessary. To fit the needles easily, lower the sample concentrator until needles are close to tubes, then gently pull the needles down a bit until they tip the inner margin of the tubes. Then lower the sample concentrator and push the needles up again into the membrane.
21. Lower the needles so that they are slightly above the liquid level
22. Open the N2 cylinder 2 turns. Note the pressure in the cylinder. Slowly open the regulator until the flow of air is sufficient to cause a dip in the surface of the liquid with no splashing. Note: an over-vigorous use of N2 can cause evaporation of esters up to C16 (i.e. the ones we are interested in!)
23. When all the liquid has evaporated put lids on the tubes, bag, label and freeze. Repeat stage 1 until all samples are extracted or go on to stage 2. Clean and rinse the needles with hexane in fume hood and leave to evaporate.

3) STAGE 2 – LIPID FRACTIONATION

Chloroform, methanol, and ammonia solution are VERY DANGEROUS if handled incorrectly. They cause eye and skin irritation and are toxic. Ammonia solution (35% ammonium hydroxide) is highly corrosive and causes severe burns and eye damage. Chloroform is potentially carcinogenic and has the

potential to cause serious irreversible damage to health if inhaled or swallowed. Inhalation causes nausea, vomiting, dizziness and possible unconsciousness. Chloroform also destroys plastic over time – never invert a pipette with a tip attached and use barrier tips where possible to avoid causing damage. Chloroform, methanol, and ammonia solution vapours must not be allowed to escape into the laboratory atmosphere.

ALL WORK MUST BE CARRIED OUT IN A FUME CUPBOARD. WEAR GLOVES, LAB COAT AND SAFETY GLASSES. NO LONE WORKING. NEVER INVERT A PIPETTE WITH A TIP ATTACHED AND USE BARRIER TIPS WHERE POSSIBLE TO AVOID CHLOROFORM AND SOLVENT DAMAGE TO PIPETTES

You need to be trained in this procedure and shown to be competent. Seek advice before starting work for the first time.

Reagents

- CHLOROFORM
- AMMONIA SOLUTION 1% in METHANOL (v/v). Partially fill a 250mL volumetric flask with methanol. Carefully pipette in 2.5mL of 35% Ammonia Solution. Remove pipette tip and place in evaporation container in fume hood, leave pipette tip out of the way in fume hood, remove gloves and place in evaporation container. Obtain fresh gloves. Fill flask to line, transfer to stoppered bottle. Leave volumetric flask out of the way in fume cupboard. Leave all things that came into contact with ammonia to evaporate overnight. Prepare solution fresh on day of use. NOTE: You need to use a glass pipette with green handler to do this! The ammonia solution drips right out of a plastic pipette tip. You do not need to be 100% accurate, just close to 2.5mL.
  - Note – If extracting < 20 samples, use a 100 mL flask and 1 mL ammonia solution
- ACETONE

Procedure

The lipids are now separated into different classes with increasing polarity: neutral lipids (hydrocarbons, free fatty acids and sterols), glycolipids and polar lipids (phospholipids). Use the repeat pipette with a 25 ml or 5 ml tip for each step below unless otherwise stated.

1. Rinse one small tube per sample with methanol
2. Place the labelled isolute column in the holder and stand it in a metal tray to catch the waste solvent.
3. Activate the column using 2.5 ml of chloroform.
4. Dissolve the dry lipid material from stage 1 in 0.5 ml chloroform.
5. Vortex carefully without cap for 5 seconds on the single tube vortexer then transfer immediately to the column using a glass pipette and green handler red squeeze thingies. Save the glass tip in the sample tube. Can be done by carefully pouring i.e. without pipettes.
6. Repeat steps 4 and 5 a total of 3 times. Dispose of the glass tips after the third transfer.
7. Add 5 ml chloroform to the column. This elutes the neutral lipids.
8. Add 20 ml acetone in 5ml portions to the column. This elutes the glycolipids.
9. When all the acetone has run to waste, carefully remove the stand from the metal tray and place over the test tube rack containing methanol-

rinsed glass tubes. Ensure the end of each column is in the top of its corresponding tube.

10. Elute the phospholipids by adding 5 ml of ammonia solution in methanol to the column.
11. Once the columns have stopped dripping evaporate solvent in tubes under N2 (see stage 1 instructions). Turn the heating block ON to 40˚C. Turn block OFF before samples are completely dry to avoid baking the dried phospholipid fraction.
    Note: This takes about 1hr 15mins per batch of 18/36/40+ samples! If possible, borrow LEC sample concentrator to speed up the process!
12. Cap the tubes, label and freeze. Repeat stage 2 for the next batch of samples or proceed to stage 3.

4) STAGE 3 - MILD ALKALINE METHANOLYSIS

The solvents used in this stage are DANGEROUS if handled incorrectly. They cause eye and skin irritation and are toxic and flammable. Never invert a pipette with a tip attached and use barrier tips where possible to avoid causing damage.

ALL WORK MUST BE CARRIED OUT IN A FUME CUPBOARD. WEAR GLOVES, LAB COAT AND SAFETY GLASSES. NO LONE WORKING. NEVER INVERT A PIPETTE WITH A TIP ATTACHED AND USE BARRIER TIPS WHERE POSSIBLE TO AVOID CHLOROFORM AND SOLVENT DAMAGE TO PIPETTES

You need to be trained in this procedure and shown to be competent. Seek advice before starting work for the first time.

Table V1-P3.1: Calculate the needed amounts before you start

Reagent	ml / sample	ml to prepare
MeOH:TOLUENE (1:1, v/v)	1	
0.2M KOH in methanol (1.122 g in 100ml Prepare fresh on day of use, transfer to beaker with parafilm cover	1	
HEXANE:CHCl3 (4:1, v/v)	4	
1M ACETIC ACID (Dilute 5.7 ml of acetic acid with Milli-Q water and make up to 100 ml)	0.3	
FRESH MILLI-Q WATER	2 + X to prepare 1M acetic acid	

Procedure

1. Rinse one small tube per sample with hexane
2. Using a filtered tip, dissolve the sample in 1 ml MeOH:toluene. Vortex carefully on single tube vortexer 5 seconds if sample is not fully covered.
3. Add 1 ml KOH. Cap tubes, place in metal rack and incubate in water bath at 37ºC for 15 min.
4. Then add:

- 2.0 ml hexane:CHCl3
- 0.3 ml acetic acid
- 2.0 ml Milli-Q water

5. Cap. Vortex for 1 minute

6. Centrifuge at 480 RCF for 5 minutes.
7. Transfer the upper (organic) phase to a clean hexane rinsed glass tube.
8. Wash the lower layer with 2 ml of hexane:CHCl3 (4:1, v/v), vortex and centrifuge as before.
9. Transfer the upper phase to the small test tube.
10. Evaporate under a stream of N2 (see stage 1 – heating block turned OFF).
11. Re-suspend samples and transfer directly to GC inserts by
12. Add 0.5 ml hexane to each tube.
13. Cover with foil and leave to stand for 10 mins.
14. Returning to sample tubes, vortex gently (can carefully do without cap) on single tube vortexer and transfer sample to GC vial using a Pasteur pipette. Place the Pasteur pipette back into the centrifuge tube.
15. Repeat steps 3 and 5 two more times to ensure complete sample transfer to GC insert.
16. Evaporate under a stream of N2. Freeze in GC vials.

Re-suspending samples for GC analysis
Reagents

- C13/C21 STANDARD Mix (22.67 mg C13, 25.00 mg C21 in 100ml in hexane), transfer to amber glass bottle. Replace at beginning of your set of extractions, or every 2 months if made up recently. Store in fridge.

Procedure

1. Work in small batches (enough for 2 or so days analysis)
2. Allow samples to thaw out in the dark for 30 mins (fume cupboard covered in foil), or for a couple hours in the flam-free fridge
3. Using the glass Hamilton syringe, add 30 µl of C13/C21 mix to each vial. Be very accurate ensuring that there are no bubbles in the syringe. Rinse out the syringe three times with hexane, then pipette in 120 µl hexane and store in fridge for analysis on the GC-FID. Keep in the fridge for no longer than ONE week. If you are not going to analyse them within this time, evaporate and store them at –20ºC.

Note: If you choose to store them in the freezer, you will need to use 150 µl (30 µl + 120 µl from previous steps) hexane only when you thaw and re-suspend them as you won't add standard again.

Table V1-P3.2: Final assignment of biomarkers to functional groups and some of their uncertainty. There is a possibility, that this assignment underestimates bacteria, as other authors have assigned several of the here unspecified biomarkers to bacteria.. As with all PLFA analysis, results therefore have to be interpreted with the highest caution.

Fungi	Actino-mycetes	Gram + bacteria	Gram – bacteria	Unspecified PLFAs
18:2	10Me-16:0	15:0i	16:1(n-7)	14:0i
18:1(n-9)	10Me-17:0	15:0a	cy17:0	14:0
	10Me-18:0	16:0i	18:1(n-7)	15:1i
		17:0i	cy19:0	15:1a
		17:0a	(*gram- ?)	16:1(n-9)*
(*AMF?)				16:1(n-5)*
			(*gram- ?)	16:0*
				17:1i(n-8)
		(*bacteria?)	(*bacteria?)	17:0*
				18:0i
			(*gram- ?)	18:1(n-5)*
				18:0
				19:1

V.2 Supplemental data for each chapter

V.2-C1 Plant species inventory

Many thanks to Jimmy R. Chambi P. and Kate Buckeridge for help with - and corrections of - plant identifications.

*Table V2-C1.1: Peru: Ecotone plant species composition (I:Puna, III:transition, V:forest, % cover and most abundant species, transects A – J, *see comment on page 193)*

A :patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	80	15	5	0
	Festuca orthophyla (Pilg.)	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Bromus spec.	Elaphoglossum spec.	Gaultheria vaccinoides (Wedd.)	
	Calamagrostis spec.	Huperzia spec.	Baccharis genstilloides (Lam.)Pers.	
	Alchemilla lobata (unres.)	Blechnum spec.	Baccharis spec.	
	Hypericum spec.	Leucobryum spec.		
	Gentianella spec.			
III %	20	20	45	15
	Festuca spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Hypericum spec.	Cladonia spec.	Vaccinium floribundum (Kunth)	Gynoxys induta (Cuatrec.)
	Carex spec.	Usnea spec.	Brachyotum spec.	
			Gaultheria vaccinoides	
			Miconia bullata (Turcz.)Triana	
V %	5	25	40	30
	Carex spec.	Leucobrium spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Oxalis spec.	Elaphoglossum spec.	Gaultheria vaccinoides	Clethra spec.
	Poper spec.	Cora spec.	Vaccinium floribundum (Kunth)	Polylepis pauta (Hieron.)
		Cladonia spec.	Desmostemon spec. (?)	Dendropanax spec.
		Usnea spec.	Brachyotum spec.	Gynoxys induta (Cuatrec.)

		Blechnum spec.	Miconia spec.	
			Miconia bullata (Turcz.)Triana	
			Campanulaceae	
			Myrteola spec.	
			Monnina spec.	
			Diplostephium spec.	

B: conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	80	15	5	0
	Festuca spec.	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Bromus spec.	Huperzia spec.	Gaultheria vaccinoides (Wedd.)	
	Luzula peruviana (Desv.)	Leucobryum spec.	Baccharis genstilloides (Lam.)Pers.	
	Chusquea spec.		Gaultheria erecta (Vent.)	
	Hypericum spec.			
	Halenia spec.			
III %	70	15	10	5
	Festuca spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Polylepis pauta (Hieron.)
	Hypericum spec.	Cladonia spec.	Gaultheria vaccinoides (Wedd.)	Gynoxys induta (Cuatrec.)
	Carex spec.	Huperzia spec.	Monnina spec.	
	Bromus spec.	Leucobryum spec.	Siphonandra spec.	
	Gentinella spec.		Diogenesia spec. Miconia spec.	
	Werneria nubigenia (Kunth.)			
V %	10	30	20	40
	Carex spec.	Leucobryum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Rynchospora spec.	Cladonia spec.	Gaultheria vaccinoides	Symplocos spec.
			Myrsine spec.	Polylepis pauta (Hieron.)
			Desfontainia spec.	Saracha spec.

C: patch	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	65	20	15	0
	Festuca spec.	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Jarava ichu (Ruiz&Pav.)	Huperzia spec.	Gaultheria vaccinoides (Wedd.)	
	Cortaderia spec.	Leucobryum spec.	Gaultheria erecta (Vent.)	
	Hypericum spec.	Lyopodium spec.		
		Blechnum spec.		
III %	60	20	15	5
	Festuca spec.	Huperzia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Gynoxys induta (Cuatrec.)
	Hypericum spec.	Leucobryum spec.	Brachyotum spec.	
	Jarava ichu (Ruiz&Pav.)		Miconia spec.	
	Calamagrostis spec.		Gaultheria vaccinoides (Wedd.)	
	Nertera spec.			
V %	45	20	25	10
	Carex spec.	Leucobryum spec.	Dendrospanax spec.	Gynoxis spec.
	Rynchospora spec.	Hymenophyllum spec.	Centropogon spec.	Myrsine spec.
	Luzula peruviana (Desv.)		Miconia spec.	

D: conti*	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	65	15	15	5
	Festuca spec.	Huperzia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Luzula peruviana (Desv.)	Lyopodium spec.	Brachyotum spec.	
	Hypericum spec.	Cladonia spec.	Brachyotum spec.	
	Jarava ichu (Ruiz&Pav.)	Sphagnum spec.	Pernettya postrata (Cav.)DC.	
	Nertera spec.			
	Gentinella spec.			

III %	60	15	20	5
	Hypericum spec.	Lycopodium spec.	Brachyotum spec.	Gynoxys spec.
	Halenia spec.	Cladonia spec.	Pernettya postrata (Cav.)DC.	
	Cortaderia spec.	Sphagnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Bromus spec.		Gaultheria vaccinoides (Wedd.)	
	Festuca spec.		Diogenesia spec.	
	Gentiana spec.			
V %	25	30	20	25
	Rynchospora spec.	Leucobryum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Gynoxis spec.
	Chusquea spec.	Blechnum spec.	Brachyotum spec.	Polylepis spec.
		Sphagnum spec.	Centropogon spec.	
		Cladonia spec.	Monnina spec.	
		Usnea spec.	Gaultheria erecta (Vent.)	
			Gaultheria vaccinoides (Wedd.)	
			Diogenesia spec.	

*E: conti**	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	65	20	15	5
	Festuca spec.	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Gynoxys spec.
	Hypericum spec.	Lyopodium spec.	Gaultheria erecta (Vent.)	Dendropanax spec.
	Jarava ichu (Ruiz&Pav.)	Usnea spec.	Centropogon spec.	
	Cortaderia spec.	Blechnum spec.	Brachyotum spec.	
	Rynchospora spec.			
	Halenia spec.			
	Brachiaria spec.			
III %	60	15	20	5

	Rynchospora spec.	Cladonia spec.	Brachyotum spec.	Gynoxys spec.
	Hypericum spec.	Sphagnum spec.	Pernettya postrata (Cav.)DC.	Oreocalamus spec. (bamboo)
	Cortaderia spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Jarava ichu (Ruiz&Pav.)		Gaultheria vaccinoides (Wedd.)	
	Festuca spec.		Centropogon spec.	
			Hesperomeles spec.	
V %	25	30	20	25
	Cortaderia spec.	Sphagnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia fagaroides (Kunth.)
	Oxalis spec.	Blechnum spec.	Gaultheria vaccinoides (Wedd.)	Gynoxys spec.
	Rynchospora spec.	Elaphoglossum	Hesperomeles (??)	Oreocalamus spec. (bamboo)
			Brachyotum spec.	
			Myrsine spec.	
			Diogenesia spec.	
			Centropogon spec.	

F: conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	85	10	5	0
	Calamagrostis spec.	Lycopodium spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Luzula peruviana (Desv.)	Cladonia spec.	Pernettya postrata (Cav.)DC.	
	Festuca spec.			
	Carex spec.			
	Rynchospora spec.			
	Cortaderia spec.			
	Hypericum spec.			
III %	75	15	8	2
	Luzula peruviana (Desv.)	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Calamagrostis spec.	Cladonia spec.	Gaultheria vaccinoides (Wedd.)	

	Rynchospora spec.	Huperzia spec.	Pernettya postrata (Cav.)DC.	
	Hypericum spec.	Lycopodium spec.	Gaultheria erecta (Vent.)	
			Diogenesia spec.	
V %	15	30	30	25
	Carex spec.	Sphagnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Chusquea spec.	Usnea spec.	Gaultheria vaccinoides (Wedd.)	Symplocos spec.
	Rynchospora spec.	Cladonia spec.	Diogenesia spec.	Clethra spec.
		Lycopodium spec.	Monnina spec.	Dendropanax spec.
		Blechnum spec.	Desfontainia spec.	Myrsine spec.
			Miconia setulosa (Cogn.)	
G: conti	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	80	15	5	0
	Calamagrostis spec.	Lycopodium spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Festuca spec.	Cladonia spec.	Pernettya postrata (Cav.)DC.	
	Hypericum spec.	Blechnum spec.		
	Jarava ichu (Ruiz&Pav.)			
	Bromus spec.			
III %	70	15	13	2
	Calamagrostis spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Hypericum spec.	Cladonia spec.	Gaultheria vaccinoides (Wedd.)	
	Festuca spec.	Huperzia spec.	Pernettya postrata (Cav.)DC.	
	Hypericum spec.	Lycopodium spec.	Diogenesia spec.	
	Jarava ichu (Ruiz&Pav.)		Brachyotum spec.	
V %	20	20	25	35
	Carex spec.	Sphagnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Chusquea spec.	Cladonia spec.	Gaultheria vaccinoides (Wedd.)	Symplocos spec.

	Rynchospora spec.	Blechnum spec.	Diogenesia spec.	Clethra spec.
	Cortaderia spec.		Myrsine spec.	
			Miconia setulosa (Cogn.)	
			Brachyotum spec.	
			Diplostephium spec.	
			Demosthencia spec.	
*H: patch**	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	85	10	5	0
	Calamagrostis spec.	Lycopodium spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Rynchospora spec.	Cladonia spec.	Pernettya postrata (Cav.)DC.	
	Cortaderia spec.	Sphagnum spec.	Diplostephium spec.	
	Festuca spec.		Gaultheria vaccinoides (Wedd.)	
	Hypericum spec.		Gaultheria erecta (Vent.)	
	Luzula spec.		Myrteola spec.	
	Chusquea spec.			
III %	60	15	35	0
	Calamagrostis spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Weinmannia mircrophylla (Kunth)
	Festuca spec.	Cladonia spec.	Diplostephium spec.	
	Rynchospora spec.	Huperzia spec.	Myrteola spec.	
	Cortaderia spec.	Lycopodium spec.	Hesperomeles latifolia (Kunth)M.Roem.	
	Hypericum spec.		Gaultheria vaccinoides (Wedd.)	
V %	25	30	25	20
	Chusquea spec.	Usnea spec.	Diplostephium spec.	Gynoxys spec.
	Rynchospora spec.	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Dendropanax spec.
		Lycopodium spec.	Hesperomeles latifolia (Kunth)M.Roem.	
			Diogenesia spec.	
			Monnina spec.	
			Gaultheria vaccinoides (Wedd.)	

			Myrsine spec.	
I: patch	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	80	15	5	0
	Festuca spec.	Cladonia spec.	Escallonia myrtoides (Gill.exHook&Arn.)	
	Calamagrostis spec.	Lyopodium spec.	Pernettya postrata (Cav.)DC.	
	Luzula peruviana (Desv.)			
	Hypericum spec.			
	Alchemilla pinnata (Rui&Pav.)			
III %	70	15	13	2
	Festuca spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Miconia bullata (Turcz.)Triana
	Calamagrostis spec.	Sphagnum spec.	Diplostephium spec.	
	Luzula peruviana (Desv.)	Cladonia spec.	Gaultheria vaccinoides (Wedd.)	
	Hypericum spec.		Diogenesia spec.	
	Gentiana spec.			
V %	25	25	20	30
	Carex spec.	Blechnum spec.	Miconia bullata (Turcz.)Triana	Weinmannia mircrophylla (Kunth)
	Rynchospora spec.	Sphagnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Miconia setulosa (Cogn.)
	Piper spec.	Elaphoglossum spec.	Desmostemon spec. (??)	Clethra spec.
			Gaultheria erecta (Vent.)	Myrsine spec.
			Gaultheria vaccinoides (Wedd.)	Symplocos spec.
			Diogenesia spec.	

*J: patch**	Grasses	Cryptogams	Shrubs & Angiosperms	Trees
I %	35	30	35	0
	Festuca spec.	Sphagnum spec.	Diplostephium haenkei (DC.)Wedd.	
	Luzula peruviana (Desv.)	Leucobryum spec.	Gynoxys spec.	
	Bromus spec.	Lycopodiella spec.	Gaultheria vaccinoides (Wedd.)	

	Jarava ichu (Ruiz&Pav.)	Huperzia spec.		
	Oxalis spec.	Blechnum spec.		
	Cortaderia spec.	Thelypteris spec.		
III %	35	15	35	15
	Cortaderia spec.	Blechnum spec.	Escallonia myrtoides (Gill.exHook&Arn.)	Miconia bullata (Turcz.)Triana
	Festuca spec.	Lycopodiella spec.	Myrteola spec.	
		Leucobryum spec.	Gaultheria vaccinoides (Wedd.)	
			Desmostemon spec. (??)	
V %	15	20	30	35
	Chusquea spec.	Elaphoglossum spec.	Desmostemon spec. (??)	Weinmannia fagaroides (Kunth)
	Carex spec.	Sphagnum spec.	Diogenesia spec.	Escallonia myrtoides (Gill.exHook&Arn.)
			Escallonia myrtoides (Gill.exHook&Arn.)	Miconia bullata (Turcz.)Triana
			Miconia bullata (Turcz.)Triana	
			Gaultheria vaccinoides (Wedd.)	

* Treelines were differentiated between conti: continuous forest with closed cover to the lowlands, patch: forest patches above the continuous treeline with several metres disjunction. Please note that transects H, J, D and E were not included for analysis of N-mineralisation and plant species composition, because environmental factors distinguished them from the average treeline ecotone as described in table below:

Peruvian treelines (transects A – J)	
Continuous	**Patchy**
A	B
C	F
I	G
H (disturbed: potentially landslide, storm?)	D (shrubby Puna)
J (disturbed: potentially landslide, storm?)	E (shrubby forest)

Table V2.C1.2: Sweden: Ecotone plant species composition (I:Tundra, III:transition, V:forest, % cover and most abundant species, transects A – F)

A: conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %		60	40	0
		Cladonia spec.	Betula nana	
		Nephroma (arcticum)	Empetrum nigrum	
		Stereocaulon spec.	Vaccinium vitis-idea	
III %		30	40	30
		Pleurozium spec.	Vaccinium vitis-idea	Betula pubescens
		Dicranium spec.	Empetrum nigrum	
		Cladonia spec.	? "spec"	
V %		20	40	40
		Dicranium spec.	Vaccinium vitis-idea	Betula pubescens
		Cladonia spec.	Empetrum nigrum	
		Moss "spec"	Ericacea spec.	

B: conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	20	35	35	10
	Graminoides spec.1	Cladonia spec.	Empetrum nigrum	Betula spec.
	Graminoides spec.2	Pleurozium spec.	Betula nana	
	Graminoides spec.3	Nephroma (arcticum)	Kalmia procumbens	
III %	5	35	30	30
	Graminoides spec.1	Moss "spec" (Polytrichium?)	Empetrum nigrum	Betula spec.
		Dicranium spec.	Vaccinium uliginosum	
			Trientalis europea	
			Graminoides spec.2	
			Vaccinium vitis-idea	
V %	0	30	30	40

		Dicranium spec.	Vaccinium vitis-idea	Betula spec.
		Polytrichum spec.	Empetrum nigrum	
			Vaccinium uliginosum	

C:patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	15	37	45	3
	Graminoides spec.1	Moss "spec 1" in dense carpet	Empetrum nigrum	Betula nana
	Graminoides spec.2	Cladonia spec.	Rubus spectabilis ("Salmonberry")	
	Graminoides spec.3		Rubus chamaemorus	
III %	5	25	40	30
	Graminoides spec.2	Moss "spec 2"	Empetrum nigrum	Betula spec.
		Lycopodium spec. ("Club moss")	Vaccinum myrtilis	
		Hylocomium splendens (?)	Vaccinium vitis-idea	
		Dicranium spec.		
V %	0	25	35	40
		Moss "spec 2"	Salix gauca	Sorbus aucuparia ("Mountain Ash")
		Trollius europaeus		
		Rumex spec.		
		Forb "spec"		

D:conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	0	30	55	5
		Folios lichen (Physconia/Leptogium?)	Forb "spec" (N-fixer)	Betula nana
		Decranium spec.	Empetrum nigrum	
			Vaccinium uliginosum	
			Cassiopea spec.	
III %	0	35	50	15
		Moss "spec 4"	Trientalis europea	Betula nana

		Equisetum spec.	Angiosperms spec 1 (yellow rosette)	Betula pubescens
		Fern "spec 1"	Alpine Mint Bush (alike Prostanthera cuneatea??)	
			Vaccinium uliginosum	
			Vaccinium vitis-idea	
			Salix glauca	
V %	0	40	40	20
		Moss "spec 3"	Bassia spec?? (Amaranthaceae)	Betula pubescens
		Lycopodium spec.	Angiosperms "spec 2" (rosette)	
		Fern "spec 1"	Angiosperms "spec 3"	
		Fern "spec 2"	Salix glauca	
		Fern "spec 3"	Salix spec.	
			Vaccinium myrtillus	

E:patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %		40	60	0
		Moss "spec 3"	Equisetum	
		Stereocaulon spec.	Graminoides spec.1	
			Graminoides spec.2	
			Empetrum nigrum	
			Salix spec 1 (small, dark green leaves)	
			Salix spec 2 (light green leaves, same in forest)	
			Vaccinium uliginosum	
III %		35	55	10
		Hylocomium spec .	Equisetum	Betula pubescens
		Lycopodium spec.	Angiosperms spec 1 (yellow rosette)	
			Angiosperms "spec 2" (rosette)	

			Salix glauca	
			Salix spec (red stem)	
			Vaccinium vitis-idea	
V %		30	50	20
		Moss "spec 3"	Salix spec 2	Betula pubescens
			Equisetum	
			Forb (4leaf)	
			Juniper spec.	
			Salix glauca	
			Rubus spec.	

F:patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %		35	65	0
		Heurozium spec.	Empetrum nigrum	
		Cladonia spec.	Vaccinium uliginosum	
		Stereocaulon spec.	Salix glauca	
		Grassy ferny	Rubus chamaemorus	
III %	5	30	50	15
	Grass "spec 5"	Polytrichum	Salix spec.	Betula nana
		Stereocaulon spec.	Angiosperms spec 1 (yellow rosette)	
		Lycopodium	Rubus spec.	
V %		40	40	20
		Polytrichum	Salix glauca	Betula nana
		Rhytidia	Salix spec (red stem)	Betula pubescens
		Lycopodium	Angiosperms spec 1 (yellow rosette)	

In addition to the six treelines included in edaphic studies (A-F), plant species were characterised in a similar approach in 25 m x 25 m plots 30 m apart at the end points each 15 m into tundra and forest (G-J)

G:patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	10	50	40	0
	Graminoides spec.2	Stereocaulon	Empetrum nigrum	
	Graminoides spec.3	Folios lichen (green)	Salix glauca	
		Cladonia	Rubus chamaemorus	
		Very fine moss "spec 4"	Vaccinium uliginosum	
			Vaccinium vitis-idea	
			Loiseloria procumbens	
V %	0	30	35	35
		Hylocomium splendis	Salix glauca	Betula nana
		Folios lichen (green)	Juniper	Betula pubescens
		Polytrichum	Empetrum nigrum	
			Angiosperms spec 1 (yellow rosette)	
			Pinguicula spec. ("butterwort")	

H: patch	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %		55	45	0
		Very fine moss "spec 4"	Empetrum nigrum	
		Folios lichen (green)	Vaccinium vitis-idea	
		Cladonia spec.	Vaccinium uliginosum	
V %	5	35	35	25
	Grass "spec 5"	Very fine moss "spec 4"	Empetrum nigrum	Betula nana
		Folios lichen (green)	Vaccinium vitis-idea	Betula pubescens
		Cladonia spec.	Vaccinium uliginosum	

I:conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %	5	35	60	0
	Grass "spec 5"	Moss "spec 3"	Empetrum nigrum	

		Folios lichen (green)	Vaccinium vitis-idea	
		Cladonia spec.	Vaccinium uliginosum	
			Salix glauca	
			Equisetum spec.	
V %		35	40	25
		Very fine moss "spec 4"	Empetrum nigrum	Betula nana
		Lycopodium	Vaccinium vitis-idea	Betula pubescens
		Cladonia spec.	Vaccinium uliginosum	
		Folios lichen (green)		

J:conti	*Grasses*	*Cryptogams*	*Shrubs & Angiosperms*	*Trees*
I %		50	50	0
		Rhytidia spec.	Empetrum nigrum	
		Cladonia spec.	Vaccinium vitis-idea	
			Vaccinium uliginosum	
			Vaccinium myrtillus	
			Andromeda spec.	
			Cassiopea spec.	
V %	5	35	40	20
	Grass "spec 4"	Hylocmium spec.	Empetrum nigrum	Betula pubescens
		Pleurozium spec.	Vaccinium vitis-idea	Betula nana
		Folios lichen (green)	Vaccinium uliginosum	
			Vaccinium myrtillus	
			Salix glauca	

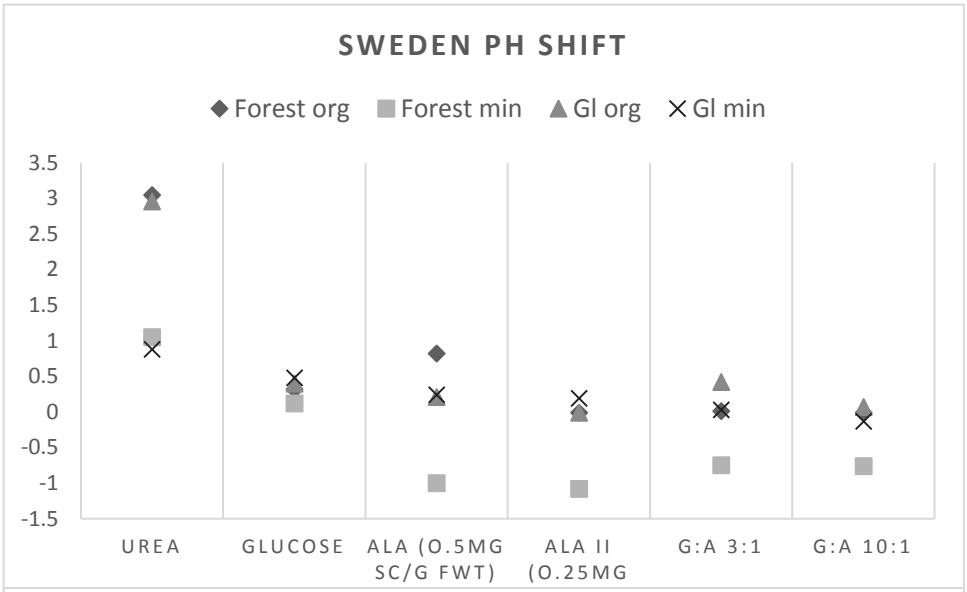
V.2-C2-A1 Nutrient solution and pH shifts

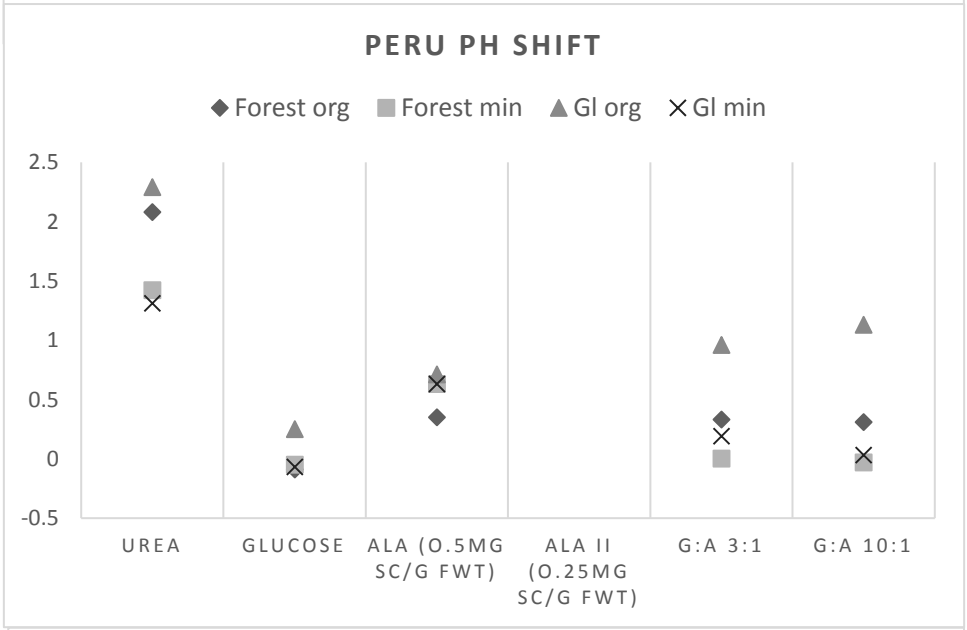
Table V2-C2.1: Detailed composition of Hoagland's solution No. 2, prepared as 10x diluted stock solution

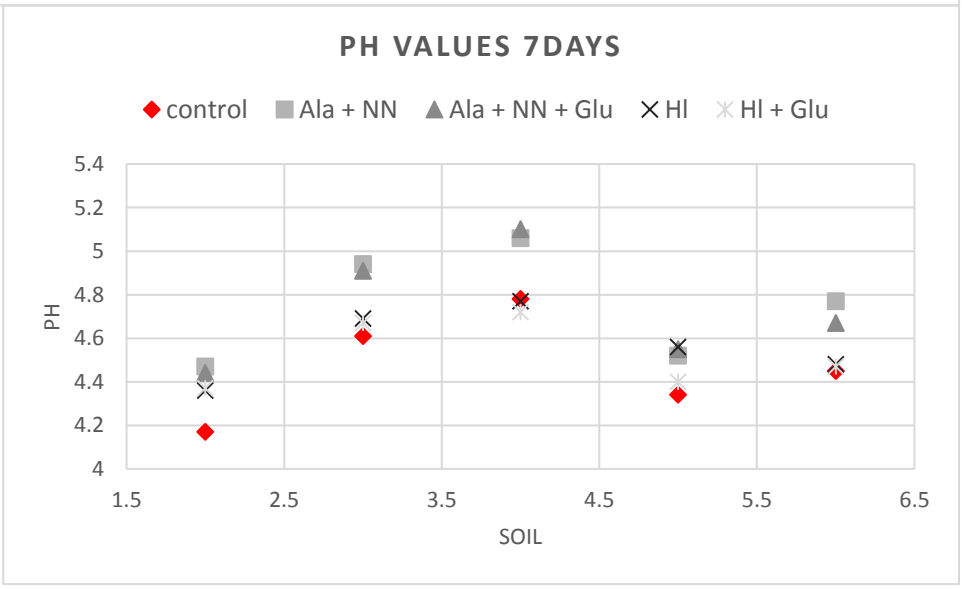
Hoagland's No. 2 basal salt mixture (H2395 Sigma Aldrich)	**Chemical formula**	**Component** (mg litre $^{-1}$ stock solution)
Ammonium phosphate monobasic	$(NH_4)_3PO_4$	11.50
Boric acid	H_3BO_3	0.29
Calcium nitrate	$Ca(NO_3)_2$	65.64
Cupric sulphate	$CuSO4 \cdot 5H_2O$	0.01
Ferric tartrate	$C_{12}H_{12}Fe_2O_{18} \cdot 2H_2O$	0.53
Magnesium sulphate	$MgSO_4 \cdot H_2O$	24.08
Manganese chloride	$MnCl_2 \cdot 4H_2O$	0.18
Molybdenum trioxide	MoO_3	0.00
Potassium nitrate	KNO_3	60.66
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	0.02
Total Nitrogen	N (various)	22.85 (0.022852 µg N µl $^{-1}$)

Shifts of soil pH were tested under substrate addition for the highest nutrient addition (C:N = 7:1) on a sub-sample of each replicate of all different soil types (n = 6 x 4 = 24 for each country, see supplementary information). The used Hoagland's Solution had a pH of 4.7 at room temperature. In most cases substrate additions lowered the pH, although an occasional pH increase was observed, both irrespective of substrate treatment C:N. Average pH shifts were less than -0.15 for all soil types, but greater for mineral forests soils of Peru (-0.33 ± 0.19) and Sweden (-0.26 ± 0.14), and for Swedish mineral tundra soils (-0.23 ± 0.11). pH was measured in water (which provided different results compared to KCl with a larger variation between organic forest soils and mineral Puna soils). Yet soil WHC was adjusted using water and that still is the form of precipitation in most ecosystems, hence this medium was used throughout.

Figure V2-C2.1: pH shift of four soil types after 3(7) days of adding different test substrates as 1ml solution in water to 5 g soil each. Abbreviations: Gl is Puna grassland in Peru and Tundra heath in Sweden, org: organic soil horizon, min: mineral soil horizon, ALA: alanine, G: glucose, SC: substrate-C, FWT: soil fresh weight







V.2-C2-A2: Cumulative fluxes

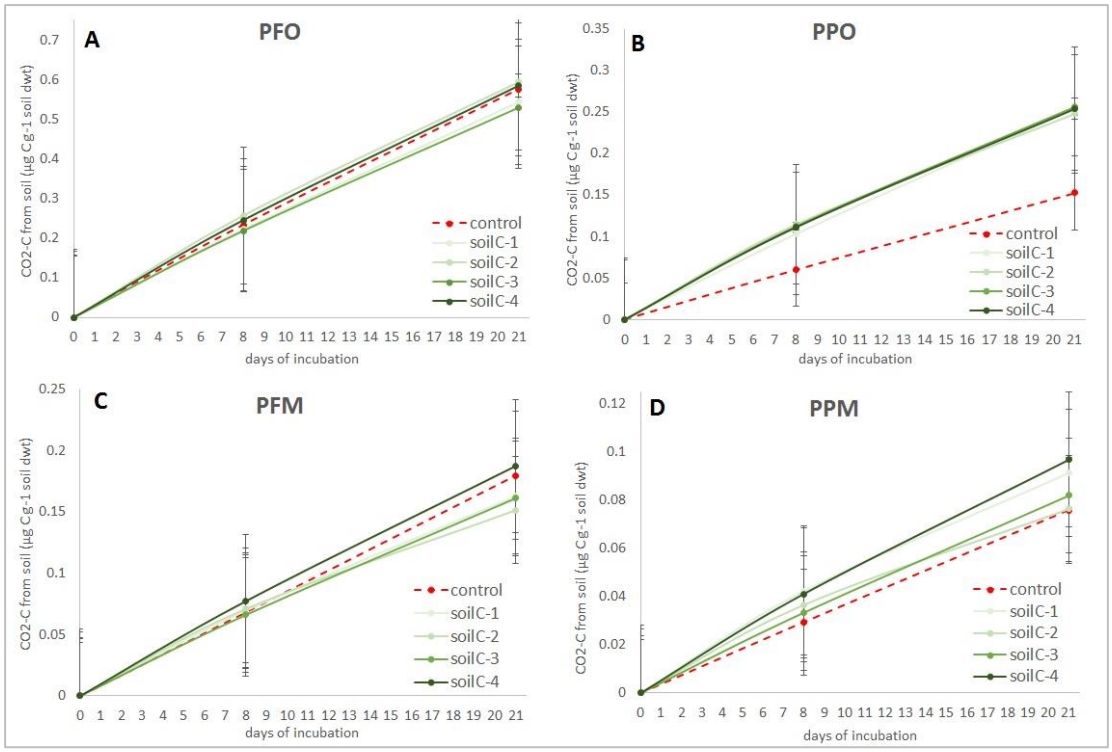


Figure V2-C2.2: Cumulative fluxes (CO_2-C ug g dwt) for Peruvian soils during 21 days of incubation, A: Peru Forest Organic, B: Peru Puna Organic, C: Peru Forest Mineral, D: Peru Puna Mineral, each four substrate treatments + control (red dotted line)

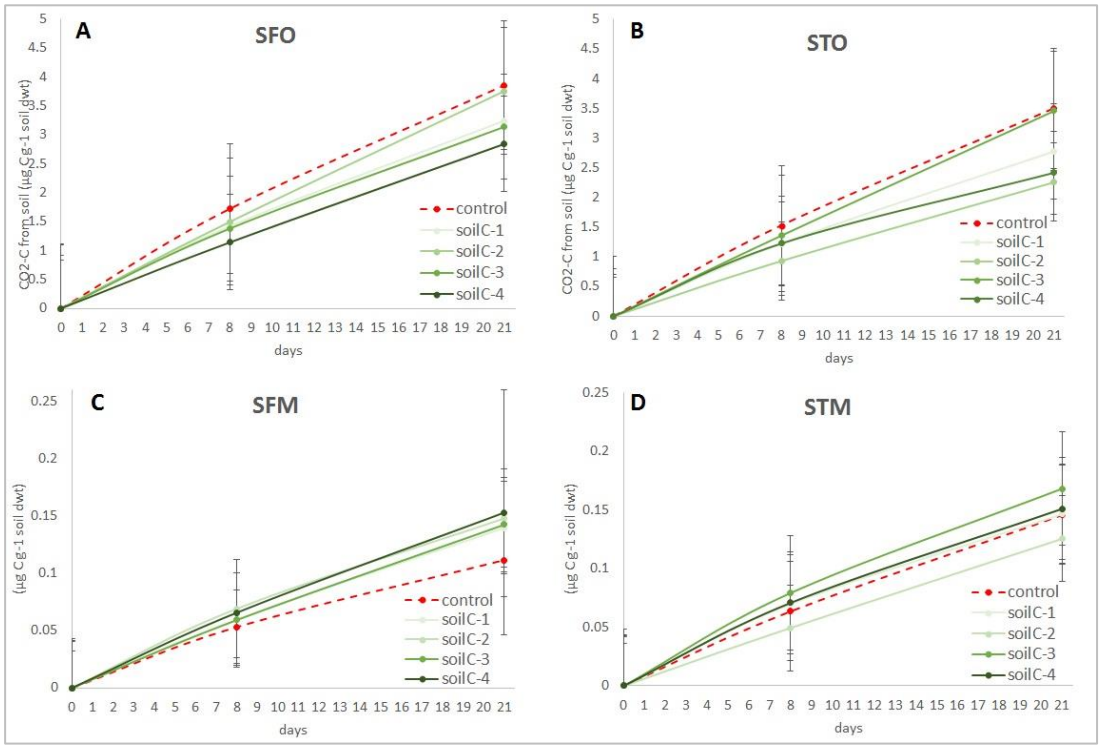


Figure V2-C2.3: Cumulative fluxes (CO_2-C ug g dwt) for Swedish soils during 21 days of incubation, A: Sweden Forest Organic, B: Sweden Tundra Organic, C: Sweden Forest Mineral, D: Sweden Tundra Mineral, four substrate treatments + control (red dotted line)

V.2-C2-A3: Case study: microbial priming in contrasting soils

Mineral soils from the Peruvian Puna (PPM) and organic soils from the Swedish boreal forest (SFO) showed opposing priming patterns, according to substrate-N content. For the Peruvian soil, increased substrate-N led to increasingly more positive PEs, even reversing negative to positive priming. The Swedish soils showed negative PE under C-only addition, which was raised to slight positive priming under lowest N-addition, but then inverted to negative priming again under increasing substrate-N.

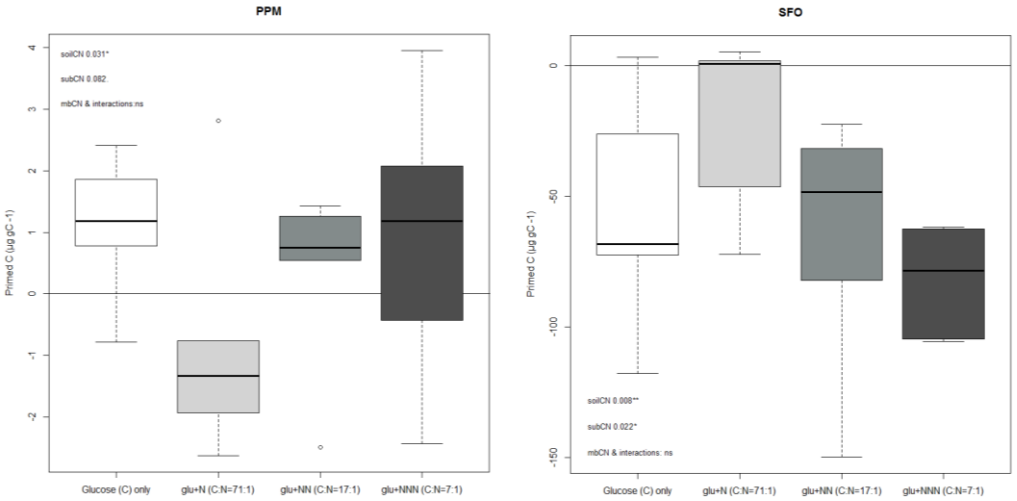


Figure V2-C2.4: Priming effects in two contrasting soils: Peru Puna Mineral (PPM) and Sweden forest organic (SFO)

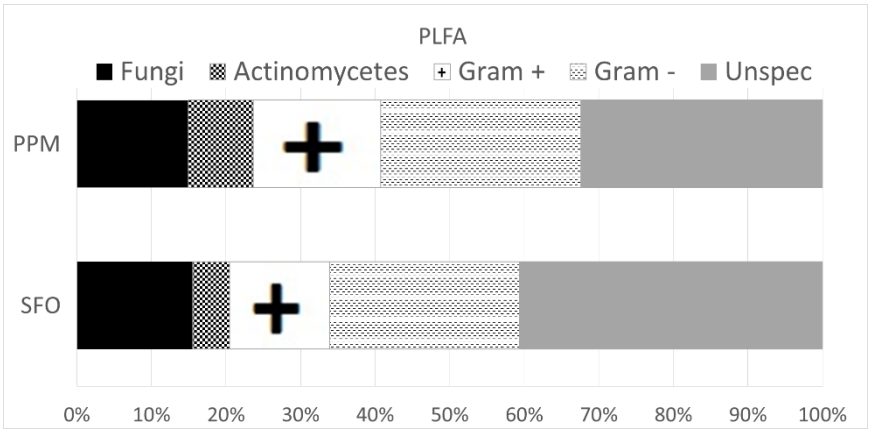


Figure V2-C2.5: PLFAs of PPM and SFO indicating relative proportions of key functional groups of soil microbes as assigned to biomarkers for fungi, actinomycetes, other gram positive and gram negative bacteria and remaining unspecified PLFAs. Total PLFAs (µg g^{-1} dwt soil): PPM: 36.03 ± 11.82, SFO: 201 ± 29.16; fungi to bacteria (F:B) ratios: PPM: 0.3 ± 0.02, SFO: 0.35 ± 0.05; gram+ to gram- ratios: PPM: 0:64 ± 0.2, SFO: 0.54 ± 0.03. n=6.

V.2-C2-A4: magnitude of PE and substrate use amongst soils

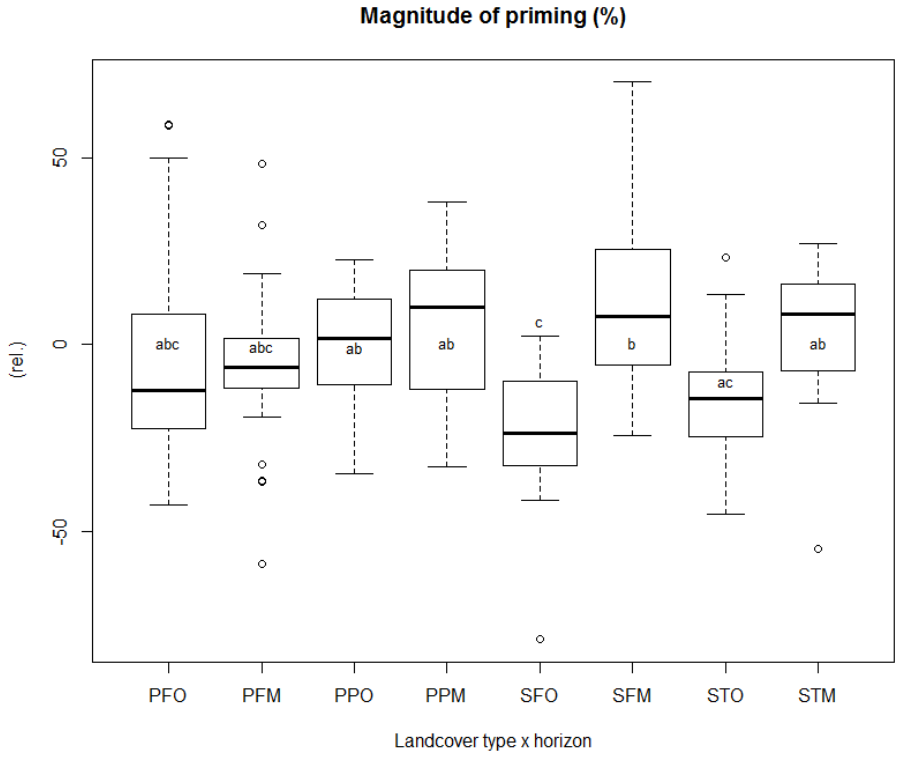


Figure V2-C2.6: Magnitude of priming effects of the different soils, letters indicate similarities of sample means following ANOVA with post-hoc Tukey test, n = 6

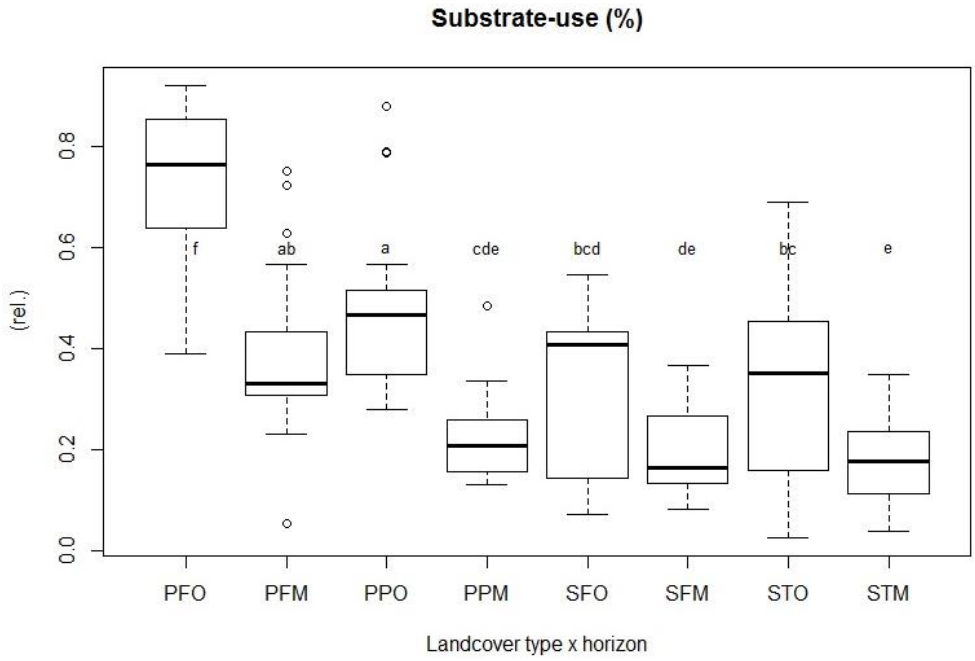


Figure V2-C2.7: Substrate-use (%) of the different soils, letters indicate similarities of sample means following ANOVA with post-hoc Tukey Test, n = 6

V.2-C3-A1 In vivo parameter

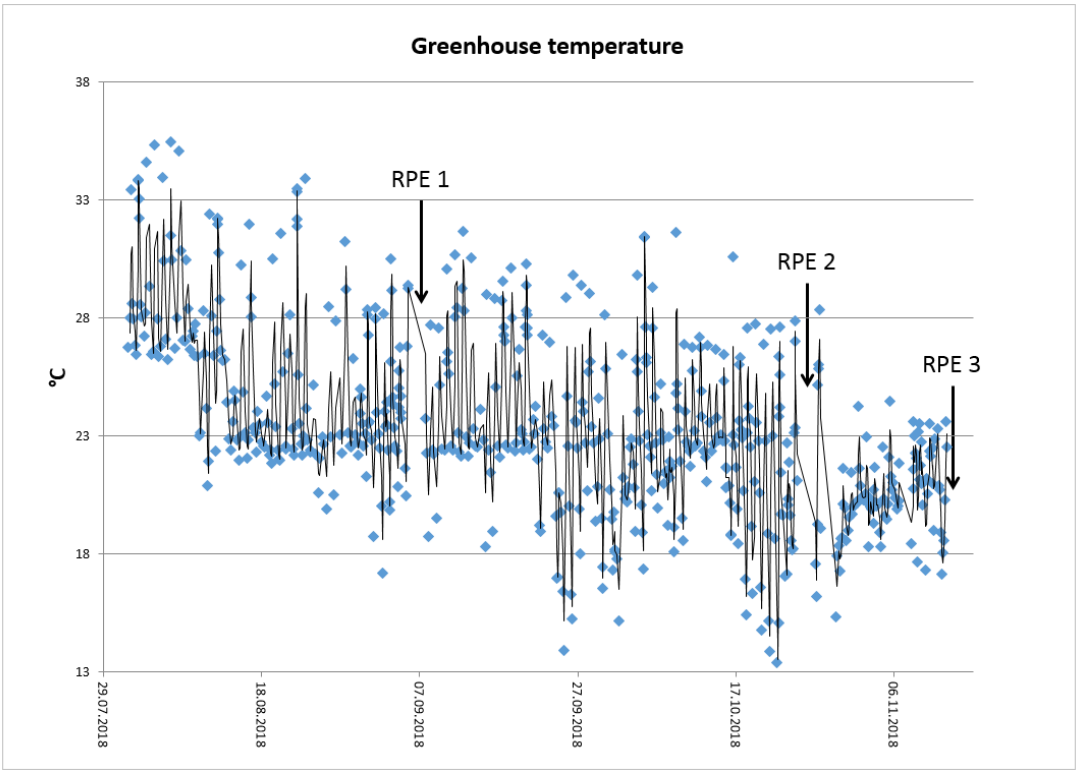
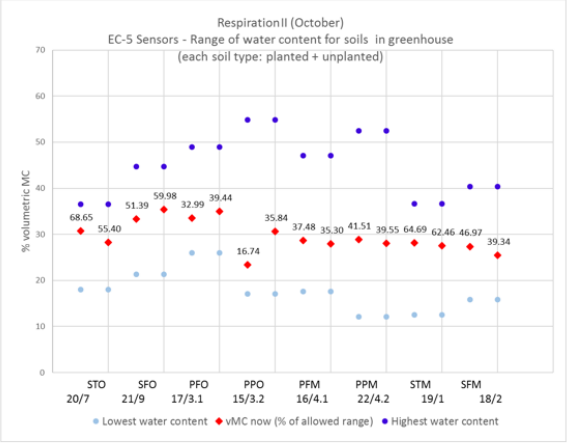
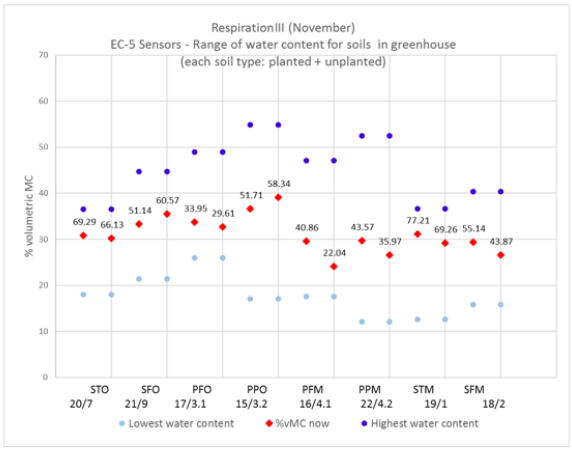


Figure V2-C3.1: Greenhouse temperature August – November





°C resp2	batch 1	batch 2
Av	21.10	21.49
Stdv	1.00	0.76
RSD	5%	4%

resp 2 Soil type	ratio CO_2 plant:soil
PFM	15.7
PFO	12.1
PPM	18.8
PPO	18.2
SFM	6.7
SFO	11.5
STM	5
STO	7.7

°C resp3	batch 1	batch 2
Av	20.71	21.10
Stdv	0.88	0.76
RSD	4%	4%

resp 3 Soil type	ratio CO_2 plant:soil
PFM	9.4
PFO	13.1
PPM	11.1
PPO	12.2
SFM	5.1
SFO	19
STM	2.2
STO	5.7

Figure V2-C3.2: Adjusted soil moisture, ratio of plant:soil-derived CO_2 in headspace and temperature during plant-soil incubations, left: RPE 2, right: RPE 3

V.2-C3-A2: RPE uncertainty in plant-soil incubations

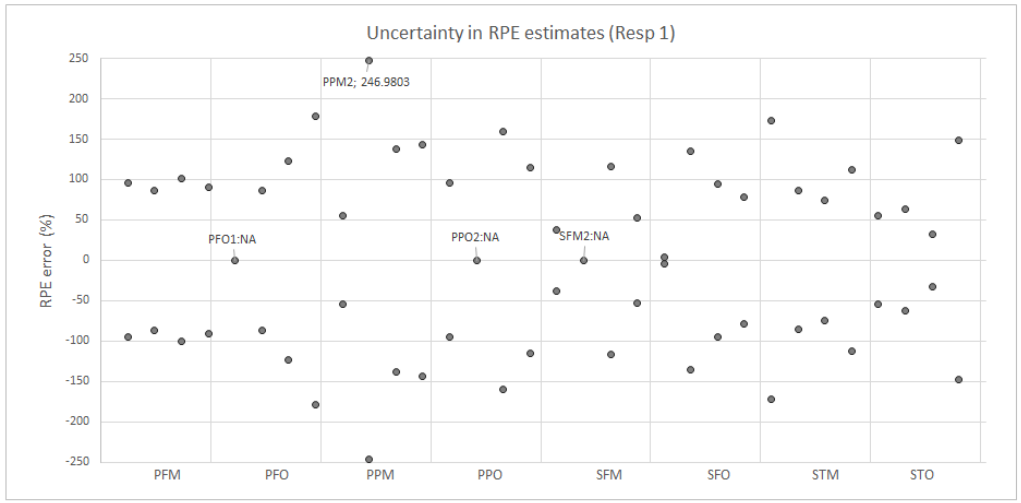
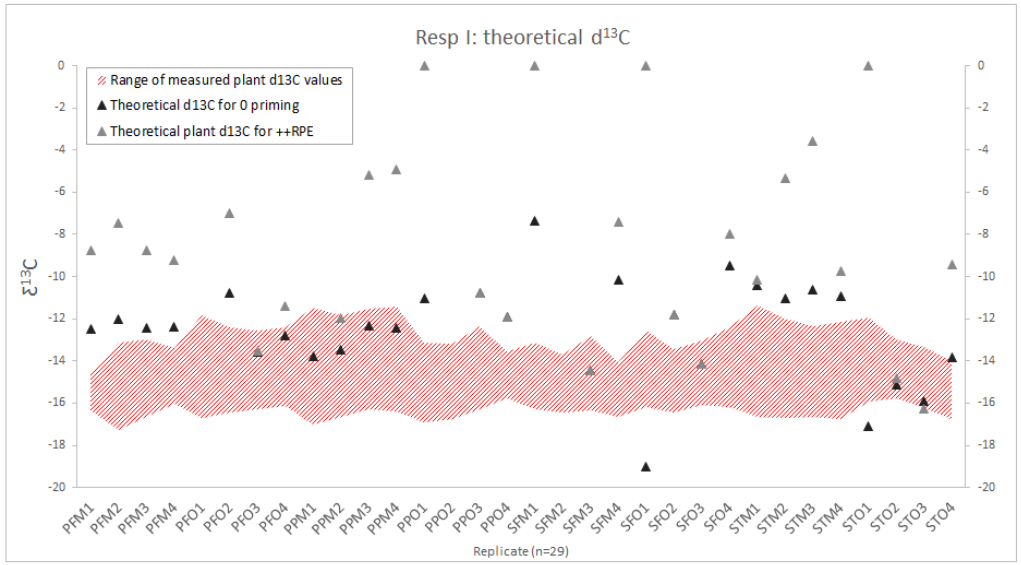
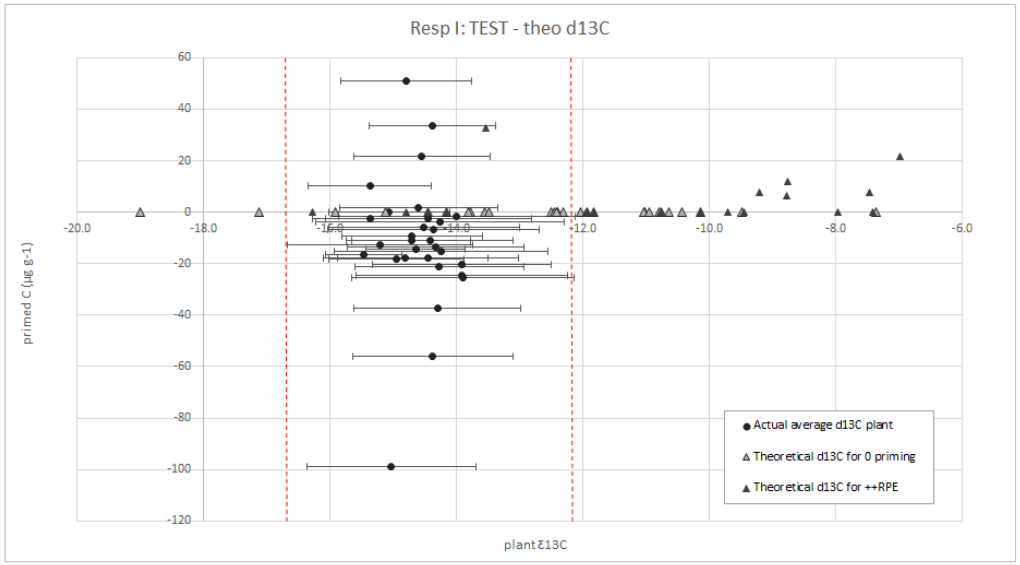
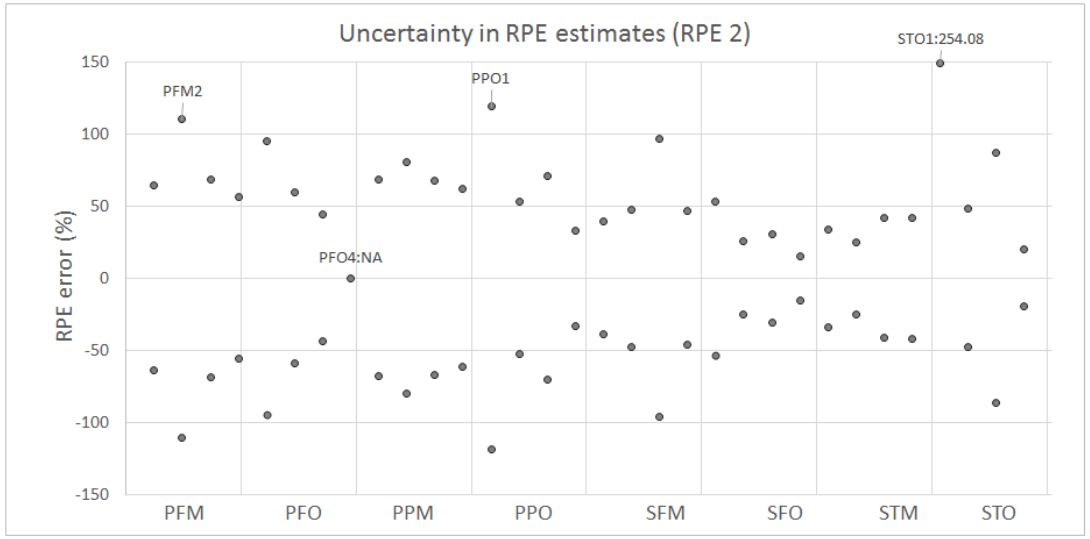
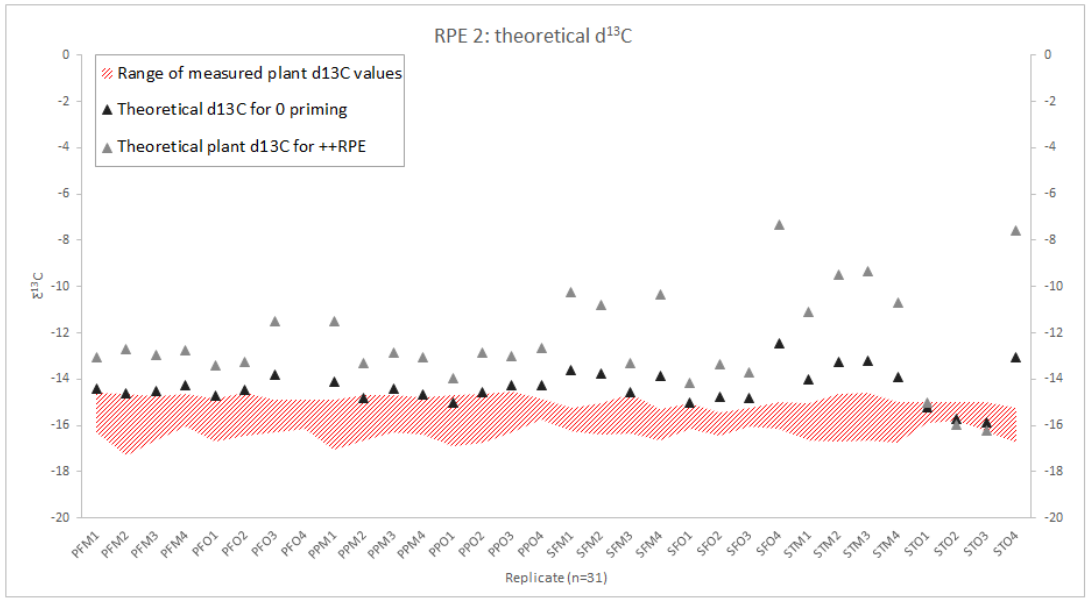


Figure V2-C3.3: Uncertainty, theoretical $d^{13}C$ and their test of RPE1 (September)
Note 1: variation of possible $d^{13}C$'s from plant derived from methodological shortcomings causing larger difference between respiration and bulk values (see methods)
Note 2: Some theoretical $d^{13}C$ would have needed to be positive in order to cause the opposed priming effects to originally calculated values and have all been set = 0 for this figure.









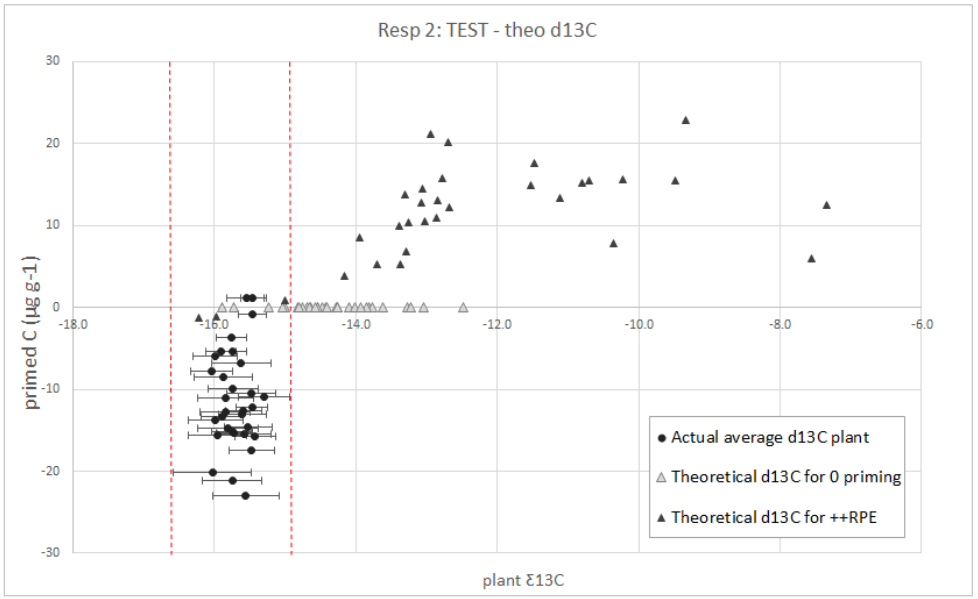


Figure V2-C3.4: Uncertainty, theoretical d^{13}C and their test of RPE 2 (October)

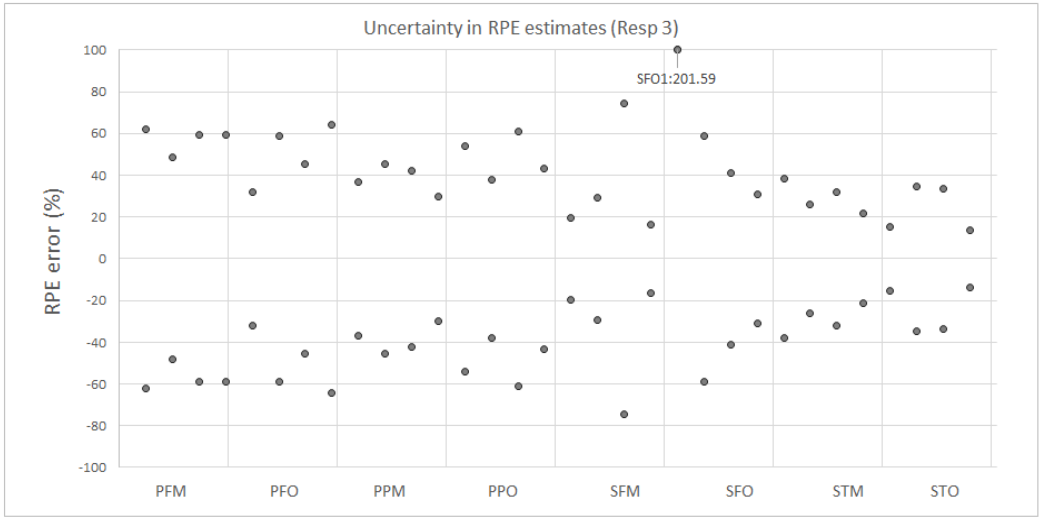
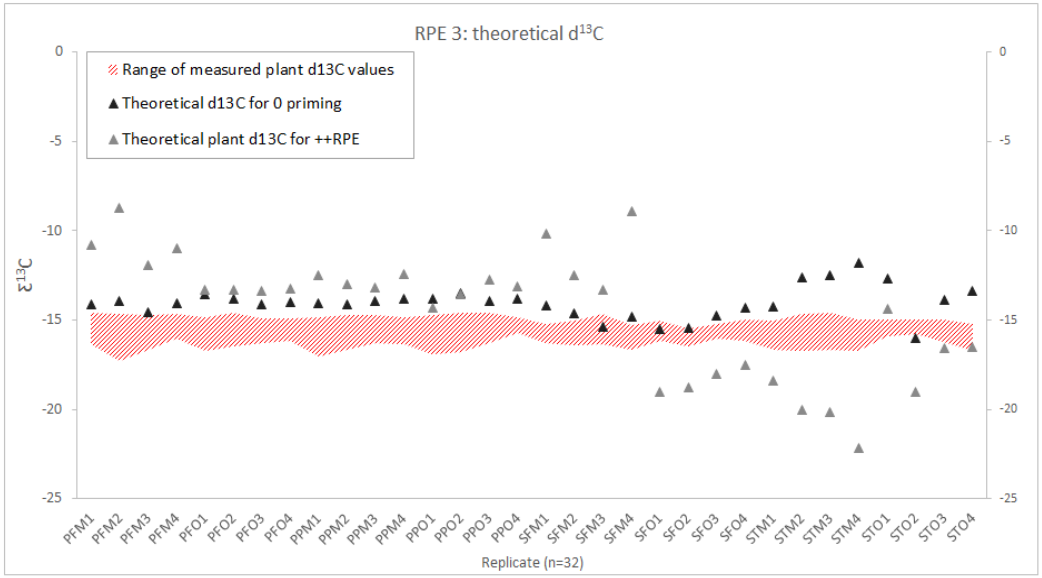
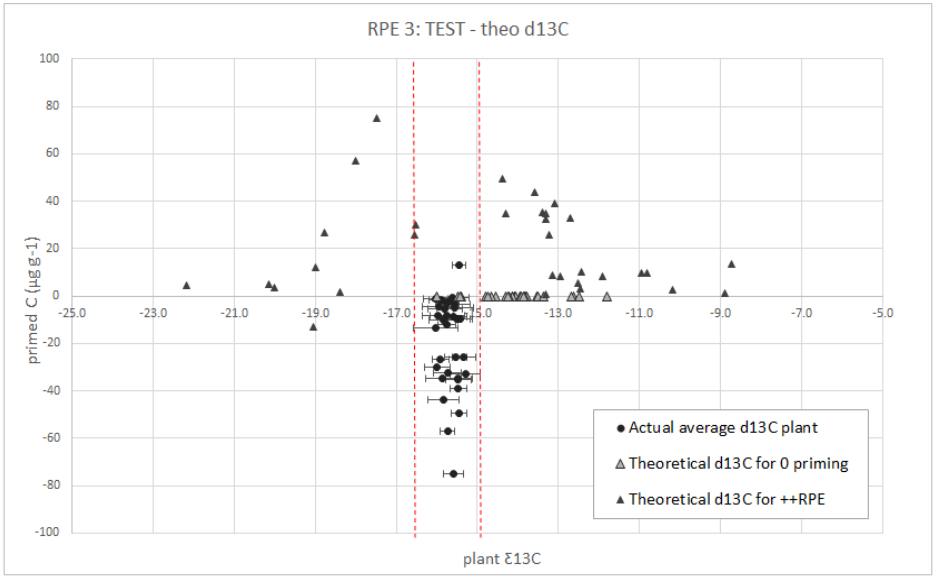


Figure V2-C3.5: Uncertainty, theoretical d^{13}C and their test of RPE 3 (November)





V.2-C3-A3: Further microbial parameter

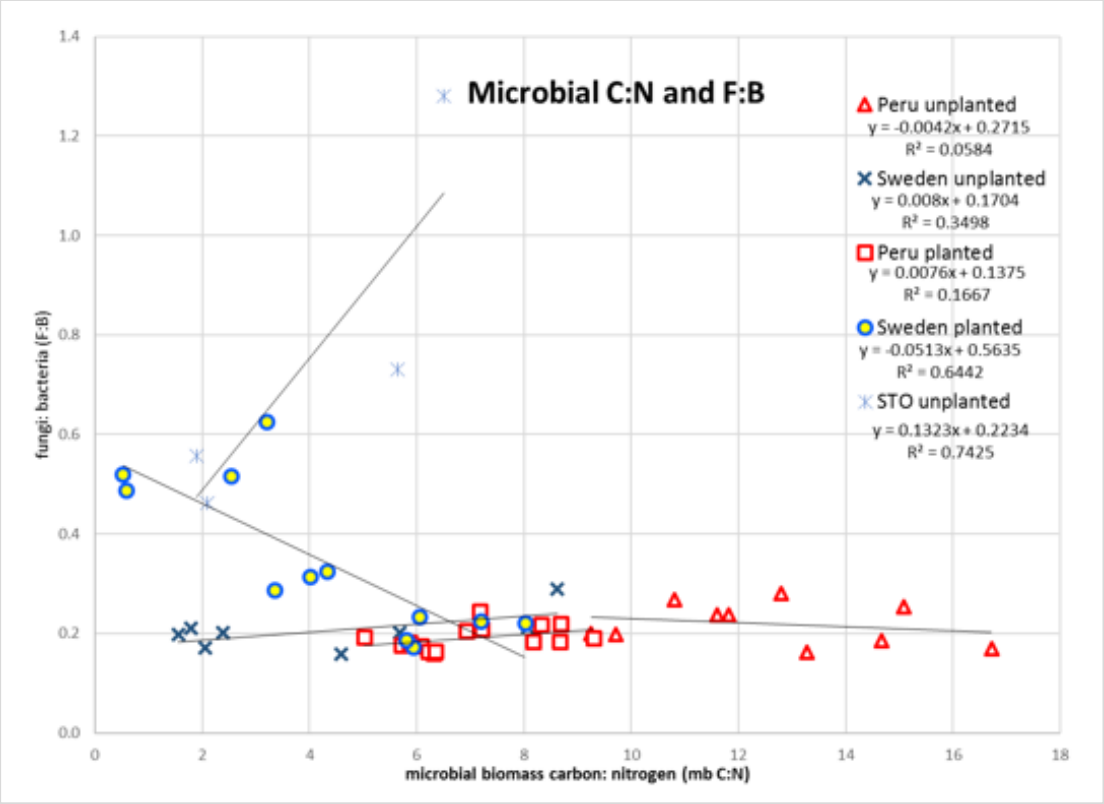


Figure V2-C3.6: Correlations of microbial C:N and F:B: Different extraction efficiencies depending on soil type (presuming because of different mineral fractions and aggregate stability (clay/silt/sand)).

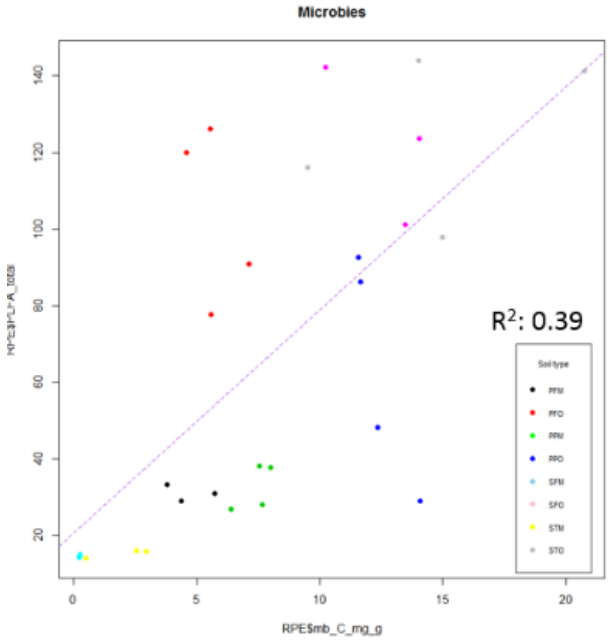


Figure V2-C3.7: Correlation of total PLFAs and microbial C by fumigation (p-value = 0.0002755, Adjusted R-squared: 0.3936)

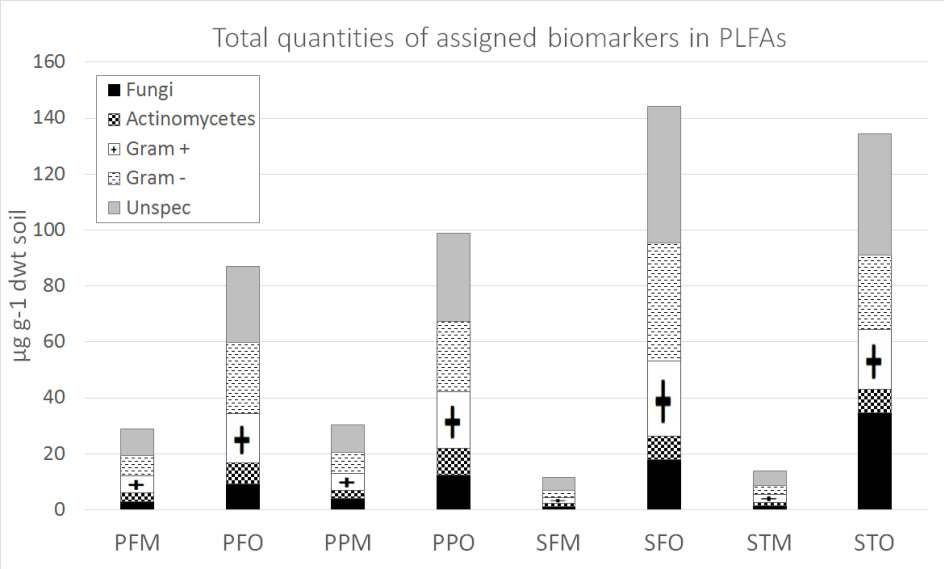


Figure V2-C3.8: *Total quantities of PLFAs (µg g^{-1} soil dry weight) for the eight soil types studied*

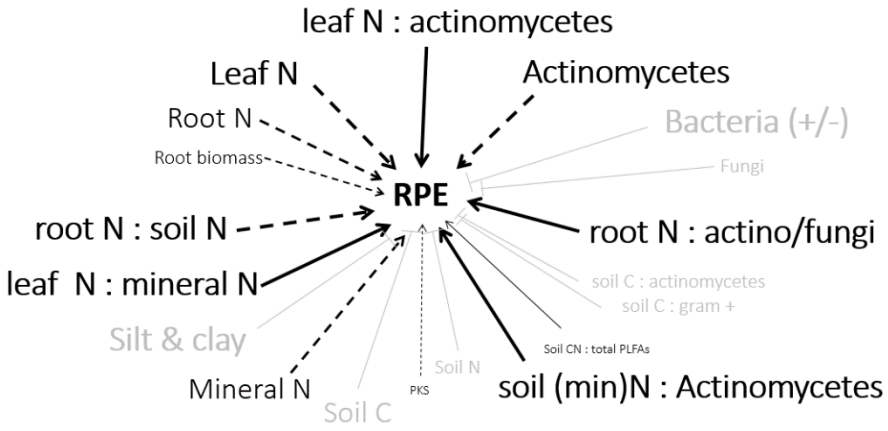


Figure V2-C3.9: *Potential supporters and antagonists of (negative) rhizosphere priming based on the models in Table 3.7, ":" indicates interactive effects*

V.2-C3-A4: *Table V2-C3.1: $\delta^{13}C$ values of all plant and soil compartments and respiration measured on the different soil types during the course of the experiment.*

country x land cover x horizon			Soil ID	Cynodon dactylon or just soil	Plant						Soil					
					Leaves				Roots		Bulk					Rhizosphere
					Respiration			Tissue	Respiration	Tissue	Respiration				Bulk	Respiration
					RPE 1	RPE 2	RPE 3		RPE 0		RPE 1	RPE 2	RPE 3	RPE 0		RPE 0
PERU	forest puna	min	PFM	plant-soil	-13.15 ± 0.11	-15.48 ± 0.43	-16.39 ± 0.05	-15.80 ± 0.34	-16.35 ± 0.34	-14.76 ± 0.12					-25.43 ± 0.11	-17.98 ± 1.1
				soil only							-20.84 ± 0.10	-22.97 ± 0.86	-24.74 ± 0.17	-25.19 ± 0.10	-25.6 ± 0.03	
		org	PFO	plant-soil	-12.29 ± 0.16	-15.34 ± 0.16	-16.27 ± 0.13	-15.59 ± 0.31	-15.94 ± 0.24	-14.8 ± 0.09					-26.06 ± 0.06	-17.37 ± 3.6
				soil only							-22.68 ± 0.00	-24.29 ± 1.12	-25.5 ± 0.55	-27.17 ± 0.53	-26.21 ± 0.04	
		min	PPM	plant-soil	-11.57 ± 0.09	-16.09 ± 0.14	-16.1 ± 0.01	-15.87 ± 0.25	-16.39 ± 0.21	-14.77 ± 0.03					-25.01 ± 0.12	-16.24 ± 1.74
				soil only							-18.70 ± 0.74	-23.3 ± 0.85	-25.18 ± 0.4	-24.65 ± 0.9	-25.19 ± 0.09	
		min	PPO	plant-soil	-13.04 ± 0.25	-16.03 ± 0.16	-16.84 ± 0.05	-15.61 ± 0.27	-15.67 ± 0.12	-14.67 ± 0.07					-25.18 ± 0.03	-18.06 ± 0.58
				soil only							-17.59 ± 0.11	-23.63 ± 0.63	-25.26 ± 0.67	-25.91 ± 0.16	-25.38 ± 0.02	
SWEDEN	forest tundra	min	SFM	plant-soil	-13.43 ± 0.28	-15.4 ± 0.43	-15.98 ± 0.16	-16.08 ± 0.15	-16.26 ± 0.03	-15.26 ± 0.03					-26.95 ± 0.17	-19.51 ± 0.81
				soil only							-21.18 ± 0.35	-24.01 ± 0.87	-24.51 ± 0.87	-26.29 ± 0.46	-27.27 ± 0.17	
		org	SFO	plant-soil	-12.87 ± 0.23	-15.5 ± 0.19	-16.31 ± 0.1	-15.92 ± 0.05	-16.01 ± 0.03	-15.31 ± 0.08					-28.75 ± 0.05	-24.21 ± 0.78
				soil only							-24.89 ± 1.09	-28.35 ± 0.41	-27.06 ± 2.3	-27.43 ± 1.12	-28.94 ± 0.03	
		min	STM	plant-soil	-11.97 ± 0.22	-15.48 ± 0.43	-16.68 ± 0.03	-15.91 ± 0.44	-15.97 ± 0.23	-15.13 ± 0.15					-25.97 ± 0.13	-17.87 ± 1.21
				soil only							-20.79 ± 1.54	-23.96 ± 0.83	-24.4 ± 0.4	-25.09 ± 0.62	-26.17 ± 0.13	
		org	STO	plant-soil	-13.05 ± 0.43	-15.34 ± 0.16	-15.85 ± 0.05	-15.71 ± 0.32	-15.71 ± 0.39	-15.49 ± 0.24					-27.06 ± 0.03	-22.92 ± 1.27
				soil only							-22.21 ± 2.15	-25.08 ± 0.99	-25.16 ± 0.98	-26.18 ± 0.76	-27.18 ± 0.04	

V.2-C3-A5: Detailed model outputs complementing Table 3.8

Table V2-C3.2: part 1: average RPE

Coefficients:	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*	*Sig.Code*
(Intercept)	6.89E+02	5.49E+02	1.256	0.25587	
soil_C	3.37E+01	6.66E+00	5.064	0.0023	**
soil_N	-4.50E+02	8.29E+01	-5.432	0.00161	**
soil_minN	-1.21E+02	2.44E+01	-4.936	0.00262	**
soil_siltclay	3.82E+01	9.42E+00	4.057	0.00667	**
soil_PKS	-1.72E+00	1.93E+00	-0.893	0.40636	
Fungi	5.88E+03	4.50E+03	1.305	0.23979	
Actinomycetes	-2.77E+04	5.38E+03	-5.149	0.00212	**
Gram_plus	-2.93E+03	6.34E+02	-4.62	0.00362	**
root_N	-1.68E+05	8.22E+04	-2.048	0.08654	.
leaf_N	-1.24E+05	2.37E+04	-5.214	0.00199	**
root_g_dwt	-1.32E+02	4.49E+01	-2.942	0.02587	*
LAI	1.72E+01	1.76E+01	0.973	0.36807	
soil_N:root_N	-9.20E+03	2.83E+03	-3.254	0.01737	*
soil_minN:leaf_N	5.80E+03	1.13E+03	5.125	0.00217	**
soil_C:root_g_dwt	3.81E+00	7.86E-01	4.848	0.00286	**
Fungi:root_N	1.89E+06	7.10E+05	2.668	0.03712	*
root_g_dwt:total.PLFAs	-2.71E+00	8.48E-01	-3.195	0.01871	*
Actinomycetes:leaf_N	1.37E+06	2.83E+05	4.856	0.00284	**
soil_C:Gram_plus	-1.09E+01	7.96E+00	-1.363	0.22186	
soil_C:Actinomycetes	-2.98E+02	5.81E+01	-5.126	0.00216	**
soil_N:Fungi	-1.01E+03	2.46E+02	-4.116	0.00624	**
soil_N:Actinomycetes	5.63E+03	1.03E+03	5.452	0.00158	**
total.PLFAs:soil_CN	5.96E-02	7.86E-02	0.758	0.47708	
soil_CN:F_B	-1.91E+02	3.45E+01	-5.523	0.00148	**

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 403.1856)

Null deviance: 113121.2 on 30 degrees of freedom

Residual deviance: 2419.1 on 6 degrees of freedom

AIC: 275.05

Table V2-C3.3: part 2: RPE 0

Coefficients:	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*	*Sig.Code*
(Intercept)	2.09E+04	4.56E+03	4.584	0.0195	*
soil_C	-4.52E+01	1.02E+01	-4.427	0.0214	*
soil_N	4.62E+02	1.54E+02	2.999	0.0577	.
soil_minN	-1.97E+02	3.81E+01	-5.171	0.014	*
soil_siltclay	-6.74E+01	1.41E+01	-4.782	0.0174	*
soil_PKS	-1.38E+01	3.71E+00	-3.718	0.0339	*
Fungi	-4.32E+03	5.14E+03	-0.84	0.4627	
Actinomycetes	-1.74E+05	5.13E+04	-3.392	0.0427	*
Gram_plus	1.75E+04	2.01E+04	0.874	0.4464	
Gram_neg	-2.66E+04	8.15E+03	-3.266	0.0469	*
root_N	-2.17E+06	5.03E+05	-4.309	0.023	*
root_g_dwt	-1.62E+03	5.60E+02	-2.884	0.0633	.
soil_N:root_N	2.30E+04	4.42E+03	5.202	0.0138	*
soil_C:root_g_dwt	-3.45E+00	8.65E-01	-3.992	0.0282	*
Fungi:root_N	1.32E+06	6.97E+05	1.889	0.1553	
Gram_plus:root_N	1.27E+07	2.86E+06	4.451	0.0211	*
Gram_plus:root_g_dwt	7.17E+03	2.69E+03	2.666	0.0759	.
Gram_plus:root_C	-2.42E+05	6.23E+04	-3.883	0.0303	*
Gram_neg:root_C	5.27E+04	1.64E+04	3.22	0.0486	*
Actinomycetes:root_N	-8.97E+06	2.32E+06	-3.862	0.0307	*
Actinomycetes:root_C	4.86E+05	1.23E+05	3.952	0.0289	*
soil_C:Actinomycetes	4.40E+01	2.47E+01	1.782	0.1728	
soil_C:Gram_neg	5.02E+01	1.14E+01	4.39	0.0219	*
soil_C:Gram_plus	7.77E+01	2.94E+01	2.643	0.0774	.
soil_N:Gram_plus	-1.56E+03	6.81E+02	-2.288	0.1062	
soil_minN:Actinomycetes	2.50E+03	4.96E+02	5.051	0.015	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 400.2487)

Null deviance: 109767.5 on 28 degrees of freedom

Residual deviance: 1200.7 on 3 degrees of freedom

AIC: 244.28

Table V2-C3.4: part 3a: Root-soil respiration (CO2)

Coefficients:	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*	*Sig.Code*
(Intercept)	-1.07E+05	6.72E+04	-1.6	0.16079	
soil_C	-4.12E+02	2.03E+02	-2.026	0.08915	.
soil_N	-2.19E+03	2.25E+03	-0.971	0.36895	
soil_minN	-6.43E+02	2.35E+02	-2.739	0.03376	*
soil_siltclay	-6.41E+02	2.45E+02	-2.617	0.03976	*
soil_PKS	-1.58E+02	1.02E+02	-1.549	0.17245	
Actinomycetes	-4.62E+05	1.71E+05	-2.698	0.03566	*
Gram_plus	1.17E+06	4.64E+05	2.518	0.0454	*
Gram_neg	-6.95E+04	2.75E+04	-2.529	0.04472	*
total.PLFAs	-8.86E+01	9.78E+01	-0.905	0.40014	
Fungi	-1.20E+05	8.52E+04	-1.402	0.21033	
mb_C	-7.39E+02	1.85E+02	-3.996	0.00715	**
root_N	-9.40E+06	2.65E+06	-3.552	0.01204	*
root_g_dwt	1.70E+03	1.35E+03	1.255	0.25629	
root_C	5.71E+05	2.09E+05	2.73	0.03418	*
soil_N:root_N	3.23E+05	1.01E+05	3.196	0.01869	*
soil_C:Gram_plus	1.68E+03	8.55E+02	1.962	0.0974	.
soil_C:Gram_neg	6.13E+02	3.52E+02	1.744	0.13174	
soil_C:total.PLFAs	-1.19E+00	7.56E-01	-1.572	0.16704	
soil_C:Actinomycetes	-3.36E+03	1.52E+03	-2.219	0.06827	.
total.PLFAs:soil_CN	2.31E+01	1.44E+01	1.599	0.16088	
soil_N:Fungi	1.65E+04	7.82E+03	2.112	0.07913	.
soil_N:Actinomycetes	2.99E+04	2.38E+04	1.258	0.25498	
Actinomycetes:root_N	7.07E+07	2.19E+07	3.231	0.01788	*
Gram_plus:root_C	-2.84E+06	1.08E+06	-2.619	0.03966	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 708491.2)

Null deviance: 448167070 on 30 degrees of freedom

Residual deviance: 4250947 on 6 degrees of freedom

AIC: 506.66

Table V2-C3.5: part 3b: control soil respiration (CO2_control)

Coefficients:	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*	*Sig.Code*
(Intercept)	6977.549	2936.448	2.376	0.028798	*
soil_C	56.023	9.993	5.606	2.55E-05	***
soil_N	-163.736	216.943	-0.755	0.460165	
soil_siltclay	39.277	18.384	2.136	0.04663	*
Actinomycetes	-59421.306	11869.379	-5.006	9.16E-05	***
Gram_plus	14264.231	13137.877	1.086	0.291924	
Gram_neg	-21084.771	3169.151	-6.653	3.04E-06	***
Fungi	-8463.957	6342.291	-1.335	0.198668	
mb_C	-72.008	31.6	-2.279	0.035106	*
soil_C:Gram_plus	-214.636	48.565	-4.42	0.000331	***
soil_C:Actinomycetes	-196.972	134.748	-1.462	0.161041	
soil_N:Actinomycetes	4719.385	2698.292	1.749	0.097318	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 89494.72)

Null deviance: 81604038 on 29 degrees of freedom

Residual deviance: 1610905 on 18 degrees of freedom

AIC: 437.87

VI Bibliography

Accoe, F; Boeckx, P; Van Cleemput, O; et al. Characterization of soil organic matter fractions from grassland and cultivated soils via C content and delta C-13 signature Rapid Communications in Mass Spectrometry Volume: 16 Issue: 23 Pages: 2157-2164 2002

ACIA Arctic Climate Impact Assessment Cambridge Univ. Press Ch. 7 2005

Akermann, J.&Johansson, M. Permafrost and periglacial processes Wiley InterScience 19: 279-292 2008

Alster, C.J., Baas, P., Wallenstein, M.D., Johnson, N.G., von Fischer, J.C. Temperature Sensivity as a Microbial Trait Using Parameters from macromolecular Rate Theory frontiers in Microbiology doi.org/10.3389/fmicb.2016.01821 2016

Angst, G., Mueller, C.W., Prater, I., Angst, S., Frouz, J., Jílková, V., Peterse, F., Nierop, K.G.J. Earthworms as biochemical reactors to conveert labile plant compounds into stabilized soil microbial necromass Nature Communications Biology doi.org/10.1038/s42003-019-0684-z 2019

Arcus, V.L., Prentice, E.J., Hobbs, J.K., Mulholland, A.J., van der Kamp, M.W., Pudney, C.R., Parker, E.J., Schipper, L.A. On the temperature dependence of enzyme-catalysed rates Biochemistry 55(12):1681-1688 2016

Cruz, A.F. & Ishii, T.Arbuscular mycorrhizal fungal spores host bacteria that affect nutrient biodynamics and biocontrol of soil-borne plant pathogens Biology Open 1: 52-57; doi: 10.1242/bio.2011014 2012

Averill, C. & Finzi, A. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem d15N Ecology 92(2), 883-891 2011

Averill, C., Turner, B.L., Finzi, A.C. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage Nature 505, 543e545 2014

Averill,C. & Waring, B. Nitrogen limitation of decomposition and decay: How can it occur? Global hange Biology 24:1417–1427. 2017

Aye, N.S., Butterly, C.R., Sale, P.W.G., Tang C. Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocellulose Soil Biology and Biochemistry 123: 33-44 2018

Ayres, E., Steltzer, H., Berg, S. & Wall, D.H. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them Journal of Ecology 97, 901–912 2009

Azam, F; Muller, C; Weiske, A; et al. Nitrification and denitrification as sources of atmospheric nitrous oxide - role of oxidizable carbon and applied nitrogen Biology and Fertility of Soils Volume: 35 Issue: 1 Pages: 54-61 2002

Bader, M. High solar radiation hinders tree regeneration above the alpine treeline in northern Ecuador Plant Ecol 191: 33 – 45 2007

Baldrian, P. Microbial enzyme-catalysed processes in soils and their analysis Plant Soil Environment 9:370-378 2009

Barea, J-M., Pozo, M.J., Azcon,R., Azcon-Aguilar, C. Microbial co-operation in the rhizosphere Journal of Experimental Botany 56(417),1761-1778 2005

Basile-Doelsch, I., Balesdent, J., Pellerin, S. Reviews and syntheses: The mechanisms underlying carbon storage in soil Biogeosciences (in review) 2020

Bastida, F., Garcia, C. Fierer, N. Eldridge, D.J.,Bowker, M.A.,Abades,S.,Alfaro,F.D.,Berhe,A.A.,Cutler,N.A.,Gallardo,A.,Garcia-Velazquez,L.,Hart,S.C.,Hayes,P.E.,Hernandez,T.,Hseu,Z-Y.,Jehmlich,N.,Kirchmair,M.,Lambers,H.,Neuhauser,S.,Pena-Ramirez,V.M.,Perez,C.A.,Reed,S.C.,Santos,F., Siebe,C.,Sullivan,B.W.,Trivedi,P.,Vera,A.,Williams,M.A.,Moreno,J.L.,Delgado-Baquerizo,M. Global ecological predictors of the soil priming effect Nature Communications 10(3481) 2019

Baumert, V.L., Vasilyeva, N.A., Vladimirov, A.A., Meier, I.C., Kögel-Knaber, I., Mueller, C.W. Root exudates induce soil macroaggregation facilitated by fungi in subsoil frontiers in Environmental Science 6:140, 1-17 2018

Bell, J.M., Smith, J.L., Bailey,V.L., Bolton,H. Priming effect and C storage in semi-arid no-till spring crop rotations Biology & Fertility of Soils 37, 237-244 2003

Beniston, M. Climate Change in mountain regions: A review of possible impacts Climate Change 59: 5-31 2003

Berdanier, A., Global treeline position Nat. Educ. Knowl 1, 11 2010

Bergkemper,bF., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M., Schulz, S. Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems Environmental Microbiology 18(8), 2767-2767 2016

Bernal, B., McKinley, D.C., Hungate, B.A., White, P.M., Mozder T.J., Megonigal, J.P. Limits to soil carbon stability; Deep, ancient soil carbon decomposition stimulated by new labile organic inputs Soil Biology and Biochemistry 98:85-94 2016

Bilyera, N.; Blagodatskaya, E.; Yevdokimov, I. Towards a conversion factor for soil microbial phosphorus European Journal of Soil Science Volume: 87 Pages: 1-8 2018

Bingeman CW, Varner JE, Martin WP The effect of the addition of organic materials on the decomposition of an organic soil Soil Science Society of America Journal 17, 34–38 1953

Blagodatskaya, E.V.,Blagodatsky,S.A.,Anderson,T.H.,Kuzyakov,Y. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies Applied Soil Ecology 37, 95e105 2007

Blagodatskaya, E.V., Kuzyakov, Y., Mechanisms of real and apparent priming effect and their dependence on soil microbial biomass and community structure:critical review Biology and Fertility of Soils 45, 115–131 2008

Blagodatskaya, E.V., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y. evated atmospheric CO2 increases microbial growth rates in soils: results of three CO2 enrichments experiments El. Glob. Change Biol 16, 836-848 2009

Blagodatskaya, E.V.,Yuyukina,T.,Blagodatsky,S.,Kuzyakov,Y. Turnover of soil organic matter and of microbial biomass under C3-C4 vegetation change: Consideration of 13C fractionation and preferential substrate utilization Soil Biology and Biochemistry 43(1) 159-166 2011

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov Y. Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition Soil Biology and Biochemistry 42, 1275-1283 2010

Bofinger, P. Grundzüge der Volkswirtschaftslehre: Eine Einführung in die Wissenschaft von Märkten Person Studium 2003

Bonan, G.B., Lombardozzi, D.L., Wieder, W.R., Oleson, K.W., Lawrence,D.M., Hoffman, F.M., Collier, N. Model structure and climate data uncertainty in historical simulations oft he terrestrial carbon cycle (1850-2014) Global Biochemical Cycles 33, 1310-1326 2019

Bond-Lamberty, B., Wang, C. & Gower, S. T. A global relationship between the heterotrophic and autotrophic components of soil respiration? Glob. Change Biol. 10, 1756–1766 2004

Bowden, R.,D., Newkirk, K.,M., Rullo, G.,M., Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions Soil Biology and Biochemistry 30, 1591–1597 1998

Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M.,Mohan, J. E., ... Wallenstein, M. D. Thermal adaptation of soil microbial respiration to elevated temperature Ecology Letters 11(12),1316–1327 2008

Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., & Strickland, M. S. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth Biogeochemistry 113, 271–281. 2013

Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W. Managing uncertainty in soil carbon feedbacks to climate change Nature climate change DOI: 10.1038/NCLIMATE3071 2016

Brito, Patricia; Soledad Jimenez, Maria; Morales, Domingo; et al. Assessment of ecosystem CO2 efflux and its components in a Pinus canariensis forest at the treeline Trees-structure and function Volume: 27 Issue: 4 Pages: 999-1009 2013

Broadbent, P., Baker, K., F., Waterworth, Y. Bacteria and Actinomycetes antagonisticto fungal root pathogens in australian soils Australian journal of biological science 4(5),925-44 1971

Bruijnzeel, LA Hydrological functions of tropical forests: not seeing the soil for the trees? Agriculture ecosystems & environment Volume: 104 Issue: 1 Pages: 185-228 2004

Bruslind L. Introduction to Microbiology LibreTexts_Biology licensed by CC BY-NC-SA 3.0. via https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Bruslind) 2019

Buckeridge, K.M, Banerjee, S., Siciliano, S.D., Grogan, P. The seasonal pattern of soil microbial community structure in mesic low arctic tundra Soil Biology and Biochemistry 65:338-347 2013

Buckeridge, K.M. & McLarren, J.R. Does plant community plasticity mediate microbial homeostasis? Ecology and Evolution DOI: 10.1002/ece3.6269 2020

Burges, A. & Raw, F. Soil Biology Academic Press Inc. 1967

Burns, R.G. Enzyme activity in soil : Location and a possible role in microbial ecology Soil Biology and Biochemistry 14(5):423-427 1982

Calvet R. Le sol, propriétés et fonctions France Agricole / Dunod Volumen 2, p 154-155 2003

Campbell, E. E., Paustian, K. Current developments in soil organic matter modeling and the expansion of model applications:A review Environmental Research Letters 10(12), 123004 2015

Carrillo, Y., Bell, C., Koyama, A., Canarini, A., Boot, C. M., Wallenstein, M. & Pendall, E. Plant traits, stoichiometry and microbes as drivers of decomposition in the rhizosphere in a temperate grassland Journal of Ecology 105, 1750-1765 2017

Chaparro, J,M, Badri, D.V., Vivanco, J.M. Rhizosphere microbiome assemblage is affected by plant development The ISME journal 8, 790-803 2013

Chapin III, F.S, McFarland, J., McGuire, D.A., Euskirchen, E.S., Ruess, R.W., Kielland, K. The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences Journal of Ecology https://doi.org/10.1111/j.1365-2745.2009.01529.x 2009

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X.,Blagodatskaya, E., Kuzyakov, Y. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric mineralization theories Global Change Biology 20, 2356–2367 2014

Cheng, W. Rhizosphere feedbacks in elevated CO2. Tree Physiology 19. 313 - 320 1999

Cheng, W. & Gershenson, A. Carbon Fluxes in the Rhizosphere in The Rhizosphere: An Ecological Perspective, Chapter 2, editors Cardon, Z.G. & Whitbeck, J.L. Academic Press 2007

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S, Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2 Science Aug 31;337(6098):1084-7 2012

Cheng, L. Liu, L., Qin, S., Yang, G., Fang, K., Zhu, B., Kuzyakov, Y., Chen,P., Xu, Y., Yang, Y. Nature Communications 10: 5112 https://doi.org/10.1038/s41467-019-13119-z 2019

Christian, J.R. & Anderson, T.R. Modelling DOM Biogeochemistry, chapter 16 in Biogeochemistry of Marine Dissolved Organic Matter, ed. Hansell, D.A. & Carlson, C.A. Academic Press 2002

Clark, D. A., Piper, S. C., Keeling, C. D. & Clark, D. B. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000 Proceedings of the National Academy of Sciences 100, 5852–5857 2003

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A. M., Cregger, M. A.,Moorhead, L. C.,Patterson, C. M. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6(8):130 2015

Clein, J., Schimel, J. Nitrogen turnover and availability during succession from alder to poplar in Alaskan taiga forests Soil Biology and Biochemistry 27(6), 743–752 1995

Clemmensen, K., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H.,Stenlid, J., Finlay, R., Wardle, D., Lindahl, B., Roots and associated fungi drive long-term carbon sequestration in boreal forest Science 339, 1615–1618 2013

Cleveland, C.C., Liptzin, D. C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235–252 2007

Conant, R.T. Temperature and soil organic matter decomposition rates: Synthesis of current knowledge and a way forward Global Change Biology 17, 3392–3404 2011

Conde, E., Cardenas, M., Ponce-Mendoza, A., Luna-Guido, M.L., Cruz-Mondragon, C., Dendooven, L. The impacts of inorganic nitrogen application on mineralization of C-14-labelled maize and glucose, and on priming effect in saline alkaline soil Soil Biol Biochem 37,681–691 2005

Constantinides, M, Fownes, J.H. Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations Soil Biology and Biochemistry 26(1),49-55 1994

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988-995 2013

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J. Formation of soil organic matter via biochemical and physical pathways of litter mass loss Nature geoscience DOI: 10.1038/NGEO2520 2015

Craine, J.M., Morrow, C., Fierer, N. Microbial nitrogen limitation increases decomposition Ecology 88, 2105–2113 2007

Cramer, W., Bondeau, A., Schaphoff, S., Lucht, W., Smith, B., & Sitch, S. Tropical forests and the global carbon cycle: impacts of atmospheric carbon dioxide, climate change and rate of deforestation Philosophical Transactions of the Royal Society B: Biological Sciences 359(1443), 331–343 2004

Cramer, W., Bondeau, A., Woodward, F. I., Prentice, I. C., Betts, R. A.,Brovkin, V., Cox, P. M., Fisher, V.,…,Young-Molling, C. Global response of terrestrial ecosystem structure and function to CO2 and climate change: results from six dynamic global vegetation models Global Change Biology 7, 357–373 2001

Crawford, J.A., Lopez-Sangil, L., Medina-Barcenas, E., Baxendale, C., George, C., Brikett,A., Bréchet, L., Sayer, E. The effects of scale and context-dependency on the outcome of experiments investigating soil carbon dynamics Thesis, University of Lancaster 2017

Crawford, K.M., Knight, T.M. Competition overwhelms the positive plant-soil feedback generated by an invasive plant Oecologia 183, 211–220 2017

Cros, C., Alvarez, G., Keuper, F., Fontaine, S. A new experimental platform connecting the rhizosphere priming effect with CO2 fluxes of plant-soil systems Soil Biology and Biochemistry 130: 12-22 2019

Danby, R., & Hik, D Responses of white spruce (Picea glauca) to experimental warming at a subarctic alpine treeline Global Change Biology 13(2), 437–451 2007a

Danby, R., & Hik, D Variability, contingency and rapid change in recent subarctic alpine tree line dynamics Journal of Ecology 95(2), 352–363 2007b

Davidson, E.A., Belk, E. & Boone, R.D. Soil water and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest Global Change Biology 4, 217–227 1998

Davidson, E.A., Janssens, I.A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change Nature 440, 165–173 2006

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., Living in a fungal world: impact of fungi on soil bacterial niche development FEMS Microbiol. 29,795–811 2005

de Deyn, G.B., Cornelissen, J.H.C. & Bardgett, R.D. Plant functional traits and soil carbon sequestration in contrasting biomes Ecology Letters 11, 516-531 2008

de Deyn, G.B., Quirk, H., Oakley, S., Ostle, N., Bargett, R.D. Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands Biogeoscience 8, 1131-1139 2011

de Graaff, M.A., Van Groenigen, K.J., Six, J., Hungate, B., van Kessel, C. Interactions between plant growth and soil nutrient cycling under elevated CO2: a meta-analysis Global Change Biology 12, 2077–2091 2006

de la Riva, E.G., Marañón, T., Pérez-Ramos, I.M., Navarro-Fernández, C.M., Olmo, M., Villar, R. Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum? Plant Soil 424:35-48 2018

de Long, J.R., Semchenko, M., Pritchard, W.J., Cordero, I., Fry, E.L., Bartgett, R.D. Drought soil legacy overrides maternal effects on plant growth Functional Ecology 33,1400–1410 2019

de Luca, T.H., Nilsson, M.-C., Zackrisson, O. Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden Oecologia 133, 206–214 2002

de Marco, J., Mack, M.C., Bret-Harte, M.S. The Effects of Snow, Soil Microenvironment, and Soil Organic Matter Quality on N Availability in Three Alaskan Arctic Plant Communities Ecosystems 14: 804–817 2011

de Nobili, M., Contin, M., Mondini, C., Brookes, Soil microbial biomass is triggered into activity by trace amounts of substrate Soil Biology and Biochemistry 33, 1163–1170. 2001

Denison, R., Bledsoe, C., Kahn, M., O' Gara, F., Simms, E., & Thomashow, L. Cooperation in the Rhizosphere and the "free rider" problem Ecology 84(4), 838–845 2003

Dickie, I.A. Mycorrhiza of Forest Ecosystems in Encyclopedia of Soil Science, Second Edition, ed. Lal, R. Taylor & Francis 2006

Dictor, M., Tessier, L., & Soulas, G. Reassessment of the K ec coefficient of the fumigation–extraction method in a soil profile Soil Biology and Biochemistry 30(2), 119–127 1998

Diekmann, M; Falkengren-Grerup, U Prediction of species response to atmospheric nitrogen deposition by means of ecological measures and life history traits Journal of Ecology Volume: 90 Issue: 1 Pages: 108-120 2002

Dignac, M.F., Derrien, D., Barré, P., Barot, S., Cécillon, L., Chenu,C., Chevallier, T., Freschet, G.T., Garnier, P., Guenet,B., Hedde, M., Klumpp, K., Lashermes, G., Maron, P.A., Nunan, N., Roumet, C., Basile-Doelsch, I. Increasing soil carbon storage:mechanisms, effects of agricultural practices and proxies. A review Agron. Sustain. Dev. 37: 14 2017

Dijkstra, F. A., Blumenthal, D.,Morgan, J. A., Pendall, E., Carrillo,Y., and Follett, R. F. Contrasting effects of elevated CO 2 and warming on nitrogen cycling in a semiarid grassland New Phytol. 187, 426–437 2010

Dijkstra, F.A., Carrillo, Y., Pendall, E., Morgan, J.A. Rhizosphere priming: a nutrient perspective Front.Microbiol 4.16 2013

Dohi, M., & Mougi, A A coexistence theory in microbial communities Royal Society Open Science 5(9), 180476 2018

dtv-Atlas zur Biologie, Tafeln und Texte, Band 2 Vogel G. & Angermann H. Deutscher Taschenbuch Verlag GmbH & Co. KG 1990

Edmeades, DC; Smart, CE; Wheeler, DM Aluminium toxicity in New-Zealand Soils- Preliminary results on the development of diagnostic criteriaNew Zealand Journal of Agriultural Research Volume: 26 Issue: 4 Pages: 493-501 1983

Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil Soil Biology and Biochemistry 42: 896-903 2010

Fajardo, A., & Piper, F An assessment of carbon and nutrient limitations in the formation of the southern Andes tree line Journal of Ecology 105(2), 517–527 2017

Fan, LianLian; Li, Yan; Tang, LiSong; et al. Combined effects of snow depth and nitrogen addition on ephemeral growth at the southern edge of the Gurbantunggut Desert, China Journal of Arid LandVolume: 5 Issue: 4 Pages: 500-510 2013

Fang, C. & Moncrieff, J.B. The dependence of soil CO 2 efflux on temperature Soil Biology & Biochemistry 33, 155–165 2001

Fang, C., Smith, P., Smith, J.U. Is resistant soil organic matter more sensitive to temperature thanthe labile organic matter? 11, 1092–1100 2006

Fanin, N., Fromin, N., Buatois, B., Hättenschwiler, S. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter–microbe system Ecology Letters 16, 764–772 2013

Fanin, N., Hattenschwiler, S., Schimann, H., Fromin, N. Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest Functional Ecology 29, 140–150 2014

Fanin, N., Hättenschwiler, S., Schimann, H., Fromin, N., 2015 Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest Functional Ecology 29, 140–150 2015

Fanin, N., Kardol, P., Farrell, M., Nilsson, M-C., Gundale, M.J., Wardle, D.A. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils Soil Biology and Biochemistry 128 (2019) 111–114 2019

FAO Measuring and modelling soil carbon stocks and stock changes in livestock production systems – Guidelines for assessment Livestock Environmental Assessment and Performance (LEAP) Partnership FAO Rome, Italy via: www.fao.org/3/I9693EN/i9693en.pdf 2018

Feeley, K. J. Compositional shifts in Costa Rican forests due to climate-driven species migrations Global Change Biol. 19, 3472 – 3480 2013

Feeley, K.J. & Silman, M.R. The data void in modeling current and future distributions of tropical species Global Change Biology 17, 626–630 2011

Fernandez, C.W. & Kennedy, P.G. Moving beyond the black box: fungal traits, community structure, and carbon sequestration in forest soils New Phytologist 205:1378-1380 2015

Fierer, N. & Schimel, J.P. A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid

Rewetting of a Dry Soil Soil Science Society of America Journal 67(3), 798-805 2003a

Fierer. N. & Schimel, J.P. Effects of dry-rewetting frequency on soil carbon and nitrogen transformations Soil Biology and Biochemistry 34:777-787 2003b

Fierer, N., Schimel, J.P., Holden, P.A. Variations in microbial community composition through two soil depth profiles Soil Biology and Biochemistry 35, 167–176 2003c

Fitter, A.H., Graves, J.D., Watkins, N.K., Robinson. D., Scrimgeour, C. Carbon transfer between plants and its control in networks of arbuscular mycorrhizas Functional Ecology 12, 406–412 1998

Fontaine, S., Bardoux, G., Benest, D., Verdier, B., Mariotti, A., Abbadie, L. Mechanisms of the priming effect in a Savanah soil amended with cellulose Soil Sci. Soc. Am. J. 68, 125-131 2004

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C. Stability of organic carbon in deep soil layers controlled by fresh carbon supply Nature 450, 277–280 2007

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B.,Revaillot, S., Maron, P.A. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect Soil Biology and Biochemistry 43, 86–96 2011

Fontaine, S., Mariotti, A., Abbadie, L. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem 35, 837-843 2003

Frankenberger. W.T. & Johanson, J.B. Effect of pH on enzyme stability in soils Soil Biology and Biochemistry 14(5):433-437 1982

Frey, S.D., Six, J., Elliott, E.T Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soilelitter interface Soil Biology and Biochemistry 35,1001-1004 2003

Frey-Klett,P., Garbaye, J., Tarkka, M. The mycorrhiza helper bacteria revisited New Phytologist 176:22-36 2007

Friggens, N.L., Aspray, T.J., Parker, T.C., Subke, J-A, Wookey, P.A. Spatial patterns of soil organic matter dynamics are shaped by mycorrhizosphere interactions in a treeline forest Plant and Soil 447, 521-535 2020

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

Frostegård, Å., Tunlid, A., Bååth, E. Microbial biomass measured as total lipid phosphate in soils of different organic content Journal of Microbiological Methods 14, pp. 151-163 1991

Gadd, G.M. Fungi in Biogeochemical Cycles Cambridge University Press 2006

Garbeva, P., van Elsas, J.D., van Veen, J.A. Rhizosphere microbial community and its response to plant species and soil history Plant and Soil 302, 19-32 2008

Garbeva, P., & Boer, W. Inter-specific Interactions Between Carbon-limited Soil Bacteria Affect Behavior and Gene Expression Microbial Ecology 58(1), 36–46 2009

Garcia-Pausas, J. & Paterson, E. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon Soil Biology and Biochemistry 43, 1705-1713 2011

Gebauer, G; Meyer, M N-15 and C-13 natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association New Phytologist Volume: 160 Issue: 1 Pages: 209-223 2003

Gibbon, A., Silman, M.R.; Malhi, Y., Fisher, J.B., Meir, P., Zimmermann, M., Dargie, G.C., Farfan, W.R., Garcia, K.C. Ecosystem Carbon Storage Across the Grassland-Forest Transition in the High Andes of Manu National ParkEcosystems 13(7),1097-1111 2010

Gilbert, M., Pammenter, N., Ripley, B. The growth respinses of coastal dune species are determined by nutrient limitation and sand burial Oecologia 156, 169-178 2008

Girardin, C.A.J., Malhi, Y., Aragao, L.E.O.C., Mamani, M., Huasco, W.H.,Durand, L., Feeley, K.J., Rapp, J., Silva-Espejo, J.E., Silman, M. Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes Global Change Biology 16,3176–3192. 2010

Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N. The influence of microbial communities, management, and soil texture on soil organic matter chemistry Geoderma 150,278–286 2009

Greenwood; S. & Jump, A.S. Consequences of treeline shifts for the diversity and function of high altitude ecosystems Arctic, Antarctic and Alpine research 46(4), 829-840 2014

Greenwood, S., Chen, J-C, Chen, C-T, Jump, A.S. Community change and species richness reductions in rapidly advancing tree lines Journal of Biogeography 43, 2274-2284 2016

Griffin, E. Measuring and assessing soils Department of Primary Industries and Regional Development, Government of Western Australia 2018

Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R.D., Poly, F. Relative contributions of

plant traits and soil microbial properties to mountain grassland ecosystem services Journal of Ecology 101 (1): 47-57 2013

Grogan, P., Illeris, L., Michelsen, A., & Jonasson, S. Respiration of Recently-Fixed Plant Carbon Dominates Mid-Winter Ecosystem CO2 Production in Sub-Arctic Heath Tundra Climatic Change 50(1-2), 129–142 2001

Gunapala, N., venette, R.C., Ferris, H., Scow, K.M. Effects of management history on the rate of organic matter decomposition Soil Biology and Biochemistry 30(14):1917-1927 1998

Guenet, B., Neill, C., Bardoux, G., Abbadie, L., Is there a linear relationship between priming effect intensity and the amount of organic matter input? Applied Soil Ecology 46, 436e442 2010

Gunina, A. and Kuzyakov, Y Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate Soil Biology and Biochemistry 90, 87-100 2015

Guyonnet, J.P., Canatarel, A.A., Simon, L., el Zahar Haichar, F. Root exudation rate as functional trait involved in plant-nutrient-use strategy classification Ecology and Evolution 8(16):8573-8581 2018

Hamer, U., Marschner, B., Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions Soil Biology and Biochemistry 37, 445–454. 2004

Hamer, U., Marschner, B., Priming effects in soils after combined and repeated substrate additions Geoderma 128, 38–51 2005

Hansen, AJ; Rotella, JJ; Kraska, MPV; et al. Spatial patterns of primary productivity in the Greater Yellowstone Ecosystem Landscape Ecology Volume: 15 Issue: 6 Pages: 505-522 2000

Harsch, M. A., Hulme, P. E., McGlone, M. S., & Duncan, R. P. Are treelines advancing? A global meta-analysis of treeline response to climate warming Ecology Letters 12, 1040–1049 2009

Hartley, I.P., Heinemeyer, A., Ineson, P., Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response Global Change Biology 13,1761-1770. 2007

Hartley, I.P. & Ineson, P. Substrate quality and the temperature sensitivity of soil organic matter decomposition Soil Biology and Biochemistry 40, 1567–1574 2008

Hartley, I. P., Hopkins, D. W., Sommerkorn, M. & Wookey, P. A., The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils Soil Biol. Biochem. 42, 92-100 2010

Hartley, I. P., Garnett, M. H., Sommerkorn, M., Hopkins, D. W., Fletcher, B.J., Sloan, V. L., Wookey, P. A. A potential loss of carbon associated

with greater plant growth in the European Arctic Nature Climate Change 2, 875–879 2012

Hayes, J.M. An introduction to isotopic calculations Woods Hole Oceanographic Institution 2004

Heath, J., Ayres, E., Possell, M., Bardgett, R.D., Black, H.I.J., Grant, H., Ineson, P.,Kerstiens, G. Rising atmospheric CO2 reduces sequestration of rootderived soil carbon Science 309, 1711–1713. 2005

Hedin, L., Brookshire, E., Menge, D., & Barron, A. The Nitrogen Paradox in Tropical Forest Ecosystems Annual Review of Ecology, Evolution, and Systematics 40(1), 613–635 2009

Heimann, M. & Reichstein, M., Terrestrial ecosystem carbon storage and climate feedbacks, Nature 451, 289e292 2008

Heitkötter,J.,Heinze,S.,Marschner,B. Relevance of substrate quality and nutrients for microbial C-turnover in top-and subsoil of a Dystric Cambisol Geoderma 302, 89-99 2017

Henneron, L.,Cros, C.,Picon-Cochard, C., Rahimian, V., Fontaine, S. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition Journal of Ecology 2019

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., de Ruiter, P.C. Carbon sequestration in ecosystems: the role of stoichiometry Ecology 85, 1179–1192 2004

Hessen, Dag O.; Elser, James J.; Sterner, Robert W.; et al. Ecological stoichiometry: An elementary approach using basic principles Limnology and Oceanography Volume: 58 Issue: 6 Pages: 2219-2236 2013

Hewitt, R.E., Taylor, D.L., Genet, H., McGuire, A.D., Mack, M Below-ground plant traits influence tundra plant acquisition of newly thawed permafrost nitrogen Journal of Ecology 10.1111/1365-2745.13062 2018

Hicks, L.C., Nottingham, A., Whitaker, J., Reay, D., Meir, P. Controls on carbon cycling in tropical soils from the Amazon to the Andes: The influence of climate, plant inputs, nutrients and soil organisms Thesis, University of Edinburgh 2016

Hicks, L.C., Meir, P., Nottingham, A., Reay, D., Stott, A.W., Salinas, N., Whitaker, J. Carbon and nitrogen inputs differentially affect priming of soil organic matter in tropical lowland and montane soils Soil Biology and Biochemistry 129: 212-222 2019

Hill, B., Jicha, T., Lehto, L., Elonen, C., Sebestyen, S., & Kolka, R. Comparisons of soil nitrogen mass balances for an ombrotrophic bog and a minerotrophic fen in northern Minnesota Science of the Total Environment 550, 880–892 2016

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B. Origins of root-mediated pH changes in the rhizosphere and their responeses to environmental constraints: A review Plant and Soil 248(1):43-59 2003

Hinzmann,L.D.,Bettez,N.D.,Bolton,W.R.,Chapin,F.S.III,Dyurgerov,M.,Fastie,C., Griffith,B.,Hollister,R.,Hope,A.,Huntington,H.P.,Jensen,A.M.,Jia,G.,Jorgenson,T.,Kane,D.L.,Klein,D.R.,Kofinas,G.,Lynch,A.,Lloyd,A.,McGuire,A.D., Nelson,F.E.,Oechel,W.C.,Osterkamp,T.,Racine,C.H.,Romanovsky,V.E.,Stone,R.S.,Stow,D.A.,Sturm,M.,Tweedie,C.,Vourlitis,G.L.,Walker,M.,Walker,D.,Webber,P.J.,Welker,J.,Winker,K.,Yoshikawa,K. Evidence and Implications of Recent Climate Change in Northern Alaska and Other Arctic Regions Climatic Change 72(2),251-298 2005

Hobbie, S.E., Nadelhoffer, K.J.,Högberg,P. A synthesis: The role of nutrients as constraints on carbon balances in boreal and arctic regions Plant and Soil 242,163 2002

Hobbie, J.E. & Hobbie, E.A. N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra Ecology 87, 816–822 2006

Hobbie, S. E. Plant species effects on nutrient cycling: revisiting litter feedbacks Trends Ecol. Evol 30, 357–363 2015

Holah, J.C. & Alexander, H.C Soil pathogenic fungi have the potential to affect the co-existence of two tallgrass prairie species Journal of Ecology 87, 598-608 1999

Hopkins, F., Torn, M., & Trumbore, S. Warming accelerates decomposition of decades-old carbon in forest soils Proceedings of the National Academy of Sciences of the United States of America 109(26), E1753–61 2012

Huntington, H.P., Goodstein, E., Euskirchen, E. Towards a Tipping Point in Responding to Change: Rising Costs, Fewer Options for Arctic and Global Societies AMBIO 41:66-74 2012

Huo, C., Luo, Y. & Cheng, W. Rhizosphere priming effect: A meta-analysis Soil Biology and Biochemistry 111, 78-84 2017

Iffis, B., St-Arnaud, M., Hijri, M. Bacteria associated with arbuscular mycorrhizal fungi within roots of plants growing in a soil highly contaminated with aliphatic and aromatic petroleum hydrocarbons FEMS Microbiology Letters Volume: 358 Issue: 1 Pages: 44-54 2014

International Panel on Climate Change (IPCC) AR4: Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the IPCC 2007

International Panel on Climate Change (IPCC) Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the

Fifth Assessment Report of the Intergovernmental Panel on Climate Change 2013a

International Panel on Climate Change (IPCC) Chapter 6: Carbon and Other Biogeochemical Cycles. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change 2013b

International Panel on Climate Change (IPCC) AR5: Climate Change 2014: Synthesis Report Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change 2014

International Panel on Climate Change (IPCC): Technical Summary (TS) on Climate Change and Land 2019

Janssens, I.A., Dieleman, W. Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, J.A., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E-D, Tang, J., Law, B.E. Reduction of forest soil respiration in response to nitrogen deposition Nature Geoscience 3(5),315–322 2010

Jenkinson, D.S.,Fox,R.H.,Rayner,J.H Interactions between fertilizer nitrogen and soil nitrogen - the so-called 'priming' effect European Journal of Soil Science 36(3),425-444 1985

Jilling, A., Keiluveit, M., Contosta, A.R., Frey, S., Schimel, J., Schnecker, J., Smith, R.G., Tiemann, L., Grandy, A.S. Minerals in the rhizosphere: overlokked mediators of soil nitrogen availability to plants and microbes Biogeochemistry 139, 203-122 2018

Jingguo, W. & Bakken, L.R. Competition for nitrogen during decomposition of plant residues in soil: effect of spatial placement of N-rich and N-poor plant residues Soil Biology & Biochemistry 29,153–162 1997

Jobbagy, E.G. & Jackson, R.B., Global controls of forestline elevation in the northern and southern hemispheres Global Ecology and Biogeography 9, 253–268 2000

Jobbágy, E.G., Jackson, R.B. The uplift of soil nutrients by plants: Biogeochemical consequences across scales Ecology 85(9), 2004, pp. 2380–2389 2004

Jones, D.L., Nguyen, C. & Finlay, R.D. Carbon flow in the rhizosphere: carbon trading at the soil–root interface Plant and Soil 321, 5-33 2009

Kallenbach, C.M., Frey, S.D., Grandy, A.S. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls Nature communications DOI:10.1038/ncomms13630 2016

Kramer, A., Hagedorn, F., Shevchenko, I., Leifeld, J., Guggenberger, G., Goryacheva, T., Rigling, A., and Moiseev, P. Treeline shifts in the Ural

mountains affect soil organic matter dynamics Glob. Change Biology 15, 1570–1583 2009

Ke, P-J & Wan, J. Effects of soil microbes on plant competition: a perspective from modern coexistence theory Ecological Monographs 90(1):e01391 2019

Keeney, DR Prediction of soil-nitrogen availability in forest ecosystems - a literature review Forest science Volume: 26 Issue: 1 Pages: 159-171 1980

Kellert, H. In the wake of chaos: unpredictable order in dynamical systems The University of Chicago Press 1993

Keiluveit, M., Bougoure. J.J., Nico, P.S., Pett-Ridge. J., Weber, P.K., Kleber, M., Mineral protection of soil carbon counteracted by root exudates. Nature Clim. Change 5(6): 588-595. 2015

Keiser, A.D., Strickland, M.S., Fierer, N., Bradford, M.A. The effect of ressource history on the functioning of soil microbial communities is maintained across time Biogeoscience 8, 1477-1486 2011

Keuskamp,J.A., Dingemans, B.J.J., Lehtinen, T., Satneel, J.M., Hefting, M.M. Tea Bag Index: a novel approach to collect uniformdecomposition data across ecosystems Methods in Ecology and Evolution doi: 10.1111/2041-210X.12097 2013

Kielland, K Amino-acid-absorption by arctic plants - implications for plant nutrition and nitrogen cycling Ecology Volume: 75 Issue: 8 Pages: 2373-2383 1994

Klumpp, K., Fontaine, S., Attard, E., Le Roux, X., Gleixner, G., Soussana, J.F., Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community Journal of Ecology 97, 876e885 2009

Kolář, L, Kužel, S., Horáček, J., Čechová, V., Borová-Batt, J., Peterk, J. Labile fractions of soil organic matter, their quantity and quality Plant Soil Environment 5 (6): 245–251245 2009

Kome, Georges Kogge; Enang, Roger Kogge; Yerima, Bernard Palmer Kfuban; et al. Models relating soil pH measurements in H2O, KCl and CaCl2 for volcanic ash soils of Cameroon Geoderma RegionalVolume: 14 Article Number: UNSP e00185 2018

Körner, C. Alpine treelines: Functional ecology of the global high elevation tree limits Springer, Basel 2012

Körner, C., & Paulsen, J. A world-wide study of high altitude treeline temperatures Journal of Biogeography 31, 713–732 2004

Körner,C. A re-assessment of high elevation treeline positions and their explanation Oecologia 115(4), 445-459 1998

Körner,C.,Paulsen,J. A climate-based model to predict potential treeline position around the globe Alpine Botany 124(1),1-12 2014

Kramer, S., Marhan, S., Ruess, L., Armbuster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems Pedobiologia 55,111-119 2012

Kuzyakov, Y., Domanski, G. Review: Carbon input by plants into the soil. J. Plant Nutr. Soil Sci. 163, 421-431 2000

Kuzyakov,Y. & Cheng,W. Photosynthetic controlsof rhizosphere respiration and organic matter decomposition Soil Biology and Biochemistry 33, 1915-1925 2001

Kuzyakov, Y. Review: Factors affecting rhizosphere priming effects J. Plant Nutr. Soil Sci. 165, 382-396 2002

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling Soil Biology and Biochemistry 41, 210–219 2009

Kuzyakov, Y. Priming effects: interactions between living and dead organic matter Soil Biology & Biochemistry 42, 1363e1371 2010

Kuzyakov, Y. How to link soil C pools with CO2 fluxes? Biogeosciences 8,1523–1537 2011

Kuzyakov, Y., Bogomolova I, Glaser B. Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific 14C analysis Soil Biology and Biochemistry 70, 229–236 2014

Kuzyakov, Y. & Blagodatskaya, E. Microbial hotspots and hot moments in soil: Concept & review Soil Biology and Biochemistry 83, 184-199 2015

Kyker-Snowman, E., Wieder, W.R., Frey, S., Grandy, A.S. Stoichiometrically coupled carbon and nitrogen cycling in the MIcrobial-Mineral Carbon Stabilisation model (MIMICS-CN) Geoscientific Model Development https://doi.org/10.5194/gmd-2019-320 2019

Lambers, H., Mougel, C., Jaillard, B., Hinsinger, P. Plantemicrobeesoil interactions in the rhizosphere: an evolutionary perspective Plant and Soil 321, 83-115 2009

Lange, Stefan; Rockel, Burkhardt; Volkholz, Jan; et al. Regional climate model sensitivities to parametrizations of convection and non-precipitating subgrid-scale clouds over South America Climate Dynamics Volume: 44 Issue: 9-10 Pages: 2839-2857 2015

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N. The influence of soil properties on the structure of bacterial and fungal communities across land-use types Soil Biol Biochem 40, 2407–2415 2008

Leake, J.R. The biology of mycoheterotrophic (‘saprophytic’) plants New Phytol 127:171–216 1994

Lehmann, J. & Kleber, M. The contentious nature of soil organic matter Nature 528, 60-68 2015

Lenoir, J., Gégout, J. C., Marquet, P. A., de Ruffray, P., & Brisse, H. A significant upward shift in plant species optimum elevation during the 20th century Science 320, 1768–1771 2008

Li, L.J, Zhu-Barker, X., Ye, R., Doane, T.A., Horwath, W.R. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability Soil Biology and Biochemistry 119: 41-49 2018

Liang, C., Cheng, G., Wixon, D.L., Balser T.C. An Absorbing Markov Chain approach to understanding the microbial role in soil carbon staboilisation Biogeochemistry 106:303-309 2011

Liang, C., Schimel, J.P., Jastrow, J.D., The importance of anabolism in microbial control over soil carbon storage Nature Microbiology 2 2017

Liang, C., Amelung, W., Lehmann, J., Kästner, M. Quantitative assessment of micorbial necromass contribution to soil organic matter Global Change Biology DOI: 10.1111/gcb.14781 2019

Liang, J., Zhou, Z., Huo, C., Shi, Z., Cole, J.R., Huang , L., Konstantinidis, K.T., Li, X., Liu, B., Luo, Z., Penton, C.R., Schuur, E.A.G., Tiedje, J.M., Wang, Y.P., Wu, L., Xia, J., Zhou, J., Luo, Y. More replenishment than priming loss of soil organic carbon with additional carbon input Nature Communications 9(1):3175 2018

Liljeroth, E., Kuikman, P., Van Veen, J. A. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels Plant and Soil Volume 161, Issue 2, pp 233–240 1994

Lloyd, J. & Taylor, J.A. On the temperature-dependence of soil respiration Functional Ecology 8, 315–323 1994

Lockwood, M. Solar change and climate: an update in the light of the current exceptional solar minimum Royal Society 466(2114),1471-2946 2010

Löhnis, F. Nitrogen availability of green manure Soil Science 22,253-290 1926

Louis, B.P., Maron, P-A-, Viaud, V., Leterme,P., Menasseri-Aubry, S. Soil C and N models that integrate microbial diversity Environ Chem Lett 14:331-344 2019

Lugo, M.A., Negritto, M.A., Jofré, M., Anton,A., Galetto, L. Colonization of native Andean grasses by arbuscular mycorrhizal fungi in Puna: a matter of altitude, host photosynthetic pathway and host life cycles FEMS Microbial Ecology 81:455-456 2012

Lugo, M.A. & Menoyo, E. Southern Highlands: Fungal EndosymbioticAssociations in Mycorrhizal Fungi in South America, ed. Pagano, M.C. & Lugo, M.A. Springer 2019

Luo, Y. & Zhou, X. Soil Respiration and the Environment Academic Press 328 pp 2006

Lutz, D. A., Powell, R.L., Silman, M. R. Four decades of Andean timberline migration and implications for biodiversity loss with climate change PLoS ONE 8(9),e74496 2013

Lynch,L.M.,Machmuller,M.B.,Cotrufo,F.M.,Paul,E.A.,Wallenstein,M.D. Tracking the fate of fresh carbon in the Arctic tundra: Will shrub expansion alter responses of soil organic matter to warming? Soil Biology and Biochemistry 120,134-144 2018

Mack, M.C., Bret-Harte, M.S., Hollingsworth, T.N., Jandt, R.R., Schuur, E.A.G., Shaver, G.R., Verbyla, D.L. Carbon loss from an unprecedented Arctic tundra wildfire Nature 475(7357), 489–492 2011

Malik, A.A., Martiny, J.B.H., Brodie, E.L., Martiny, A.C., Treseder, K.K., Allison, S.D. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change The ISME Journal 14:1-9 2020

Männistö, M., Ganzert, L. Tiirola, M., Häggblom, M.M., Stark,S. Do shifts in life strategies explain microbial community responses to increasing nitrogen in tundra soil? Soil Biology and Biochemistry 96,216-228 2016

Manzoni S, Taylor P, Richter A, Porporato A, Agren GI. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196(1):79-91 2012

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L., Wiesenberg, G.L.B. How relevant is recalcitrance for the stabilisation of organic matter in soils? Journal of Plant Nutrition and Soil Science 171, 91-110 2008

Marschner, B. & Dell, B. Nutrient uptake in mycorrhizal symbiosis Plant and Soil 159, 89-104 1994

Mason-Jones, K., Schmücker, N., Kuzyakov, Y. Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming Soil Biology and Biochemistry 124: 38-46 2018

Maynard, D.S., Crowther, T.W., Bradford, M.A. Fungal interactions reduce carbon use efficiency Ecology Letters 20: 8, 1034-1042 2007

Mayor, J.R., Sanders, N.J., Classen, A.T., Bardgett, R.D., Clément, J-C., Fajardo, A., Lavorel, S., Sundqvist, M.K., Bahn, M., Chisholm, C., Cieraad, E., Gedalof, Z., Gridulis, K., KudoG., Oberski, D.L., Wardle, D.A. Elevation alters ecosystem properties across temperate treelines globally Nature letters 542, 91-95 2017

McLaren, J.R., Buckeridge, K.M Decoupled above- and belowground responses to multi-decadal nitrogen and phosphorus amendments in two tundra ecosystems Ecosphere 10(7): e02735.10.1002/ecs2.2735 2019

Meier, C.L., Keyserling, K. & Bowman, W.D. Fine root inputs to soil reduce growth of a neighbouring plant via distinct mechanisms dependent on root carbon chemistry J. Ecol. 97,941–949 2009

Meier, I.C., Finzi, A.C., Phillips, R.P. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools Soil Biology and Biochemistry 2016

Melillo,J.M.,Butler,S.,Johnson,J.,Mohan,J.,Steudler,P.,Lux,H.,Burrows,E.,Bowles,F.,Smith,R.,Scott,L.,Vario,C.,Hill,T.,Burton,A.,Zhou,Y-M.,Tang,J. Soil warming, carbon-nitrogen interactions, and forest carbon budgets Proceedings of the National Academy of Sciences 108(23),9508-9512 2011

Merino, C., Godoy, R., Matus, F. Soil enzymes and biological activity at different levels of organic matter stability Journal of Soil Science and Plant nutrition 16(1), 14-30 2016

Metcalfe, D.B., Asner, G.P., Martin, R.E. Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. Ecol Lett 17,324–332 2014

Mikutta R, Kleber M, Torn MS, Jahn R Stabilization of soil organic matter: association with minerals or chemical recalcitrance? Biogeochemistry 77(1):25–56 2006

Moorhead, D.L. & Sinsabaugh, R.L. A theoretical model of litter decay and microbial interaction Ecological Monographs 76,151–174 2006

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources Frontiers in Microbiology 5 2014a

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstein, S., Richter, A. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling Nature communications DOI: 10.1038/ncomms4694 2014b

Morris, AEL; Stark, JM; Gilbert, BK Evaluation of isotopic fractionation error on calculations of marine-derived nitrogen in terrestrial ecosystems Canadian Journal of Forest Research - Revue Canadienne de Recherche Forestiere Volume: 35 Issue: 7 Pages: 1604-1616 2005

Morris, SJ & Blackwood, CB The Ecology of the Soil Biota and their Function Soil Microbiology, Ecology and Biochemistry DOI: 10.1016/B978-0-12-415955-6.00010-4 2015

Mouginot, C, Kawamura, R., Matulich, KL, Berlemont, R, Allison, SD, Amend, AS, Martiny, AC Elemental stoichiometry of fungi and bacteria strains from grassland leaf litter Soil Biology and Biochemistry 10.1016/j.soilbio.2014.05.011 2014

Müller, M., Oelmann, Y., Schickhoff, U., Böhner, J., Scholten, T. Himalayan treeline soil and foliar C:N:P stoichiometry indicate nutrient shortage with elevation Geoderma 291:21-32 2017

Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. Soil Biology and Biochemistry 81: 236-243 2015

Newcomb, C.J., Qafoku,N.P., Grate, J.W., Bailey, V.L., De Yoreo, J.J. Developing a molecular picture of soil organic matter-mineral interactions by quantifying organo-mineral binding Nature Communications 8:396 2017

Newsham, K.K., Upson, R., Read, D.J. Mycorrhizas and dark septate root endophytes in polar regions Fungal Ecology 2(1), 10-20 2009

Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J. Soil priming by sugar and leaf-litter substrates: a link to microbial groups Applied Soil Ecology 42, 183e190 2009

Nottingham, A. T., Turner, B. L., Chamberlain, P. M., Stott, A. W. & Tanner, E. V. J. Priming and microbial nutrient limitation in lowland tropical forest soils of contrasting fertility Biogeochemistry 01-19 2012

Nottingham, A.T., Whitaker, J., Turner, B.L., Salinas, N., Zimmermann, M., Malhi, Y.,Meir, P. Climate warming and soil carbon in tropical forests: insights from an elevation gradient in the Peruvian Andes Bioscience 65, 906–921 2015

Nottingham,A.T.,Fierer,N.,Turner,B.L.,Whitaker,J.,Ostle,N.J.,McNamara,N.P.,Bardgett,R.D.,Leff,J.W.,Salinas,N.,Silman,M.R.,Kruuk,L.E.B.,Meir,P.

Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazonas to the Andes Ecology 99:2455-2466 2018

Nottingham, A.T., Whitaker, J., Ostle, N.J., Bardgett, R.D., McNamara, N.P., Fierer, N., Salinas, N., Ccahuana,A.J.Q., Turner, B.L., Meir, P. Microbial responses to warming enhance soil carbon loss following translocation across a tropical forest elevation gradient Ecology Letters 10.1111/ele.13379 2019

Oburger, E. & Jones, D. Sampling root exudates – Mission impossible? Rhizosphere 6(10), 1016 2018

Oliveras,I., van der Eynden,M., Malhi,Y., Cahuana,N., Menor,C., Zamora,F., Haugaasen,T. Grass allometry and estimation of above-ground biomass in tropical alpine tussock grasslands Global Biogeochemical Cycles 7,785-809 2014

Oreskes, N. The Scientific Consensus on Climate Change Science 306 (5702), 2004

Oskay, A.M., Üsame, T., Cem, A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey African Journal of Biotechnology Vol 3 No9 2004

Ostle, N. J. Integrating plant–soil interactions into global carbon cycle models Journal of Ecology 97,851–863 2009

Pancotto,V.A.,Sala,O.E.,Robson,T.M.,Caldwell,M.M.,Scopel,A.L. Direct and indirect effects of solar ultraviolet-B radiation on long-term decomposition Global Change Biology 11,1982-1989 2005

Parker, T.C., Sadowsky, J., Dunleavy, H., Subke, J.-A., Frey, S.D., and Wookey P.A. Biogeochemical Cycling in Sub-arctic Birch Forest Linked to Reduced Mycorrhizal Growth and Community Change after a Defoliation Event Ecosystems 20, 316 2016

Parker, T.C., Sanderman, J., Holden, R.D., Blume-Werry, G. ,Sjögersten, S., Large, D., Castro-Díaz, M., Street, L.E., Subke J.-A., Wookey, P.A. Exploring drivers of litter decomposition in a greening Arctic: results from a transplant experiment across a treeline Ecology 0(0), pp. 1–11 2018

Parker, T.C., Subke, J.-A., & Wookey, P.A. Rapid carbon turnover beneath shrub and tree vegetation is associated with low soil carbon stocks at a subarctic treeline Global Change Biology 21, 2070–2081 2015

Pearsall WH The Soil Complex in relation to Plant Communities Journal of Ecology Vol. 26, No. 1 1938

Perveen, N., Barot, S., Alvarez, G., Klumpp, K., Martin, R., Rapaport, A., Herfurth, D., Louault, F. & Fontaine, S. Priming effect and microbial diversity in ecosystem functioning and response to global change: a

modeling approach using the SYMPHONY model Global Change Biology 20, 1174-1190 2014

Perveen, N., Barot, S., Maire, V., Cotrufo, F.M., Shahzad, T., Blagodatskaya, E., Stewarth, C.E., Ding, W., Siddiq M.R., Dimassi, B., Mary, B., Fontaine, S. Universality of priming effect: An analysis using thirty five soils with contrasted properties sampled from five continents Soil Biology and Biochemistry 134: 162-171 2019

Philips, C.A., Elberling, B., Michelsen, A. Soil Carbon and Nitrogen Stocks and Turnover Following 16 Years of Warming and Litter Addition Ecosystems 22, 110-124 2019

Plaza, C., Pegoraro, E., Bracho, R., Celis, G., Crummer, K.G., Hutchings, J.A., Hicks Pries, C.E., Mauritz,M., Natali, S.M., Salmon, V.G., Schädel., C., Webb, C.E., Schuur, E.A.G. Direct observation of permafrost degradation and rapid soil carbon loss in tundra Nature Geoscience 0.1038/s41561-019-0387-6 2019

Poirier, V., Roumet, C., Munson, A.D The root of the matter : Linking root traits with soil organic matter stablisation processes Soil Biology and Biochemistry 120, 246-259 2018

Pommer, J., Walker, T.W.N., Wanek, W., Braun, J., Zezula, D., Hu, Y., Hofhansl, F., Richter, A. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity Global Change Biology DOI: 10.1111/gcb.14777 2019

Porder, S. & Hilley, G.E. Linking chronosequences with the rest of the world: Predicting soil phosphorus content in denuding landscapes Biogeochemistry 102, 153–166 2010

Post, W.M., Peng, T-H., Emanuel, W.R., King, A.W., Dale, V.H. and DeAngelis, D.L. The Global Carbon Cycle American Scientist 87: 310-326 1990

Powers, J.S., Montgomery, R.A., Adair, E.C., Brearley, F.Q., DeWalt, S.J., Castanho, C.T., Chave, J., Deiner, E., Ganzhorn, J.U., Gilbert, M.E., Gonzalez-lturbe, J.A., Bunyavejchewin, S., Grau, H.R., Harms, K.E., Hiremath, A., Iriarte-Vivar, S., Manzane,E., de Oliveira, A.A., Poorter, L., Ramanamanjato, J.B., Salk, C., Varela, A., Weiblen, G.D., Lerdau, M.T. Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient Journal of Ecology 97, 801-811 2009

Qiao, N., Schaefer, D., Blagodatskaya, E., Zou, X.M., Xu, X.L., Kuzyakov, Y. Labile-carbon retention compensates for CO2 released by priming in forest soils Global Change Biology 20, 1943-1954 2014

Qiao, N. Xu, X, Hu, Y., Blagodatskaya, E., Liu, Y., Schaefer, D., Kuzyakov, Y. Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum Nature Scientific Reports DOI: 10.1038/srep19865 2016

Qiao, S., Fang, Y., Wu, A. Dissecting root trait variability in maize genotypes using the semi-hydroponic phenotyping platform Plant and Soil 439(1-2),75-90 2019

Qiu, Q., Wu, L., Ouyang, Z., Li, B., Xu, Y., Wu, S., Gregorich, E.G. Priming effect of maize residue and urea N on soil organic matter changes with time. Applied Soil Ecology 100, 65–74 2016

R core team R: A language and environment for statistical computing. R Foundation for Statistical Computing 2015

R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 2017

Raich, J. W., Russell, A. E., Kitayama, K., Parton, W. J. & Vitousek, P. M. Temperature influences carbon accumulation in moist tropical forests Ecology 87, 76–87 2006

Raich, J.W. & Schlesinger, W.H. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate Tellus 44B,81–99 1992

Rain Bird Cooperation ESP-Me user manual (6904178-03) 2017

Razanamalala, K., Fanomezana, R.A., Razafimbelo, T., Chevallier, T., Trap, J., Blanchart, E., Bernard, L. The priming effect generated by stoichiometric decomposition and nutrient mining in cultivated tropical soils: Actors and drivers Applied Soil Ecology 126: 21-33 2018

Reed, S. C., Vitousek, P. M. & Cleveland, C. C Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. Biogeochemistry 01-14 2011

Reeder, J., Schuman, G. Influence of livestock grazing on C sequestration in semi-arid mixed-grass and short-grass rangelands Environmental Pollution 116(3), 457–463 2002

Rehm, E.M. & Feeley, K.J. Forest patches and the upward migration of timberline in the southern Peruvian Andes Forest Ecology and Management 305,204-211 2013

Rehm, E.M. & Feeley, K.J. Freezing temperatures as a limit to forest recruitment above tropical Andean treelines Ecology 96(7),1856-65 2015a

Rehm, E.M. & Feeley, K.J. The inability of tropical cloud forest species to invade grasslands above treeline during climate change: potential explanations and consequences Ecography 38, 001–009 2015b

Reichstein, M., Falge, E., Baldocchi, D. On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm Global Change Biology 11, 1424–1439 2005

Reiners Complementary models for ecosystems Am.Nat. 127, 59-73 1986

Rillig MC & Field CB Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO2 as a function of soil depth Plant and Soil 254: 383-391 2003

Rinnan, R. & Baath, E. Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil Applied and environmental Microbiology 6,3611-3620 2009

Risch, A.C., Zimmermann, S., Ochoa-Huesco, R., Schütz, M., Firn, J.L., Fay, P.A., Hagedorn, F., Borer, E.T., Seabloom, E.W., Harpole, W.S., Knops, J.M.H., McCulley, R.L., Broadbent, A.A.D., Stevens, C.J., Silveira, M.L., Adler, P.B., Báez, S., Biederman, L.A., Blair, J.M., Brown, C.S., Caldeira, M.C., Collins, S.L., Daleo, P., di Virgilio, A., Ebeling, A., Eisenhauer, N., Esch, E., Eskelinen, A., Haganah, N., Hautier, Y., Kirkman, K.P., MacDougall, A.S., Moore, J.L., Power, S.A., Prober, S.M., Roscher, C., Sankaran, M., Siebert, J., Speziale, K.L., Tognetti, P.M., Virtanen, R., Yahdjian,L., Moser, B. Nature Communications 10.1038/s41467-019-12948-2 2019

Robertson, A.D., Paustian, K., Ogle, S., Wallenstein, M.D., Lugato, E., Cotrufo, M.F. Unifying soil organic matter formation and persistence frameworks: the MEMS model Biogeosciences 16, 1225–1248 2019

Roesti, D; Ineichen, K; Braissant, O; et al. Bacteria associated with spores of the arbuscular mycorrhizal fungi Glomus geosporum and Glomus constrictum Applied and environmental Microbiology 71(11): 6673-6679 2005

Rolando, J.L., Turin, C., Ramírez, D., Mares, V., Monerris, J., Quiroz, R Key ecosystem services and ecological intensification of agriculture in the tropical high-Andean Puna as affected by land-use and climate changes Agriculture, Ecosystems and Environment 236, 221–233 2017

Rothstein, David E. Soil amino-acid availability across a temperate-forest fertility gradient Biogeochemistry Volume: 92 Issue: 3 Pages: 201-215 2009

Rousk, J., Brookes, P.C., Ba°a°th, E. Investigating the mechanisms for the opposing pH-relationships of fungal and bacterial growth in soil Soil Biol Biochem 42, 926–934 2010

Rousk, J., Brookes, P.C., Baath, E., Fungal and bacterial growth responses to N-fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. Microbiology Ecology 76, 89–99. 2011

Rousk, J., Hill, P.W., Jones, D.L. Priming of the decomposition of ageing soil organic matter: concentration dependence and microbial control Functional Ecology 29, 285-296 2015

Rousk, K., Michelsen, A., Rousk, J. Microbial control of soil organic matter mineralization responses to labile carbon in subarctic climate change treatments Change Biology 22, 4150–4161 2016

Rumpel, C., Kögel-Knaber, I. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle Plant Soil 338:143–158 2011

Russell, M.B., Richards, L.A. The determination of soil moisture energy relations by centrifugation Soil Sci. Soc. Am. Proc. Volume 3, Pages 65-69 1938

Rustad, L.E., Campbell, J.L., Marion, G.M. A meta-analysis of the response of soil respiration, net mineralization, and aboveground plant growth to experimental ecosystem warming Oecologia 126, 543–562 2001

Saatchi, S. S., Harris, N. L., Brown, S., Lefsky, M., Mitchard, E. T., Salas, W., Zutta, B. R., Buermann, W., Lewis, S. L. & Hagen, S Benchmark map of forest carbon stocks in tropical regions across three continents. Proceedings of the National Academy of Sciences, 108, 9899-9904. 2011

Sanden, Taru; Zavattaro, Laura; Spiegel, Heide; et al. Out of sight: Profiling soil characteristics, nutrients and bacterial communities affected by organic amendments down to one meter in a long-term maize experiment Applied soil ecology Volume: 134 Pages: 54-63 2019

Schädel, C., Koven, C.D., Lawrence, D.M., Celis, G., Garnello, A.J., Hutchings, J., Mauritz, M., Natali, S.M., Pegoraro, E., Rodenhinzer, H., Salmon, V.G., Taylor, M.A., Webb, E.E., Wieder, W.R., Schuur, E.A.G. Divergent patterns of experimental and model-derived permafrost ecosystem carbon dynamics in response to Arctic warming Environmental Research letters 13, 105002 2018

Schelhas, J. & Greenberg, R. (editors) Forest Patches in Tropical Landscapes Island Island Press 1996

Schimel, J.P. & Weintraub, M.N. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model Soil Biology & Biochemistry 35, 549–563 2003

Schimel J.P., Bilbrough, C., Welker, J.M. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities Soil Biol Biochem 36:217–27 2004

Schimel, J.P. & Bennett, J. Nitrogen mineralisation: Challenges of a changing paradigm Ecology, 85(3), 591-602 2004

Schipper, L.A., Hobbs, J.K., Rutledge, S., Arcus, V.L. Thermodynamic theory explains the temperature optima of soil microbial processes and high Q10 values at low temperatures Global Change Biology 20, 3578-3586 2014

Schlesinger, W.H. & Andrews, J.A. Soil respiration and the global carbon cycle Biogeochemistry 48:7-20 2000

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse,D.P., Weiner, S., Trumbore, S.E. Persistence of soil organic matter as an ecosystem property Nature 478: 49-56 2011

Smith, S. & Read, D. Mycorrhizal symbiosis 2nd edition Academic Press 2002

Schneider T, Keiblinger KM, Schmid E Isme Journal, 6, 1749-1762. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions Isme Journal 6, 1749-1762 2012

Schrumpf, M., Schulze, E.D., Kaiser, K., Schumacher, J. How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories? Biogeoscience 8,1193-1212 2011

Schulp, C.J.E. & Verburg, P.H Effect of land use history and site factors on spatial variation of soil organic carbon across a physiographic region Agricultre, Ecosystems and Environment 133, 86-97 2009

Scott, N.A., and Binkley, D. Foliage litter quality and annual net N mineralization: comparison across North American forest sites Oecologia 111: 151–159 1997

Senapati, N.; Chabbi, A.; Gastal, F.; et al. Net carbon storage measured in a mowed and grazed temperate sown grassland shows potential for carbon sequestration under grazed system Carbon Management Volume: 5 Issue: 2 Pages: 131-144 2014

Shahzad, T., Chenu, C., Repinçay, C., Mougin, C., Ollier, J.-L. & Fontaine, S. Plant clipping decelerates the mineralization of recalcitrant soil organic matter under multiple grassland species Soil Biology and Biochemistry 51, 73-80 2012

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., Fontaine, S., Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species Soil Biology and Biochemistry 80, 146-155 2015

Silva-Sanches,A.,Soares,M.,Rousk,J. Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality 2019

Sinsabaugh, R.L., Gallo,M.E.,Lauber,C.,Waldrop,M.P.,Zak,D.R. Extracellular Enzyme Activities and Soil Organic Matter Dynamics for Northern Hardwood Forests receiving Simulated Nitrogen Deposition Biogeochemistry 75(2),201-215 2005

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling Ecology Letters 16, 930–939. 2013

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems Soil Science Society of America Journal 70:555-569 2006

Sitters, J., Cherif, M., Egelkraut, D., Giesler, R., Olofsson, J. Long-term heavy reindeer grazing promotes plant phosphorus limitation in arctic tundra Funct Ecol. 33, 1233– 1242 2019

Sitton, J.D. & Story, B.A. Estimating Soil Classification Via Quantitative and Qualitative Field Testing for Use in Constructing Compressed Earth Blocks Procedia Engineering 145: 860-867 2016

Sjögersten, S. & Wookey, P. A. The impact of climate change on ecosystem carbon dynamics at the Scandinavian mountain birch forest-tundra heath ecotone AMBIO 38, 2–10 2009

Skidmore, E.L. & Powers, D.H Dry Soil-Aggregate Stablity: Energy-Based Index Soil Science Society of America Jounral 46:6 1982

Soares, M. & Rousk, J. Microbial growth an carbon use effiecency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry Soil Biology and Biogeochemistry 131: 195-205 2019

Sollins, P., Homann, P., Caldwell, B.A. Stabilisation and destabilisation of soil organic matter: Mechanisms and controls Geoderma 74:65-105 1996

Soltner D. Les bases de la production végétale, le sol Collection: Sciences et techniques agricoles Volumen 1, p 67-71 1986

Soong, J.L., Marañon-Jimenez, S., Cotrufo, M.F., Boeckx, P., Bodé, S., Guenet, B., Peñuelas, J., Richter, A., Stahl, C., Verbruggen, E., Janssens, I.A. Soil microbial CNP and respiration responses to organic matter and nutrient additions: Evidence from a tropical soil incubation Soil Biology and Biochemistry 122 (2018) 141–149 2018

Speed, J. D. M., Martinsen,V.,Hester, A.J., Holand, Ø., Mulder, j., Mysterud, A., Austrheim, G. Continuous and discontinuous variation in ecosystem carbon stocks with elevation across a treeline ecotone Biogeosciences 12, 1615–1627 2015

Spohn, M., Klaus, K., Wanek, W., Richter, A. Microbial carbon use efficiency and biomass turnover times depending on soil depth - implications for carbon cycling Soil Biology and Biochemistry 96, 74-81 2016

Sterner, R.W. & Elser, J.J. Ecological Stoichiometry The Biology of Elements from Molecules to the Biosphere Princeton University Press. ISBN 9780691074917 2002

Street, L., Subke, J.A., Baxter, R., Dinsmore, K.J., Knoblauch, C., Wookey, P.A. Ecosystem carbon dynamics differ between tundra shrub types in the western Canadian Arctic Environmental Research Letters 13: 084014 2018

Stock, S.C., Köster, M., Dippold, M.A., Nájera, F., Matus, F., Merino, C., Boy, J., Spielvogel, S., Gorbishina, A., Kuzyakov, Y. Environmental drivers and stoichiometric constraints on enzyme activities in soils from rhizosphere to continental scale Geoderma 337, 973-982 2019

Subke, J.A., Heinemeyer, A., Vallack, H.W., Leronni, V., Baxter, R., Ineson, P. Fast assimilate turnover revealed by in situ $^{13}CO_2$ pulse-labelling in Subarctic tundra Polar Biology 35, 8, 1209-1219 2012

Subke, J.A., Inglima, I., Cotrufo, M.F. Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review Global Change Biology 12, 921–943 2006

Sylvester, S.P., Heitkamp, F., Sylvester, M.D.P.V., Jungkunst, H.F., Sipman, H.J.M., Toivonen,J.M., Gonzales Inca, C.A., Ospina, J.C., Kessler, M. Relict high-Andean ecosystems challenge our concepts of naturalness and human impact Scientific reports 7:3334 DOI:10.1038/s41598-017-03500-7 2017

Talbot, J.M., Allison, S.D., Treseder, K.K. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change Functional Ecology 22, 955-963 2008

Tamm, CO Denitrification in forest ecosystems - preface Biological Bulletin Volume: 181 Issue: 2 Pages: 335-336 1991

Tanner, E.V.J., Vitousek, P.M., Cuevas, E. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains Ecology 79, 10-22 1998

Tape, K., Sturm, M., Racine, C., The evidence for shrub expansion in northern Alaska and the Pan-arctic Global Change Biology 12, 686–702 2006

Thébault, A., Clément, J-C., Ibanez, S., Roy, J., Geremia, R.A., Pérez, C.A., Buttler, A., Estienne, Y., Lavorel, S. Nitrogen limitation and microbial diversity at the treeline Oikos 123:729-740 2014

Todd-Brown, K. E. O. Changes in soil organic carbon storage predicted by Earth system models during the 21st century Biogeosciences 11,2341–2356 2014

Treseder, K.K. & Holden, S.R. Fungal Carbon Sequestration Science 339, 1528 2013

Treonis, AM; Wall, DH Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs

Integrative and comparative Biology Volume: 44 Issue: 6 Pages: 653-653 2004

van de Weg, M.J. Gross primary productivity of a high elevation tropical montane cloud forest Ecosystems 17(5), 751-764 2014

van Der Heijden, M. G., Bardgett, R. D. & Van Straalen, N. M. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology letters, 11, 296-310. 2008

van der Putten,W.H., Macel,M.,Visser,M.E., Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels Royal Society 365(1548),1471-2970 2010

Vance, E., Brookes, P., Jenkinson, D An extraction method for measuring soil microbial biomass C Soil Biology and Biochemistry 19: 703-707 1987

Vásquez, E., Ladd, B., Borchard, N. Carbon storage in a high-altitude Polylepis woodland in the Peruvian Andes. Alp. Botany 124, 71-75 2014

VD LUFA Methodenbuch I, 2. Teillfg. VDLUFA-Verlag, Darmstadt ISBN 978-3-941273-13-9 1997

Vidal, A., Hirte, J., Bender, S.F., Mayer, J., Gattinger, A., Höschen, C., Schädler, S., Iqbal, T.M., Müller, C.W. Linking 3D structure and plant-microbe-soil carbon transfer in the rhizosphere frontiers in Environmental Science doi.org/10.3389/fenvs.2018.00009 2018

von Humboldt, A. Entwurf einer physischen Weltbeschreibung (1860) in Enzensberger, H.M. Die andere Bibliothek Band 17 2004

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. Stabilisation of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions – a review European Journal of Soil Science 57:426-445 2006

Voríšková, J., & Baldrian, P. Fungal community on decomposing leaf litter undergoes rapid successional changes The ISME Journal 7(3), 477–486 2013

Walker, L. R., & del Moral, R. Primary succession and ecosystem rehabilitation Cambridge University Press 2003

Wallander H, Göransson H, Rosengren U. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types Oecologia 139:89–97 2004

Walther, G.R., Beissner, S. & Burga, C.A. Trends in the upward shift of alpine plants Journal of Vegetation Science 16, 541–548 2005

Wang, B., Qiu Y.L. Phylogenetic distribution and evolution of mycorrhizas in land plants Mycorrhiza 16: 299–363 2006

Wang, G., Jia, Y., Wei, L Effects of environmental and biotic factors on carbon isotopic fractionation during decomposition of soil organic matter Sci Rep 5, 11043 2015

Wang, X., Tang, C., Severi, J., Butterly, C. R. & Baldock, J. A. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation New Phytologist. 211, 3: 864-873 2016

Wardle, D.A., Zackrisson, O., Hörnberg, G., Gallet, C. The influence of island area on ecosystem properties Science 277, 1296–1299 1997

Waring, B.G., Averill, C., Hawkes, C.V., Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models Ecology Letters 16, 887–894. 2013

Watson, J.D., Crick, F.H.C. Molecular structure of nucleic acids Nature 171,737-738 1953

Wayolle, A.A.J., Williams, M., Wilson, C., Wookey, P. Multiscale Soil Carbon Distribution in Two Sub-Arctic Landscapes Thesis, University of Sterling 2011

Whitaker, J., Ostle, N., McNamara, N.P., Nottingham, A.T., Stott, A.W., Bardgett, R.D., Salinas, N., Ccahuana, A.J.Q., Meir, P. Microbial carbon mineralization in tropical lowland and montane forest soils of Peru Microbiology 5.72 2014a

Whitaker,J..,Ostle,N.,Nottingham,A.T.,Ccahuana,A.,Salinas,B.,Bardgett,R.D.,Meir,P.,McNamara,N.P. Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient Ecology 102,1058-1071 2014b

White, A., Cannell, M. G. R. & Friend, A. D. CO 2 stabilization, climate change, and the terrestrial carbon sink Global Change Biology 6, 817–833 2000

White, I, Smiles, DE, Perroux, KM Absorption of water by soil - constant flux boundary-condition Soil Science Society of America Journal Volume: 43 Issue: 4 Pages: 659-664 1979

Wieder, W. R., Allison, S. D., Davidson, E. A., Georgiou, K., Hararuk, O.,He, Y., ... Xu, X. Explicitly representing soil microbial processes in Earth system models Global Biogeochemical Cycles 29(10),1782–1800 2015

Wieder, W.R., Sulman, B.N., Hartman, M.D., Koven, C.D., Bradford M.A. Arctic Soil Governs Whether Climate Change Drives Global Losses or

Gains in Soil Carbon Geophysical Research Letters 46 GeophysicalResearch Letters,46. 10.1029/2019GL085543 2019

Wieser, R.; Caciuc, V.; Lazo, C.; et al. A theoretical study of the dynamical switching of a single spin by exchange forces New Journal of Physics Volume: 15 Article Number: 013011 2013

Wild, B., Li, J., Pihlblad, J., Bengtson, P., Rütting, T. Decoupling of priming and microbial N mining during a short-term soil incubation Soil Biology and Biochemistry 129: 71-79 2019

Wild, B., Schnecker, J., Alves, R.J., Barsukov, P., Barta, J., Capek, P., Gentsch, N.,Gittel, A., Guggenberger, G., Lashchinskiy, N., …., Watzka, M., Zrazhevskaya, G., Richter A Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil Soil Biol Biochem 75,143–151 2014

Williams, Alwyn; Borjesson, Gunnar; Hedlund, Katarina The effects of 55 years of different inorganic fertiliser regimes on soil properties and microbial community composition Soil Biology and Biochemistry Volume: 67 Pages: 41-46 2013

Wilmking, M., Harden, J. & Tape, K. Effect of tree line advance on carbon storage in NW Alaska J. Geophys. Res. 111, G02023 2006

Wolf, D. & Lehmann, J. Microbial models with minimal mineral protection can explain long-term soil organic carbon persistence Scientific Reports 9:6522 https://doi.org/10.1038/s41598-019-43026-8 2019

Wood, T.E., Cavaleri, M.A., Reed, S.C. Tropical forest carbon balance in a warmer world: A critical review spanning microbial- to ecosystem-scale processes Biological Reviews 87, 912–927 2012

Wookey P.A. Experimental approaches to predicting the future of tundra plant communities Plant Ecology and Diversity 1(2), 299-307 2008

Wookey,P.A.,Aerts,R.,Bardgett,R.D.,Baptist,F.,Brathen,K.A.,Cornelissen,J.H.,Gough,L.,Hartley,I.P.,Hopkins,D.W.,Lavorel,S.Shaver Ecosystem feedbacks and cascade processes: understanding their role in the responses fo Arctic and alpine ecosystems to environmental change Global Change Biology 15,1153-1172 2009

Xu, X., Thornton, P.E., Post, W.M. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems Global Ecology and Biogeography 22, 737–749 2013

Yamamoto, Y; Chung, JK; Sekino, T; et al. Self-organized regular array microstructure of LiNbO3-based crystal composites Journal of Alloys and Compounds Volume: 385 Issue: 1-2 Pages: 252-256 2004

Yang, Songyu; Cammeraat, Erik; Jansen, Boris; et al. Soil organic carbon stocks controlled by lithology and soil depth in a Peruvian alpine

grassland of the Andes Catena Volume: 171 Pages: 11-21 2018

Yin, L., Dijkstra Feike, A., Wang, P., Zhu, B. & Cheng, W. Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition New Phytologist 218, 1036-1048 2018

Zak, D.R., Groffman, P.M., Pregitzer, K.S., Christensen, S., Tiedje, J.M. The vernal dam: Plant-microbe competition for nitrogen in nothern hardwood forests Ecology 71(2), 651-656 1990

Zelles, L. Phospholipid fatty acid profiles in selected members of soil microbial communities, Chemosphere 35, 275-294 1997

Zelles, L., Palojarvi, A., Kandeler, E., VonLutzow, M., Winter, K., Bai, Q. Y Changes in soil microbial properties and phospholipid fatty acid fractions after chloroform fumigation Soil Biology and Biochemistry 29, 1325-1336 1999

Zhang, Jun; Bruijnzeel, L. Adrian; Marie Quinones, Cecille; et al. Soil physical characteristics of a degraded tropical grassland and a 'reforest': Implications for runoff generation Geoderma Volume: 333 Pages: 163-177 2019

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., Wanek, W. Growth explains microbial carbon use efficiency across soils differing in land use and geology Soil Biology and Biochemistry 128,45-55 2019

Zhu, B., Cheng, W. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition Global Change Biology 2011

Zhu, Z., Ge, T., Luo, Y., Liu, S., Xu, X., Tong, C., Shibistova, O., Guggenberger, G., Wu, J. Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil Soil Biology and Biochemistry 121: 67-76 2018

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient European Journal of Soil Science 60:895–906 2009

Zimmermann, M., Meir, P., Silman, R.M., Fedders, A., Gibbon,A., Malhi, Y., Urrego,D.H., Bush, M. B., Feeley, K.J., Garcia, K.C., Dargie, G.C., Farfan, W.R., Goetz, B.P., Johnson, W.T., Kline, K.M., Modi, A.T., Rurau,N.M.Q., Staudt, B.T., Zamora, F. No Differences in Soil Carbon Stocks Across the Tree Line in the Peruvian Andes Ecosystems 13(1):62-74 2010a

Zimmermann, M., Meir, P., Bird, M.I., Malhi, Y., Ccahuana, A.J.Q., Temporal variation and climate dependence of soil respiration and its components

along a 3000 m altitudinal tropical forest gradient Global Biogeochemical Cycles 24.4012 2010b

Zimmerman, M., Bird, M.I. Temperature sensitivity of tropical forest soil respiration increase along an altitudinal gradient with ongoing decomposition Geoderma 187-188,8-15 2012