ACTIVITY REPORT

MARSHALL PLAN SCHOLARSHIP

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1 INTRODUCTION

Within the scope of my one semester abroad at the Columbia University (New York, USA), I could gather international work experience in the group of Prof. Griffin (Earth Institute) within a common project on the dynamics of forest ecosystems affected by environmental changes. The focus of this project changed from primarily intended study of belowground changes in soil organic matter to the investigation of changes in soil microbial community because this part of the study complement better to the ongoing soil respiration study in the Black Rock Forest. In addition, I got the opportunity to work together with Prof. McGuire (Biological sciences, Bernard College) and benefit a lot from her expertise in microbial ecology. I also was responsible for the set up of the laboratory and the PLFA method. Furthermore, I was involved in dissertation work of Jennifer Levy, who is working on quantification of carbon fluxes in an oak removal experiment by investigation of changes in soil microbial community composition along a soil profile.

Additional, I participated on the lab meeting weekly and could thereby get good inside in the daily routine at one of the leading Universities worldwide.

2 PROJECT OBJECTIVES & MILESTONES

2.1 Objectives

Objective	Status
Investigate changes of soil microbial community as well as bacterial and fungal species diversity as a ecosystem responses to loss of a foundation taxon	Thirteen sites were selected. A variety of chemical, physical and biological parameters were analyzed. Two manuscripts are in preparation.
The general set up of the laboratory and PLFA method	The set up of a soil-microbiological laboratory as well as PLFA method and teaching was performed.
Future research collaboration with the *Earth and Environmental Sciences Department* at the Columbia University	Next meeting has been planed for the beginning of May in Vienna.

2.2 Milestones

Milestone	Anticipated completion (project month)	Status
Sites for soil analysis selected and a research design developed	0.5	completed
Set up of the laboratory and PLFA method	2	completed
Spatial soil variability characterized	1	completed
Data processing and analysis of soil microbial community	2	completed
Publication in scientific journals	2.5	submitted
Work presentation at the Institute of Soil Science, Vienna on April 26, 2010		completed

3 PROJECT SUMMARY

A large portion of the forests of eastern North America are dominated by just a few species of oaks (genus *Quercus*) for the past ten millennia (Maenza-Gmelch 1997, Foster et al. 2002). Foundation or dominant species play critical roles in ecosystem structure and function (Ellison et at., 2005), but environmental change can lead to the loss of such species. Invasive plant pathogens can eliminate dominant host species that play key roles in forest ecosystem structure and function (Thrall and Burdon, 1999). Chestnut blight (*Cryphonectria parasitica*) and Dutch elm disease (*Ophiostoma ulmi*) in North America and Eucalyptus (jarrah) dieback (*Phytophthora cinnamomi*) in Western Australia are well-known examples where major taxon losses have caused cascading ecological impacts (Anagnostakis, 1987; Brasier, 2001; Weste and Marks, 1987). Loss of key taxa in communities can drastically impact biogeochemistry, ecosystem processes, the provision of ecosystem services, and even human health (Paine, 1996; Groom and Schumaker, 1993; Lerdau at al., 1997; Chivian, 2001; Orwig, 2002). In many cases, such as in the loss of American chestnut (*Castanea dentate* L.) and American elm (*Ulmus americana* L.), such events have not been accompanied by a rigorous before-and-after analysis that would enable full quantification of impacts and investigation of causal mechanisms. Understanding long-term implications of such losses requires monitoring the impacts of such changes in dominant species as they occur, ideally also using appropriate data-based models. But the rapidity of catastrophic losses of dominant species often impairs our ability to initiate studies in advance of the cascade of changes that follow (Gilbert, 2002; MCPherson, et al., 2002; Rizzo and Garbelotto, 2003). *Quercus* species are the most widespread and important hardwood trees in northern temperate forests (Pavlik et al., 1991). In the widespread, oak-dominated portion of the North American eastern deciduous forest, several species of oaks (e.g. *Quercus*

rubra, *Q. alba*, *Q. prinus*, *Q. velutina*) have dominated forest community composition for the past ten millennia (Webb, 1988; Maenza-Gmelch, 1997; Foster et al., 2002). Oak forest provide several important ecosystem services including basic support (oxygen, soil formations, nutrient cycling), provisioning (timber, fiber and pulp, water, food, fuel), regulating (air purification, preventing flooding and erosion, carbon storage) and cultural (recreation, spiritual). Eastern North American oak forests in particular yield benefits such as water and watershed protection, grazing, recreation, and wood products (Kliejunas, 2003; Logan, 2005), are known for their scenic beauty, contribute to tourism and high property values, and are valued for shelter and food for wildlife. The future of oak forest is uncertain. Due to a suite of factors including human-mediated changes in disturbance regimes (esp. fire) and trophic structures (predator removal resulting in excessive herbivory), oak trees have not been regenerating in the majority of these forests (Nowacki et al., 1990; Abrams, 1992; Loftis and McGee, 1992). The remaining oak canopy trees are aging, and factors such as insect herbivory, pathogen introduction, storm damage, extractive logging and fires pose significant threats. Additional oak canopy tree mortality will almost certainly result from increasing levels of extractive logging, since the commercial value of oak forests increases dramatically above a threshold (roughly 40 cm dbh/ 60 years age; Johnson, 2992) that has now been realized in many areas. Of the many “pests” that now threaten oak forests, a previously unknown pathogen which causes “sudden oak death” (SOD), may be the most likely factor to cause massive future oak mortality in eastern North American forests. SOD is caused by a brown alga, *Phytophthora ramorum* (Phylum Oomycota) related to the pathogen that caused the Irish potato famine (Rizzo et al., 2002). Large groups of oaks can die together within periods as short as a few weeks. The disease affects dozens of plant species, but its effects are particularly pronounced in oaks, often resulting in large

stands with dead canopies and many downed trees. *P. ramorum* is currently confined to coastal locations in California and Oregon, but has been dispersed via shipments of wood products, ornamental, and nursery stock. APHIS has already discovered it once (in 2004) in a park in New York State and three times in residential areas in Georgia and South Carolina, with a total of 176 confirmed positive sites in 21 states, including in many tree nurseries in the eastern U.S, (Cave et al., 2005; APHIS 2005a,b). It may be already present but undetected in eastern forests: the pathogen was probably introduced at least five years before it was first detected in the western U.S. (Rizzo and Gerbelotto, 2003). In California it is continuing to spread and is considered beyond the possibility of eradication. Much of the eastern US from eastern Texas through southern New England is considered to be at moderate to high risk for the disease (Magarey et al., 2005). Modeling results indicate that much of the eastern deciduous forest in highly susceptible to the spread of SOD due to appropriate climate, oak prevalence (eastern red and white oaks have both shown susceptibility in greenhouse trials; Brasier et al., 2002; Tooley and Kyde, 2003), and the presence of understory shrubs which typically harbour non-lethal leaf and steam infections that sustain SOD infestations (Garbelotto et al., 2003; Meentemeyer, 2004; Cave et al., 2005; Magarey et al., 2004, 2005). *P. ramorum's f*requently lethality, broad host range, and ability to disperse spores via water and air suggest it is capable of causing long-term landscape-level changes (Apigian and Allen-Diaz, 2005; Apigian et al., 2005). SOD represents a potential economic threat to commercial timber production in the U.S. exceeding $ 30 billion (Kliejunas, 2003). The export value of *Q. rubra* logs and lumber alone was over $ 300 million dollars in 2002 (USITC, 2005). Other oak mortality-inducing factors are also known to be increasing. On a series of undisturbed long-term plots in the Blac Rock Forest in south-eastern New York State, mortality of canopy oaks has averaged 4% per year

over the last five years compared to annual mortality of only 1 % between 1930 and 2000 (Schuster et al., 2005). Advancing age of the canopy trees and a series of severe droughts in the late 1990s is thought to be key factors. In other parts of the same forest insect outbreaks, fires, and storm damage have resulted in even higher canopy mortality levels. Increased canopy tree mortality has also been reported in other regional oak forests due to bacterial leaf scorch (Lashomb et al., 2003). This insect-borne disease is caused by a bacterium, *Xylella fastidiosa*, which blocks trees' abilities to draw water from the ground. It was formerly limited to southern and central regions of New Jersey, but is now found as far north as Sussex County, on the New York border. Oak leaf wilt, caused by the fungus *Ceratocystis fagacearum*, represents another serious and expanding cause of rapid, widespread oak mortality (Appel and Billings, 1995).

Large- scale manipulation provide powerful tools to develop and test our understanding, and in this study we manipulate more than 100 oaks trees in replicated forest plots to examine key ecosystem impacts the sudden loss of oaks. This will advance our knowledge of these important ecosystems in advance of the massive changes they are likely to experience in the face of the many existing threats. Important changes may result in productivity, carbon storage and cycling, water chemistry and cycling, microbial diversity, the abundance of invasive plant species, interaction with deer herbivory, and the response of ticks and insect communities. Such study will help us to predict future sates of the eastern deciduous forest, impacts on various taxa, ecosystem services such as water quality and quantity, wood production, and identity drivers behind the likely changes. The results may be scaled up to model behaviour of lager systems, such as the entire oak-dominated portion of eastern North American forests.

The main objectives of this project are to assess impacts of foundation taxon loss on 1) ecosystem productivity and carbon storage, 2) nutrient cycling and water quantity and quality, and 3) biological diversity. The study should help predict future states of the eastern deciduous forest, impacts on ecosystem services, and aims to identity key drivers behind the changes. This study will therefore examine, in advance of the loss of foundation forest species, the mechanisms that may lead to a cascade of impacts on short- and long-term ecosystem structure and function.

4 STUDY AREA

This project is being carried out at the Black Rock Forest located in the Hudson Highlands New York-New Jersey Highlands Province, USA (Figs.1, 2). The Black Rock Forest covers an area of 15.3 km^2. The experimental plots are located on the north slope of Black Rock Mountain at the different slope positions (low, intermediate, and high).

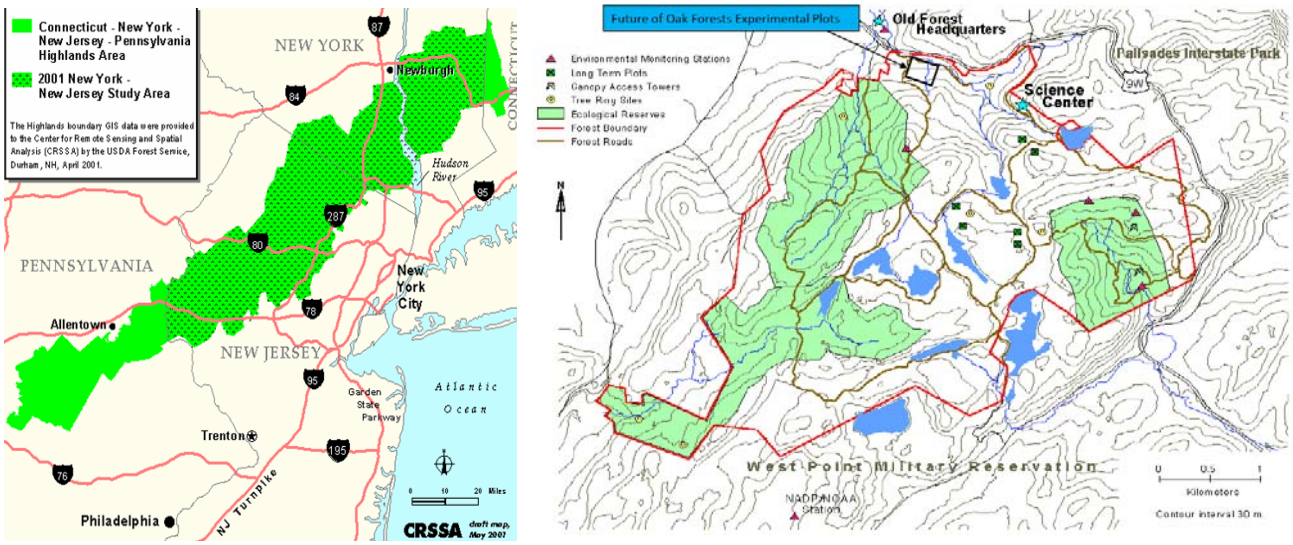


Fig.1: Location of the study area in the Hudson Highlands. Fig. 2: Location of the north slope of Black Rock Forest

4.1 Climate

Mean annual precipitation is 1190 mm and air temperature is strongly seasonal with monthly means ranging from –2.7º C in January to 23.4º C in July (Ross 1958, Turnbull *et al*. 2001, Engel *et al*. 2002).

4.2 Soil

Soils are all classified as Hollis soils (IUSS Working Group WRB, 2006) and are formed on granite gneiss bedrock or glacial till parent material (Olsson 1981). The forest soils are medium-textured loams and typically shallow in depth (0.25 - 1.0 m). The soils are acidic (pH 3.65-4.55), nutrient availability is low and the site index ranges from poor to average (Lorimer, 1981).

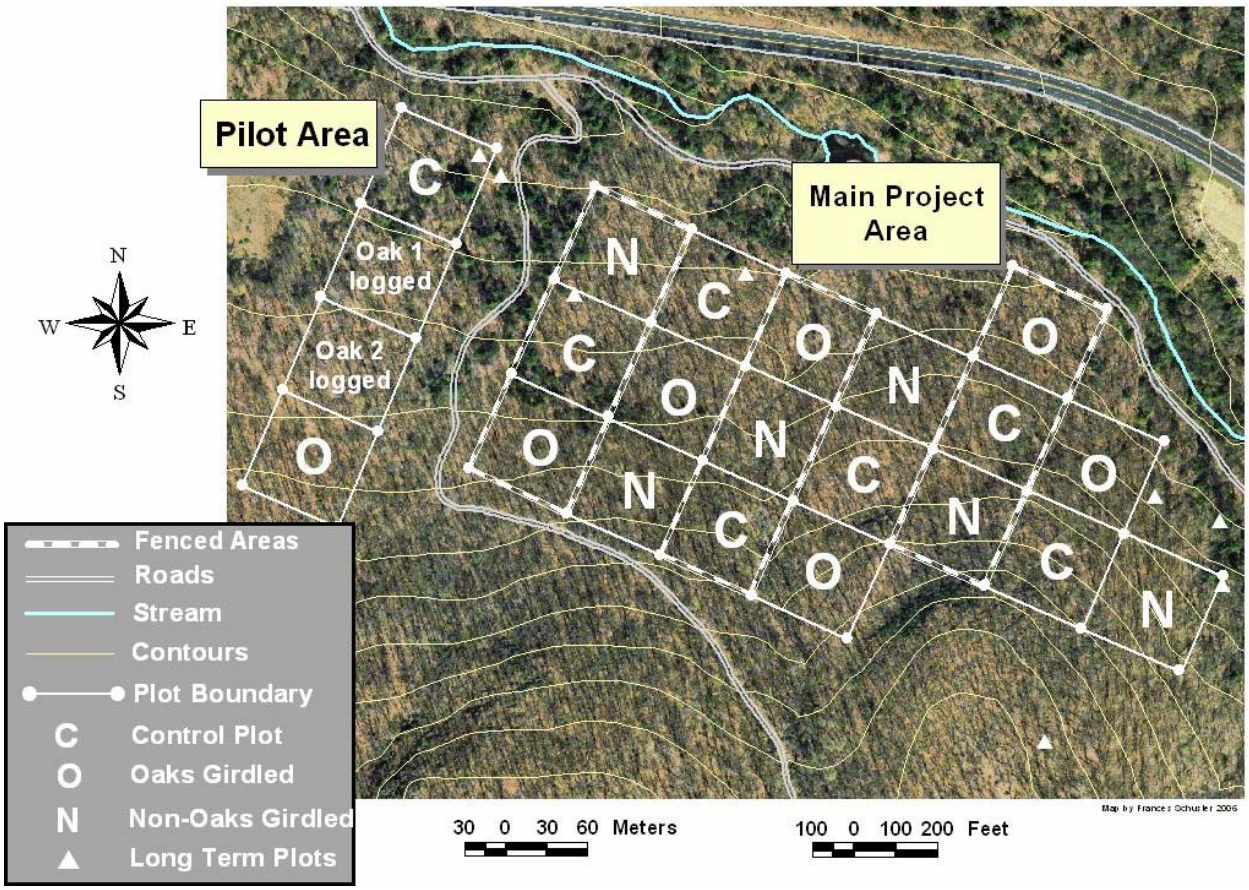
4.3 Vegetation

The canopy made up of 23 tree species, dominated by red oak (*Quercus rubra*), chestnut oak (*Quercus prinus*) - accounting for 60% of the forest's basal area (Friday and Friday 1985), and black oak (*Quercus velutina*), followed by red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), and black birch (*Betula lenta*). These are 100-120 years old oaks, typical for forests in the surrounding of the New York-New Jersey Highlands Province. Sub canopy trees were dominated by red maple (*Acer rubrum*), followed by black birch (*Betula lenta*), sugar maple (*Acer saccharum*), beech (*Fagus grandifolia*), and black gum (*Nyssa sylvatica*). The under story includes many plants in the Rhododendron family (*e.g., Kalmia, Vaccinium* spp.).

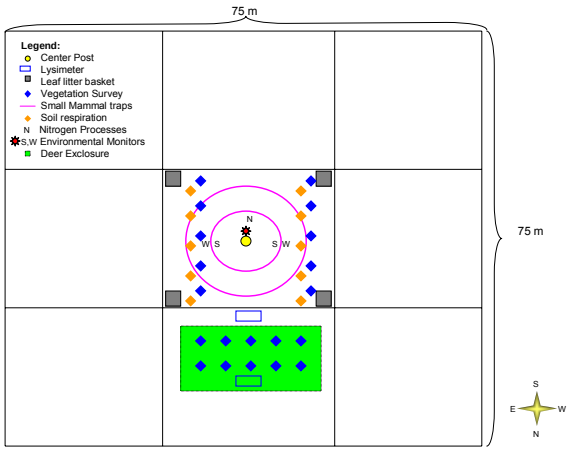
The area was clear cut in the late 1800s and since has been managed as one unit, planned for eventual timber harvest (Tryon 1939). Thinning operations that yielded firewood were made in 1932-1933 and again around 1960.

4.4 Experimental Layout and Treatments

Tree-girdling treatments intended to mimic pathogen-induced mortality were instituted in a randomized block design on twelve 0.56 hectare plots on the north slope of Black Rock Mountain (Fig. 3). One treatment involved chainsaw girdling of all oak trees (O). Girdling all mature oak will mimic pathogen/pest impacts on mature trees. A second treatment (O 50) was similar girdling of half of the oak trees. A third treatment (N) was girdling of all non-oak trees to evaluate the specific importance of the genus *Quercus* compared to loss of other forest species. A fourth set of plots (C) were left unmanipulated to represent controls. Each treatment is replicated three times. One additional treatment (ALL) was instituted where all trees were girdled, regardless of species. All the plots were girdled in 2006 and the investigation will be continued until 2013.



The plots were arranged as shown in Figure 4. Primary measurements were made on 25 X 25 meter subplots located in the center of each plot to minimize edge effects (Fig. 4). This design results in eight exterior subplots surrounding each central subplot, available for additional measurements and purposeful study of edge/neighbour-plot effects.



The characteristics of the individual study sites are described on the following pages:

Side C1 (Oaks girdled)

Altitude	165 m asl
Slope	21.7 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear slope Horizon plot size: 0.5299 ha. Position: upper back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 30.8cm, the litter layer is 5.4cm
Biotope	Oak-Maple Forest
Tree layer	*Quercus rubus* (73%), *Acer saccharinum* (17%), *Acer rubrum* (3%)
Shrub layer	scattered
Herb layer	non
Treatment	Oaks girdled

Vegetation	*Pignut hickory*
Quercus rubus	*Betula lenta*
White Oak	*Christmas Fern*
Quercus prinus	*Vaccinium angustifolium*
Acer saccharinum	*Viburnum acerfolium*
Acer rubrum	*Hamamelis virginiana*

Side C2 (Control Plot)

Altitude	165 m asl
Slope	28.5 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear slope Horizon plot size: 0.5632 ha. Position: upper back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 30.8cm, the litter layer is 5.4cm
Biotope	Oak-Maple-Birch Forest
Tree layer	*Quercus rubus* (70%), *Acer saccharinum* (6%), *Acer rubrum* (6%), *Betula lenta* (6%)
Shrub layer	scattered
Herb layer	non
Treatment	Control plot

Vegetation	*Acer rubrum*	*Rosa multiflora*
Quercus rubus	*Betula lenta*	*Marianthemun canadense*
White Oak	*Christmas Fern*	
Quercus prinus	*Aster divericatus*	
Black Oak	*Vaccinium angustifolium*	
Acer saccharinum	*Hamamelis viginiana*	

Side C3 (50% of Oaks girdled)

Altitude	165 m asl
Slope	27 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear slope Horizon plot size: 0.5567 ha. Position: upper back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 30.8cm, the litter layer is 5.4cm
Biotope	Oak-Maple Forest
Tree layer	*Quercus rubus* (70%), *Quercus prinus* (8%), *Acer rubrum* (7%), *Acer saccharinum* (6%)
Shrub layer	scattered
Herb layer	non
Treatment	50% Oak girdled

Vegetation	*Acer rubrum*
Quercus rubus	*Betula lenta*
Black Gum	*Christmas Fern*
Quercus prinus	*Aster divericatus*
Black Oak	*Vaccinium angustifolium*
Acer saccharinum	*Hamamelis virginiana*

Side C4 (Non-Oaks girdled)

Altitude	165 m asl
Slope	24.7 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear slope Horizon plot size: 0.5608 ha. Position: upper back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 30.8cm, the litter layer is 5.4cm
Biotope	Oak-Maple Forest
Tree layer	*Quercus rubus* (43%), *Quercus prinus* (36%), *Acer rubrum* (16%)
Shrub layer	scattered
Herb layer	non
Treatment	Non Oak girdled

Vegetation	*Chestnut*	*Vaccinium pallidum*
Qercus rubus	*Betula lenta*	
Black Gum	*American Beech*	
Quercus prinus	*Vaccinium angustifolium*	
Swamp White Oak	*Hamamelis virginiana*	
Acer rubrum	*Vaccinium corymbosum*	

Side B1 (50% of Oaks girdled)

Altitude	150 m asl
Slope	20 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear slope Horizon plot size: 0.5727 ha. Position: middle back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 16.5cm, the litter layer is 8.5cm
Biotope	Marple-Oak-Black gum Forest
Tree layer	*Acer saccharinum* (44%), *Quercus rubus* (16%), *Black gum* (13%), *Acer rubrum* (10%)
Shrub layer	scattered
Herb layer	non
Treatment	50% Oak girdled

Vegetation	*Acer rubrum*	*Yellow Birch*
Quercus rubus	*Tsuga canadensis*	*Tulip poplar*
White Oak	*Pignut hickory*	*Christmas Fern*
Quercus prinus	*Fraxinus americana*	*Aster divericatus*
Black Gum	*Vaccinium pallidum*	*Vaccinium angustifolium*
Acer saccharinum	*Dogwood*	*Hamamelis virginiana*

Side B2 (Oak girdled)

Altitude	150 m asl
Slope	12.3 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5442 ha. Position: middle back slope
Soil	Parent material: granite. Soil type: Hollis soils Average soil depth is 16.5 cm, the litter layer is 8.5 cm (the slope is separated in two parts; the left one has a good developed organic layer and the right one is wet and has a marginal organic layer
Biotope	Maple-Black gum-Birch Forest
Tree layer	*Black gum* (26%), *Acer rubrum* (18%), *Betula lenta* (15%), *Acer saccharinum* (14%)
Shrub layer	scattered
Herb layer	Sparsely, mainly covered with litter
Treatment	Oak girdled

Vegetation	*Acer saccharinum*	*Betula lenta*
Quercus rubus	*Acer rubrum*	*White Spruce*
White Oak	*Black Gum*	*Christmas Fern*
Quercus prinus	*Vaccinium pallidum*	*Aster divericatus*
Swamp White Oak	*Fraxinus americana*	*Hamamelis virginiana*
Yellow Birch	*Liriodendron tulipfera*	*Rhus radicans*
Fraxinus americana	*Obolaria virginica*	*Pilea pumila*

Side B3 (Non-Oaks girdled)

Altitude	150 m asl
Slope	16.7%
Apect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5686 ha. Position: middle back slope
Soil	Parent material: granite. Soil type: Hollis soils Average soil depth is 16.5cm, the litter layer is 8.5cm
Biotope	Oak-Maple Forest
Tree layer	*Quercus rubus* (37%), *Quercus prinus* (26%), *Acer saccharinum* (14%), *Acer rubrum* (12%)
Shrub layer	scattered
Herb layer	non
Treatment	Non Oak girdled

Vegetation	*Chestnut*	*Vaccinium angustifolium*
Quercus rubus	*Betula lenta*	*Hamamelis virginiana*
White Oak	*Acer saccharinum*	
Quercus prinus	*Pignut hickory*	
Acer rubrum	*American Beech*	
Black Gum	*Christmas Fern*	

Side B4 (Control Plot)

Altitude	150 m asl
Slope	11.7 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5666 ha. Position: middle back slope
Soil	Parent material: granite. Soil type: Hollis soils Average soil depth is 16.5cm, the litter layer is 8.5cm
Biotope	Oak-Maple-Birch Forest
Tree layer	*Quercus prinus* (40%), *Quercus rubus* (39%), *Acer rubrum* (11%), *Betula lenta* (8%)
Shrub layer	scattered
Herb layer	non
Treatment	Control plot

Vegetation	*Acer pensylvanicum*
Quercus rubus	*Aster divericatus*
White Oak	*Vaccinium angustifolium*
Quercus prinus	*Viburnum acerfolium*
Betula lenta	
Acer rubrum	

Side A1 (Non-Oak girdled)

Altitude	135 m asl
Slope	13.5 %
Apect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear with outcrop of bedrock Horizon plot size: 0.5752 ha. Position: low back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is <10 cm, the litter layer marginal
Biotope	Maple-Black gum-Oak Forest
Tree layer	*Acer saccharinum* (27%), *Black gum* (26%), *Acer rubrum* (11%), Quercus rubus (9%)
Shrub layer	scattered
Herb layer	Sparsely, mainly covered with litter
Treatment	Non Oak girdled

Vegetation	*Betula lenta*	*Iron Wood*
Quercus rubus	*Black Gum*	*Pignut hickory*
White Oak	*Basswood*	*Tsuga canadensis*
Quercus prinus	*Acer rubrum*	*Liriodendron tulipfera*
Vaccinium pallidum	*Pilea pumila*	Christmas Fern
Acer saccharinum	*Sassafrass*	Aster divericatus
Vaccinium angustifolium	*Fraxinus americana*	*Solidago sp.*
Rosa multiflora	*Galium boreale*	*Trillium undulatum*

Side A2 (50% Oaks girdled)

Altitude	135 m asl
Slope	11 %
Aspect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5554 ha. Position: low back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 27cm, the litter layer is 15.4 cm
Biotope	Maple-Birch-Oak Forest
Tree layer	*Betula lenta* (23%), *Acer rubrum* (22%), *Black gum* (19%), *Acer saccharinum* (15%), *Quercus rubus* (9%)
Shrub layer	non
Herb layer	non
Treatment	50% Oak girdled

Vegetation	*Acer saccharinum*	*American Beech*
Quercus rubus	*Betula lenta*	*IronWood*
White Oak	*Sassafrass*	*Liriodendron tulipfera*
Quercus prinus	*Dogwood*	*Chestnut*
Black Gum	*Pignut hickory*	
Acer rubrum	*Tsuga canadensis*	

Side A3 (Control Plot)

Altitude	135 m asl
Slope	16 %
Apect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5554 ha. Position: low back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 27cm, the litter layer is 15.4 cm
Biotope	Oak-Beech Forest
Tree layer	*Quercus prinus* (37%), *Quercus rubus* (29%), *White oak* (14%), *American beech* (10%)
Shrub layer	non
Herb layer	non
Treatment	Control plot

Vegetation	*American Beech*
Quercus rubus	*Betula lenta*
White Oak	*Black Gum*
Quercus prinus	*Hamamelis virginiana*
Black Oak	
Acer saccharinum	

Side A4 (Oak girdled)

Altitude	135 m asl
Slope	24.3 %
Apect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5475 ha. Position: middle back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 27cm, the litter layer is 15.4 cm
Biotope	Oak-Maple-Black gum Forest
Tree layer	*Quercus rubus* (34%), *Quercus prinus* (32%), *Black gum* (13%), *Acer rubrum* (11%)
Shrub layer	scattered
Herb layer	Sparsely, mainly covered with litter
Treatment	Oak girdled

Vegetation	*Acer rubrum*	*Vaccinium pallidum*
Quercus rubus	*Black Gum*	*Rhus radicans*
White Oak	*Acer saccharinum*	*Fraxinus americana*
Quercus prinus	*Pignut hickory*	*Pilea pumila*
Betula lenta	*Christmas Fern*	*Oxalis sp.*
Acer pensylvanicum	*Aster divericatus*	*Acalypha chumboidea*
Prenanthes alba	*Indian fabucus*	*Parth quinquet*

Side A5 all girdled

Altitude	135 m asl
Slope	12 %
Apect	N
Position	N:41°25’ 09“ / E: 74°01’32“ (UTM 83)
Topography	Shape of the slope: linear Horizon plot size: 0.5437 ha. Position: low back slope
Soil	Parent material: granite. Soil type: Hollis soil Average soil depth is 32cm, the litter layer is 6cm
Biotope	Oak-Maple-Birch-Black gum Forest
Tree layer	*Quercus rubus* (47%), *Acer rubrum* (17%), *Betula lenta* (15%), *Black gum* (13%), *Quercus prinus* (8%)
Shrub layer	non
Herb layer	Sparsely, mainly covered with litter
Treatment	All tree girdled

Vegetation	*Black Gum*	*Lysimachia quadrifolia*
Quercus rubus	*Liriodendron tulipfera*	
Quercus prinus	*Acer saccharinum*	
Betula lenta	*Christmas Fern*	
Acer rubrum	*Aster divericatus*	
Pignut hickory	*Hamamelis virginiana*	

5 SAMPLING AND ANALYSIS OF SOIL AND LITTER

5.1 Soil and litter sampling

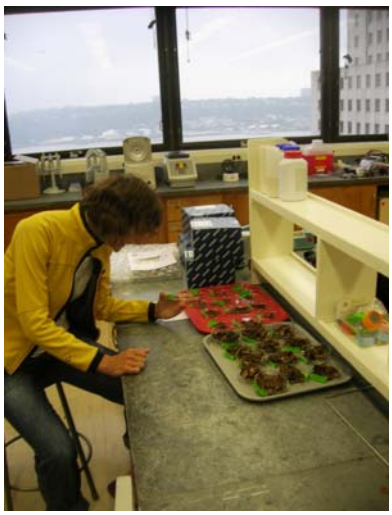
Ten replicate soil cores (2.5 cm x 0-10cm soil depth) were taken from each of the thirteen sites in August 2009. The samples for microbial analyses were transported in a cooling box, sieved (2 mm) and frozen until analysed. The remaining samples were air dried and sieved (2 mm).

5.2 Bulk soil and litter analyses

- Soil pH was measured in H_2O at a soil:solution ratio of 1:10 (*Soil Survey Staff*, 2004)
- Electrical conductivity was measured in distilled water at a 1:10 soil:solution ratio after shaking and filtration (Blum et al., 1996).
- The total carbon (C) and nitrogen (N) contents were determined by dry combustion (*Tabatabai and Bremner*, 1991).

6 LABORATORY AND METHOD SET UP

The challenge was to set up the laboratory with storage racks, ordering of lab equipment (e.g. sonicator, desiccators, pipettes, gas tanks etc.) and chemicals (after the price comparison) as well as the instruction of students in diverse analytical methods (e.g. hydrometer, pH, electrical conductivity). Furthermore, set up of the Phospho Lipid Fatty Acid (PLFA) method as well as knowledge transfer among the colleagues and draw up of the protocol for the simple use in the future.



6.1 Microbiological analyses

The soils' microbial community composition was assessed by analysing the phospholipid fatty acid (PLFA) composition. PLFAs were extracted according to the procedure of Bligh and Dyer (1959) as described by Frostegård et al. (1991). One of the main tasks was to prepare a protocol for the PLFA analysis for the independent future work of the laboratory technician. The PLFA analysis will be described on the following pages:

1) Needed Materials and Chemicals:

• SPE Silica columns: Isolute SI 3ml =	• Acetic acid p.a
• Isooctane	• KOH pellets
• Methanol	• Citric acid monohydrate
• Toluene	• Potassium citrate monohydrate
• N-Hexane	• Nonadecanoic Acid Methyl ester
• Acetone	• Tridecanoic Acid Methyl ester
• Chloroform stabilized with ethanol 99%	• Bacterial Acid Methyl Ester CP Mix

2) Equipment:

Vortex, “Vortex 2 genie”: The adjustment for the whole sample preparation: 7

Centrifuge: “TJ: 6 Table Top Centrifuges” (Rotor TH-4; radius min=86mm, Radius max=226mm: max. rotor speed = 2700rpm). The adjustment for the whole sample preparation: 2140 rpm ≈800 rcf.

N2 tank: adjust at 25 psi (The gas should be ruffling the surface of the liquid, but not very vigorously). You will need app. 3 “lines “on regulator per extraction. Notes: N2 tanks run dry with alarming frequency. Always have a back-up tank available, and if you are going to run out, be sure to store the samples under N2 gas prior to the tank running dry.

Evaporator concentrator: Company: Cole-Parmer

Gas chromatograph; Column: Agilent ULTRA2 Column (50mx0.20mmx0.33µm)

3) Preparation of the solutions:

General:

• Do not pipette chemicals direct from the big flask.

• Rinse each bottle with the chemical that should be stored in before

• The Cardinal Sins: Water, Heat, Light, Oxygen. Any of these will destroy your fatty acids. Avoid at all costs! Also, use only glass, Teflon or metal. Avoid plastics that are soluble in chloroform.

a) 0.2M Potassium hydroxide methanol solution:

Weigh 0.28g of KOH pellets in a 25ml volumetric flask and fill it with Methanol. Label it! Prepare every day before the analysis.

b) 1M Acetic acid:

In a 100 ml bottle add 50ml Millipore H2O and 5.8 ml of Acetic acid. After the dissolution, fill the bottle with H2O until the 100ml line. Label it! Store it in the refrigerator.

c) Citrate buffer:

Add 1.78g of Citric acid monohydrate and 2.11g of Potassium citrate monohydrate in a 100ml Erlenmeyer flask and fill it with Millipore H2O until the 100ml line. Close it with a plug and label it. Use always the same Erlenmeyer flask, funnel etc. for preparation of buffer. Prepare every day before the analysis.

d) Bligh and Dyer solution =chloroform: methanol: citrate buffer 1:2:0.8 (v: v: v)

Add 100ml Chloroform, 200ml Methanol and 80ml of Citrate buffer in a 500ml closable bottle, wrap it in an Aluminum foil, label it and store it in the refrigerator. Prepare once a week!

e) Methanol:Toluene solution 1:1 (v:v)

Add 100ml of Methanol and 100ml of Toluene in a 200ml closable bottle, label it and store it under the fume hood.

f) n-hexane: Chloroform solution 4:1 (v:v)

Add e.g. 50ml n-Hexane and 12.5 Chloroform in a 50 ml closable bottle, label it and store it under the fume hood.

g) Internal standard (Quantitative standard):

Add 20mg of 19:0 (Nonadecanoic acid methyl ester) and 10mg of 13:0 (Tridecanoic acid methyl ester)in a 1000ml Erlenmeyer flask and fill it with Methanol:Toluene solution 1:1 (v:v) until the 1000ml line, wrap it in an Aluminum foil, label it and store it in the refrigerator. You should use for the entire sample the same standard (be sure to prepare rather more than less). The exact weighted sample (4 decimals) is very important!

h) External standard (Qualitative standard) = Bacterial Acid Methyl Esters (BAC MIX)

Add 2µl Bacterial Acid Methyl Esters into 200µl Isooctane GC vials with 350µl insert close it and measure it. Prepare on the day of analysis. It is enough to measure it ones per "sample group measurement" (not per measurement), because it is very expensive. Close the standard and store it in the freezer. Use for taking the standard a Hamilton syringe. Clean the syringe after the use with Methanol.

4) Safety regulations:

Basic rules:

All works with organic solution may only be carried out under a switched on and functioning fume hood. Moreover, the protective gloves and glasses are to wear. Important: during the samples concentration using N2 gases the fume hood should be closed and in the meantime not used.

Collection of laboratory waste:

The chlorinated and non chlorinated hydrocarbons must be separate collected and adequate disposed. In no case drained the organic solution directly into the sink!

Specifications for handling with the hazardous chemicals:

Extract from "Merck and Fisher Material safety data sheet". See also printed sheets!

- Chloroform: If inhaled or swallowed causes respiratory tract, eye and skin irritation. This product contains a chemical known to the State of California to cause cancer. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- Acetone: This product irritates the skin, eyes, mucous membranes and tissues of the upper respiratory tract. It degreases the skin. Inhalation and ingestion may cause dizziness, weakness, fatigue, nausea, headache, vomiting or diarrhea.

- Methanol: Toxic by inhalation. Irritating to respiratory system. Irritating to eyes. Very toxic if swallowed. Toxic in contact with skin. Irritating to skin. Do not breathe vapor or mist. Do not ingest. Do not get in eyes or on skin or clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- n-Hexane: Toxic by inhalation. Irritating to respiratory system. Irritating to eyes. Toxic if swallowed. Aspi-ration hazard if swallowed. Can enter lungs and cause damage. Irritating to skin. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- Toluene: Toxic by inhalation. Irritating to respiratory system. Irritating to eyes. Very toxic if swallowed. Aspiration hazard if swallowed. Can enter lungs and cause damage. Toxic in contact with skin. Irritating to skin. Do not breathe vapor or mist. Do not ingest. Do not get in eyes or on skin or clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- Isooctane: Causes eye, skin, and respiratory tract irritation. Vapors may cause drowsiness and dizziness. Aspiration hazard if swallowed - can enter lungs and

cause damage. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

- Acetic Acid: Toxic by inhalation. Irritating to respiratory system. Corrosive to eyes. Causes burns. Very toxic if swallowed. May cause burns to mouth, throat and stomach. Corrosive to the skin. Causes burns. Do not breathe vapor or mist. Do not ingest. Do not get in eyes or on skin or clothing. Avoid contact with skin and clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- Citric Acid Monohydrate: Irritating to respiratory system. Severely irritating to eyes. Risk of serious damage to eyes. Ingestion may cause gastrointestinal irritation and diarrhea. Irritating to skin. Do not get in-eyes or on skin or clothing. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.

- Potassium citrate monohydrate: Dust may cause mechanical irritation. May cause mild skin irritation. In-gestion of large amounts may cause gastrointestinal irritation. Inhalation of dust may cause respiratory tract irritation. Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid breathing dust.

- Potassium hydroxide pellets: Hygroscopic (absorbs moisture from the air). Causes severe eye and skin burns. Causes severe digestive and respiratory tract burns. Harmful if inhaled or swallowed. Target Or-gans: Respiratory system, eyes, skin. Do not breathe dust, mist, or vapor. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Do not ingest or inhale. Use only in a chemical fume hood. Discard contaminated shoes.

- Bacterial acid methyl ester: If swallowed, wash out mouth with water provided person is conscious. If in-haled, remove to fresh air. If not breathing give artificial

respiration. In case of contact, immediately wash skin with soap and copious amounts of water. In case of contact, immediately flush eyes with copious amounts of water for at least 15 minutes.

5) PLFA Extraction:

- Sieved the soil with 2mm sieve and stored until the use at -20° C
- Freeze-dried soil samples (app. 6g). soil can be stored indefinitely freeze – dried, in a freezer
- If you work with the leafs grind it before
- Weigh into Teflon tubes

Extraction tubes and caps (36 (12mL) and 24 (4-6mL) vials) should be soaked in 10% HCL for 24h, ultrasonically washed then washed with phosphate free detergent (e.g. 7X Cleaning Solution), rinsed 8 times with tap water, 3 times with deionized water, ones with methanol. Wash the glassware with phosphate free detergent (e.g. 7X Cleaning Solution), rinsed 8 times with tap water, 3 times with deionized water.

First day:

• Label the extraction tubes (9 samples and 1 blank)

• Weigh 2g freeze-dried soil (accurate on 4 decimals) in the extraction tubes (12mL). The amount of soil used can vary. 2-8 g is good for mineral soil. Use less for highly organic soil.

wet soil (g)	dried soil (g)	Water content wet soil (%)	weighted sample (g)	(g) H2O in weighted sample	(%)Citratbuffer in B&D	(ml) Citratbuffer in 8 ml B&D	(ml) Cittratbuffer-amount	B&D (ml)	C:M (ml)
5.13	3.24	36.84	2	0.74	21.05	1.68	0.95	4.50	3.50

• Add 8mL B&D, a mixture of chloroform: methanol: citrate buffer *(1:2:0.8 volume bases). Do al-ways “pre-pipette” when you work with organic solution; otherwise you will lose solution – solution drip! If material is not freeze-dried prior, then you must adjust sample to a total water volume of the P-buffer. *The amount of citrate buffer in the B&D depends on the water content of the soil. We are working with the freeze dried soils were the water content ≈ zero. This means the amount of the buffer is the same for all samples.

• Cap the extraction tubes. Use for this step of extraction, where you have soil in the tubes, always the same caps!!

• Ad Blank: add 8 ml B&D + 2ml B&D instead of soil

• Vortex for at 7 for 20 sec

• Left overnight to separate (try to keep the same time length). Cover the tubes with e.g. a box – should be kept in the darkness. Treat the blank in the same way.

Second day:

• Take the needed chemicals from the fridge

• Turn the sample concentrator on

• Vortex for at 7 for 20 sec

• Centrifuge for 10 min at 2140 rpm (≈800 x g). Equalize weight of tubes! Handle the tubes careful – the supernatant should not be mixed while taking out from the centrifuge!!

• Transfer the supernatant to new 12ml labeled extraction tubes e.g. using the Pasteur pipette.

• Add into the tubes with supernatant: 1.5 ml citrate buffer and 1.5 ml chloroform

• Vortex for at 7 for 20 sec

• Centrifuge for 10 min at 2140 rpm (≈800 x g). Equalize weight of tubes! Handle the tubes careful – the supernatant should not be mixed while taking out from the centrifuge!!

• Transfer chloroform-supernatant (below part) to the 6ml tubes- use the long Pasteur pipette. Write down the exactly amount (~2x1.5ml) of the removed supernatant (you need it later for the calculation- The amount of removing supernatant depends on the added amount of chloroform). You should always leave a safety amount of supernatant of about 1mm to avoid mix of solution and soil. In the case you get the soil particle into the supernatant; centrifuge it again.

• Dispose the residual substance into the halogenated solution waste container.

• Dried the extract under N2 at 40 ° C. Avoid touching the needles, but if happened clean it with acetone. The needles should also not touched the solution surface (should be approximately 1 cm above the surface); please pay attention when wind the concentrator down into the tubes. N2 flow should be chosen only as much as you can see a slight movement at the surface of the solution. Do not turn the gas flow to much because the pressure can press out the solution and you lose lot of “expensive” gas.

• In the meantime clean the tubes with the residua with warm water and soak in beaker with 10% HCL and afterwards clean it with sonicator and washed with phosphate free detergent.

• When the samples are concentrated, at this stage you can stop extraction and store the sample at -20 ° C for 3 weeks! In this case, let the tubes cool off; fill the tubes with N2 gases- take silicon tubing with a pipette tip (hold the tubes with the bottom upturned – because N2 is lighter than air) and screw down the tube.

• Turn off the gas

Lipid fractionation:

• Place the 3ml Silica SPE columns in the Polyethylene sheet. Place under each column a collecting vessel.

• Conditioning of the Silica Columns: Add 2x 2.5ml Chloroform into the columns. (From this point the columns should not dry out. Be attentive, because the solution in some columns have different percolation rate).

• Transfer the concentrated extract using 500ul = 0.5ml of Chloroform into the center of silica columns (avoid to pipette extract on the wall of column) after the fluid levels in the columns have disappeared.

Elution of neutral lipids:

• When the fluid levels in the columns have disappeared, add 6x 2.5 ml Chloroform into the sil-ica columns. The first 2.5 ml of Chloroform add into the vials of concentrated samples, to get quite sure the entire sample out, and transfer into the columns.

• Change the collecting vessel! (Empty “halogenated solution” in adequate waste container!)

Elution of glycol lipids:

• When the fluid levels in the columns have disappeared, add 20ml Acetone into the silica columns. Use for this the 20ml syringes with SPE adapter!

• Change the collecting vessel! (Empty “organic solution” in adequate waste container!)

NOTE: If desired, you can also collect the other fractions. The Neutral fraction (first one) contains sterols used for traditional fungal biomass estimates. The Glycolipid

fraction has PHAs in it, for analyzing microbial carbon storage compounds. See White and Ringelberg, 1998 reference for more detail.

Collect phospholipids:

• Place under each column a collecting vessel with a labeled 12ml extraction tubes.

• When the fluid levels in the columns have disappeared, add 4x 2.5 ml of Methanol into the silica columns.

• Dry the collected extract under N2 at 40 ° C. (It takes around 1 and halve hour).

• Turn off the gas

• Soak the accumulated tubes into the beaker with the 10% HCl too and afterwards clean it with sonicator and wash with phosphate free detergent.

• Prepare the 0.2M Potassium hydroxide methanol solution.

Methylation:

• Add 1ml of internal standard (19:0 and 13:0) to the concentrated extract.

• Swing round

• Add 1 ml of 0.2M potassium hydroxide methanol solution. Chemical background: The fatty acids react with methanol molecule by exchanging the glycerin molecule of the fatty acids against me-thanol molecule of the solution (esterification). As an end-product we get a glycerin molecule and a fatty acids methyl ester (FAME) molecule.

• Cap the extraction tubes with the new caps!

• Vortex for at 7 for 20 sec.

• Heat at 40°C for 20 min; without N_2.

FAME extraction:

• Add 2ml Hexane to each sample (to extract the FAME)

• Add 0.3ml of acetic acid (for neutralization)

• Add 2 ml distilled H2O (for phase separation)

• Vortex for at 7 for 20 sec.

• Centrifuge for 10 min at 2140 rpm (≈800 x g). Equalize weight of tubes! Handle the tubes careful – the supernatant should not be mixed while taking out from the centrifuge!!

• Label 10 (6 ml) vials

• Transfer 2 ml of supernatant (upper part) into the 6ml tubes. Use for this small Pasteur pipettes. You should always leave a safety amount of supernatant of about 1mm to avoid mix of solution and soil.

• Add 2ml Hexane to the vials with the sample

• Vortex for at 7 for 20 sec.

• Centrifuge for 10 min at 2140 rpm (≈800 x g). Equalize weight of tubes! Handle the tubes careful – the supernatant should not be mixed while taking out from the centrifuge!!

• Transfer 2 ml of supernatant (upper part) into the 6ml tubes. Use for this small Pasteur pipettes. You should always leave a safety amount of supernatant of about 1mm to avoid mix of solution and soil.

• Place the 6 ml Vials with the supernatant into the concentrator and dry under N2 at 40 ° C.

• Label the GC vials with 350ul Inserts

• Dissolve the samples in Hexane 200µl Isooctane and transfer into the GC vials.

• Close the GC vials using a piece of Aluminum foil and cap

• Prepare 2 GC vials with Isooctane for GC

• Turn off the sample concentrator, N2 gas

• Store the chemicals e.g. Internal Standard, Acidic Acid, B&D (chloroform: methanol: citrate buffer) in the fridge. Empty the collected solution waste; keep in mind to separate halogenated and organic solution waste!!

• Empty the stored solution waste into the big waste container.

• Empty the pipette tips in appropriate waste.

6. Measurement:

• Turn on the Computer

• Turn on the gases: He, H2 and Synthetic Air.

You should always check the gas availability early enough before the measurement start. (E.g. one week before)!

• Turn on the GC

• Ignite the FID detector: The Sig.1 should be around 3

• If the GC has not be used for longer time, measure always at first only Isooctane, and or extend the "bake out phase" in the GC, in order to remove possibly accumulated dirt.

• Place also two vials with Isooctane for cleaning the GC needle into the GC.

At the computer:

• Open ChemStation online program. You should be in the Method Sequence part; if not choose it.

• Type the Sequence: Sequence - New Sequence. Sequence - Sequence Table. Under Vial type e.g.1-10. Under Sample name: Start always with Isooctane and end with Blank. Choose the Method. Injection Volume: the first should be 2 all other 1. ISTD amount: 20

• Sequence – Save Sequence as-Sequence – Parameters – Prefix – Subdirectory

• Ok

• Place the vials into the auto sampler in the same order as you typed the sequence!!!

• Run control – Run sequence

• Stay by the GC for the first injection, sometimes there are some alert.

• To measure 10 samples takes around 15 hour (It depends of the column length).

• If the measurement is not immediate possible, please store the FAME extract at -20°C. Before you put the extract into GC, if you have stored it usually the fatty acids moved to the bottom of the vials, bring the fatty acids into solution again e.g. palpitate on the vials wall but do not shake. It should not touch the lid of the vials!!!

• If you have to measure lot of sample at once be aware that one run takes around 1 hour. One way to protect the evaporation of sample is, to put a small piece of aluminum foils on the top of vials before you close the vials.

• After the measurement, please replace the perforated cap through new one and store the extract again at -20°C (can be stored around a half year). Be award that the total PLFA concentration decreases between one year by more than 30% (Wu et al., 2009).

7. Data evaluation: (one possibility; you can also work with the “batch”)

• Open the ChemStation “offline modus”

• Go to the View “Data and Analyses” in the “Select signal task modus”

• Open one chromatogram using the icon “Load signal and spectra of a Data file”

• Open the BacMix chromatogram too using the icon “Load Signal of data file and overlay with current signal”

• Open the Blank chromatogram too using the icon “Load Signal of data file and overlay with cur-rent signal”

• All 3 chromatogram should be visible and not superposed. If anyhow, separate it using the icon “Display separate signal”

• Delete given the time reference by using the icon “Delete time reference points from chromatogram”

• Set new start points for 13:0 and 19:0 peaks for all 3 chromatograms using icon “Set time reference points to chromatogram”. Zoom in to see good the onset point of those two peaks. And set the line in front of the onset point of the peaks.

• To adjust the chromatograms, press the icon “Align the time axis of the signal”

• Put the chromatograms on the top of each other using the icon “Display overlay of signal”

• Go to the integration modus

• Integrate every single peak e.g. using a tangential baseline from the onset to the offset of each peak. You can integrate it parallel to the baseline too. The way that you have chosen is not important; important is to stay in one direction!!!!! Use the “Zoom In or out” icon to see the on/offset peak points.

• For manual integration press the icon “Manual integration, draw Peak baseline and integrate

• To split the peaks press the icon “split manually integrated peak

• To remove the not relevant peaks press the icon “Remove integrated peak from the integration results”

• Press the icon “Display separate signal” to get separated chromatogram again.

• Select again the signal task modus

• Delete the BacMix and Blank chromatogram using the icon “Delete object from chromatogram display”

• Press “report” icon and print report

• You cannot save the made integration. But if you work with the “Batch” you can save it!!

• Ad Batch: In order to load chromatogram press Batch – Load Beach- choose the sequence- load all-OK. Integration goes similar as described above. To save the Integration chooses: Batch-Save Batch as (give different name then the sequence to can distinguish between the integrated and untreated data). To finish press: Batch – Exit Batch

• Copy the Data about the peak area in a new Excel sheet

• Subtract the area amount of Blank peaks from the corresponding Sample peaks apart from the peaks 13:0 and 19:0 (do not subtract blank here!)

• Now, your data are ready for calculation.

8. Calculation of PLFA

Chloroform (CHCl3) correction factor [ml]: Added amount of chloroform to the sample /Removed amount of chloroform from the sample

Sum of nmol/g: Sum of all peaks but 13:0 and 19:0

Dry weight [g/g]: Weight of wet soil/ weight of dried soil

Amount of internal standard in µg: Amount of GC injection is 1.2 ml. The concentration of standard is 20mg/L. That is; 20*1.2/1000 ≈0.020mg ≈2.0µg

Concentration of each peak in [µg/g]: ((the area of the peak*internal standard amount/area of the internal standard)/sample weight*CHCl3 correction factor*dry weight)

Concentration of each peak in [nmol/g]: (calculated amount of µg/g of the peak divided by the amount of mol)*1000

Concentration of each peak in [mol%]: (calculated amount of nmol/g *100)/sum of nmol/g

Ratio of 13:0 to 19:0 should be the same for all samples. If not repeat the extraction!

General: Check the results for outlier before calculate mean values and make statistic analyses!

Molecular weight of the fatty acids:

e.g. molecular weight of PLFA 13:0 with the molecular formula: $C_{14}H_{28}O_2$: 12*14+1*28+16*2=228 g/mol

PLFAs	M[g/mol]	PLFAs	M[g/mol]
13:0	228.38	i-17:0	284.48
2-OH 12:0	214.35	a-17:0	284.48
3-OH 12:0	214.35	17:1 w8	284.48
i-14:0	242.39	cy17:0	268.43
14:0	242.39	17:0	284.47
i-15:0	256.43	2-OH 16:0	310.00
a-15:0	256.43	10Me 17:0	298.50
15:0	256.43	18:2 w6,9	294.47
2-OH 14:0	282.00	18:1 w9c	296.49
3-OH 14:0	282.00	18:1 w8	296.49
i-16:0	270.45	18:1 w7c/9t	296.49
16:1 w7c	268.43	18:1 w5	296.49
16:1 w6c/7t	268.43	18:0	298.50
16:1 w5c	268.43	10Me 18:0	298.50
16:0	270.45	cy19:0	296.48
i-17:1 w8c	282.46	19:0	310.51
10Me 16:0	284.48	20:0	326.55

PLFA nomenclature:

Fatty acid nomenclature is in the form of A:BωC, where ‘A’ is the number of C atoms in the chain, ‘B’ is the number of double bonds, and ‘C’ is the position of the double bond from the methyl end of the mole-cule; cis geometry is indicated by the suffix ‘c’. The prefixes ‘i’, ‘a’, and ‘me’ refer to iso, anteiso, and midchain methyl branching, respectively, with ‘cy’ indicating a cyclopropyl ring structure (Frostegård et al., 1993). Be aware that also other type of nomenclature can be used e.g. without “ω”. I.e.

18:2(9, 12) ≈18:2 ω 6, 9 (subtract from the number of C atoms (here 18) the amount in the bracket (here 9 and 12) to get "ω" value.

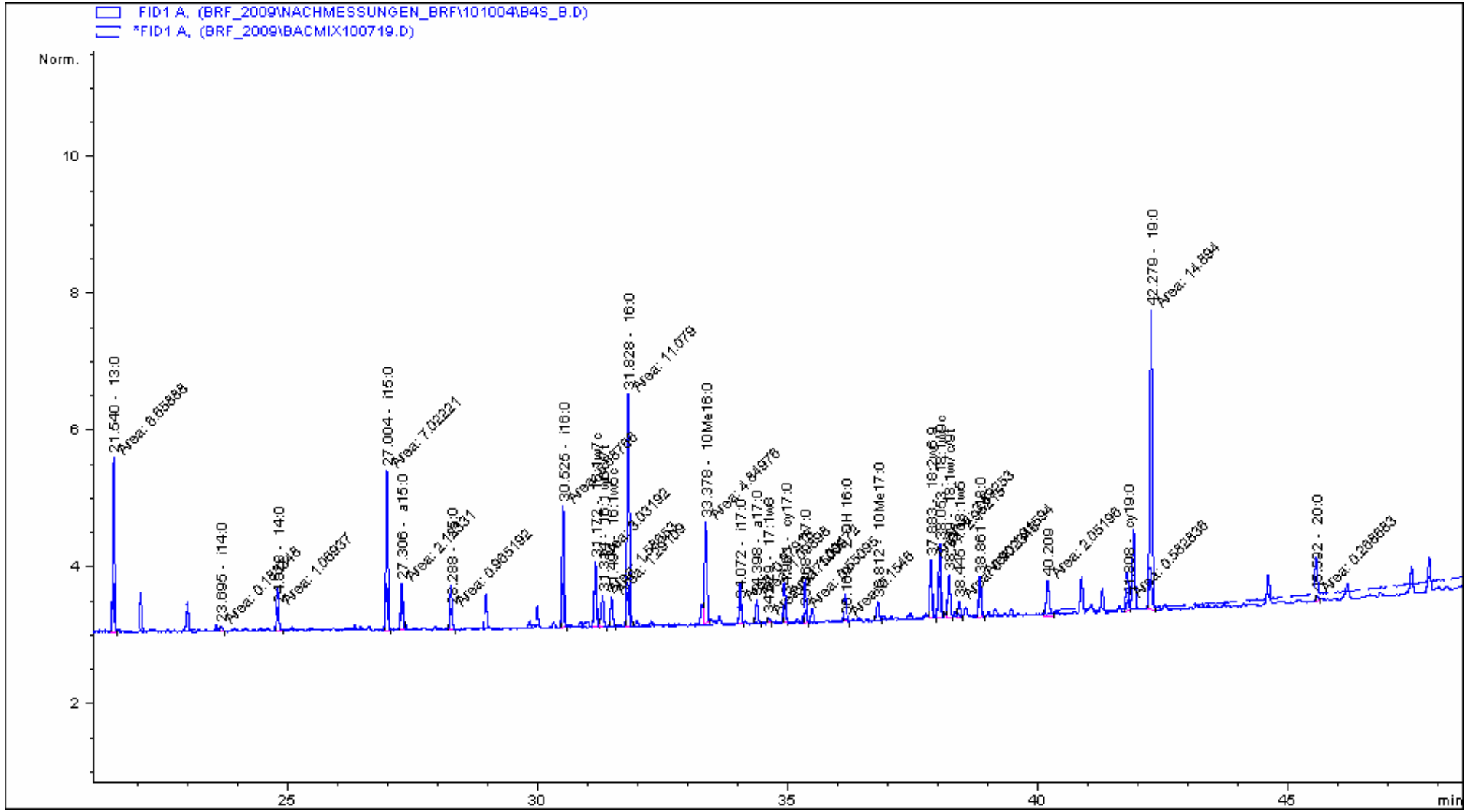
PLFA retention times:

PLFAs	Retention time	PLFAs	Retention time
i14:0	24.20	**17:0**	36.63
14:0	25.50	**10Me17:0**	38.15
i15:0	27.69	**18:2w6,9;**	39.30
a15:0	28.11	**18:1w9c**	39.38
15:0	29.11	**18:1w7c/9t**	39.67
i16:0	31.50	**18:1w5**	40.03
16:1w7c	32.20	**18:0**	40.32
16:1w7t	32.34	**10Me18:0**	41.88
16:1w5c	32.53	**cy19:0**	43.47
16:0	32.88	**19:1**	43.49
i17:0	35.25	**19:0**	43.96
a17:0	35.60	**20:0**	47.47
17:1w8	35.96	**22:0**	54.12
cy17:0	36.21		

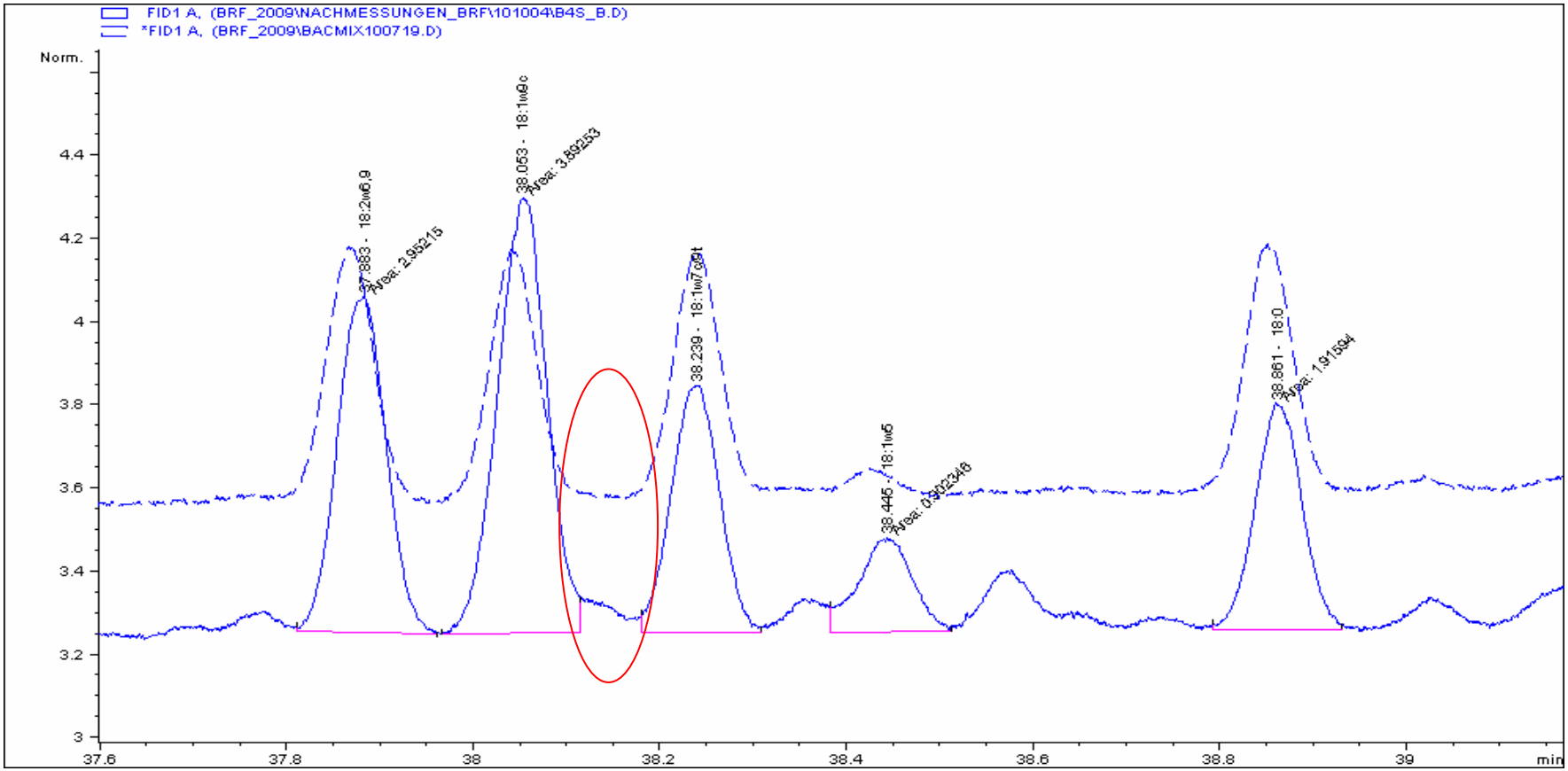
9. PLFA assignment:

PLFA biomarkers	PLFA group	Specific PLFA markers	Reference
Bacteria	Multiple groups	Sum of i14:0, i15:0, a15:0. i16:0. i17:0, a17:0, cy17:0, cy19:0, 16:1w7c, 18:1w7c, 14:0, 15:0, 17:0	Frostegård and Bååth 1996
Gram-positive bacteria	Branched PLFAs	Sum of i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	Kaur et al., 2005
Gram-negative bacteria	Cyclopropyl, monounsaturated and straight chain PLFAs	Sum of cy17:0, cy19:0, 16:1w7c, 18:1w7c, 14:0, 15:0, 17:0	Kourtev et al., 2002
Actinomycetes	10Me-PLFAs	Sum of 10Me16:0, 10Me 17:0, 10Me 18:0	Kroppenstedt R, 1985
Fungi	Polyunsaturated PLFAs	18:2w6,9, 18:1w9c	Federle et al., 2003, Frostegård and Bååth 1996, Olsson, 1999
AM Fungi	Monosaturated PLFAs	16:1w5c	Olsson, 1999
Fungi: Bacteriaratio	Multiple groups	Fungi/Bacteria	Federle et al., 2003, Frostegård and Bååth 1996
Bacteria: Actinomycetes	Multiple groups	Bacteria/Actinomycetes	
Gram neg:Gram pos bacteria	Multiple groups	Gram neg/Gram pos	
Total PLFA	Multiple groups	Total amount of extracted microbial PLFAs	

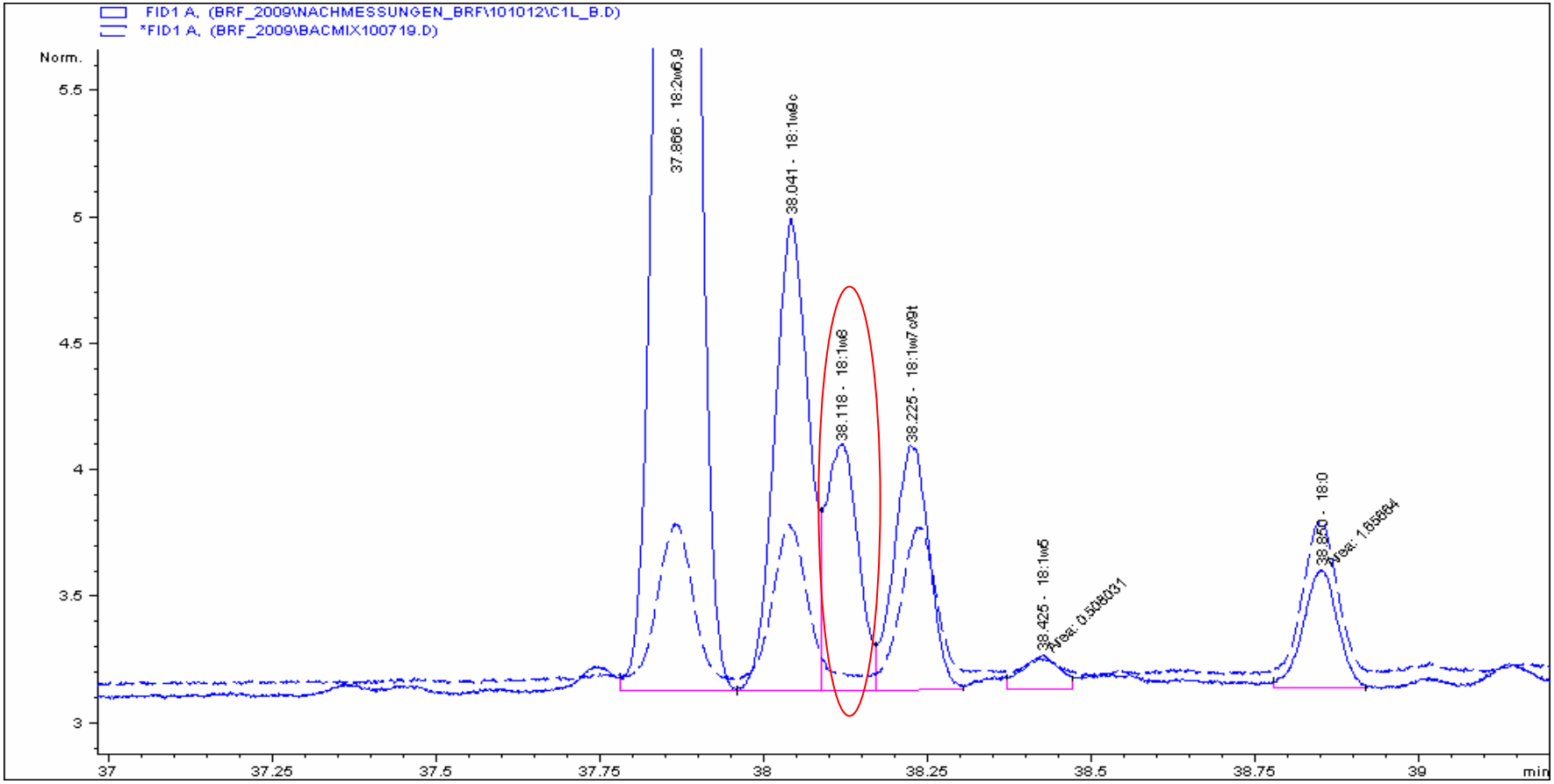
One example for the chromatogram of a soil sample- B4S.



One of the noticeable differences between the soil and litter chromatogram: Solid line represents the sample and the dashed line an internal standard. Remarkable is the absence of the fatty acid, 18:1ω8, in the soil sample (red circle)!



Litter sample. Presence of the fatty acid, 18:1ω8. Solid line represents the sample and the dashed line an internal standard.



7 RESULTS AND DISCUSSION

7.1 pH & Electric Conductivity (EC)

In most investigated soils, the pH was around 4 and in litter around 5 (Tab. 1). The soil electric conductivity (EC) varied in a narrow range but was lowest at the site with all girdled trees and highest in the litter, respectively (Tab. 1).

Tab.1: Basic characteristics of the studied soils and litter: mean values ± standard deviation. n=3. ALL = all tree girdled; O = Oaks girdled; O50 = 50% Oaks girdled; N = Non Oaks girdled; C = Control Plot

Treatment (soil)	Soil depth	pH (H_2O)	EC (µS/cm)
ALL	0-10	4.91	112.50
O	0-10	4.70 ± 0.5	143.4 ± 30.2
O50	0-10	4.38 ± 0.1	152.4 ± 24.4
N	0-10	4.42 ± 0.5	161.5 ± 42
C	0-10	4.20 ± 0.4	169.7 ± 77.8

Treatment (litter)	pH (H_2O)	EC (µS/cm)
ALL	5.24	502
O	5.32± 0.3	366.2±189.8
O50	5.43±0.3	310.7±95.5
N	5.13±0.1	435.33±74.8
C	5.31±0.1	298.7±75.1

Tab.2: Soil texture of the studied soils (n=3). ALL = all tree girdled; O = Oaks girdled; O50 = 50% Oaks girdled; N = Non Oaks girdled; C = Control Plot

Sites	Treatment	Soil texture
A4	O	silt loam
B2	O	silt loam
C1	O	silt loam
A2	O50	loamy sand
B1	O50	sandy loam
C3	O50	sandy loam
A1	N	sandy loam
B3	N	silt loam
C4	N	loam
A3	C	loamy sand
B4	C	sandy loam
C2	C	silt loam
All	ALL	loam

Soil texture is the tool used to describe the grains and mineral particle sizes in sediment. Particles are classified by the fractions (sand, silt, and clay) present in a

soil. Hollis soils formed on granite gneiss bedrock or glacial till parent material are characterized by manly sandy loam texture.

7.2 Organic C, total N, nutrients, PLFA

Tab.3: Carbon and nitrogen content of the studied soils (n=3). ALL = all tree girdled; O = Oaks girdled; O50 = 50% Oaks girdled; N = Non Oaks girdled; C = Control Plot

Plot	Treatment	N (%)	C(%)
A4_S	O	0.81	16.79
B2_S	O	0.61	10.41
C1_S	O	0.51	8.57
A2_S	O50	0.67	12.42
B1_S	O50	0.64	11.64
C3_S	O50	0.67	12.3
A1_S	N	0.42	6.55
B3_S	N	0.92	21.35
C4_S	N	0.98	21
A3_S	C	1.25	28.23
B4_S	C	0.85	19.58
C2_S	C	0.61	10.44
All_S	ALL	0.3	5.04

The highest organic carbon (OC) content was observed on the control site (19.4 % ± 8.9) and lowest on the site where all trees has been girdled (5%). Among the plots with different girdled trees the highest OC content was observed at the sites where non-oaks trees have been girdled (16.3% ± 8.45) followed by 50% oak girdled plots (12.12 % ± 0.42) and all oak girdled plots (11.9% ± 4.3). The C:N ratios ranged from 18 to 21, with the lowest ratios at the site with all girdled tree .

Tab.4: Nutrient content of the studied soils (n=3). ALL = all tree girdled; O = Oaks girdled; O50 = 50% Oaks girdled; N = Non Oaks girdled; C = Control Plot

Plot	Treatment	Ca	K	Mg	P	Al	B	Cd	Cr	Cu	Fe	Mn	Na
		%				ppm							
A4_S	O	4.72	4.02	0.87	2	2560	58	0.5	10	175	1777	723	16064
B2_S	O	2.27	2.73	0.55	1.12	1674	38	<0.1	6	139	1259	474	11084
C1_S	O	3.82	3.19	0.81	1.85	4470	51	0.3	13	135	2072	643	13180
A2_S	O50	3.21	3.03	0.72	1.59	5924	48	1	12	84	3455	803	13061
B1_S	O50	3.53	3.41	0.76	1.71	5016	53	<0.1	10	97	2717	623	15620
C3_S	O50	2.97	3.56	0.76	1.65	4088	54	2	10	99	2029	628	15391
A1_S	N	5.34	3.97	0.85	1.85	2013	57	1	9	223	1524	652	15818
B3_S	N	2.71	2.69	0.62	1.33	1475	40	<0.1	6	88	1142	517	10918
C4_S	N	3.11	3.06	0.74	1.6	7956	49	1	13	134	4439	708	13649
A3_S	C	3.21	3.44	0.73	1.58	2329	50	<0.1	7	140	1791	615	15739
B4_S	C	2.02	2.3	0.47	0.98	1595	34	<0.1	4	61	1139	450	9607
C2_S	C	4.15	3.79	0.85	1.91	2828	59	<0.1	10	129	1860	696	15353
All_S	ALL	<0.1	<0.1	<0.1	<0.1	37	2	<0.1	<0.1	10	28	5	184

The nutrient amount showed no constant trends in relation to plot treatments.

Fig. 5: Relative abundance of bacterial, actinomycetal and fungal phospholipid fatty acids (PLFAs) in soil (0-10 cm depth) within different tree treatments. Values are arithmetic means ± standard deviation (n = 3). AM – arbuscular mycorrhiza.

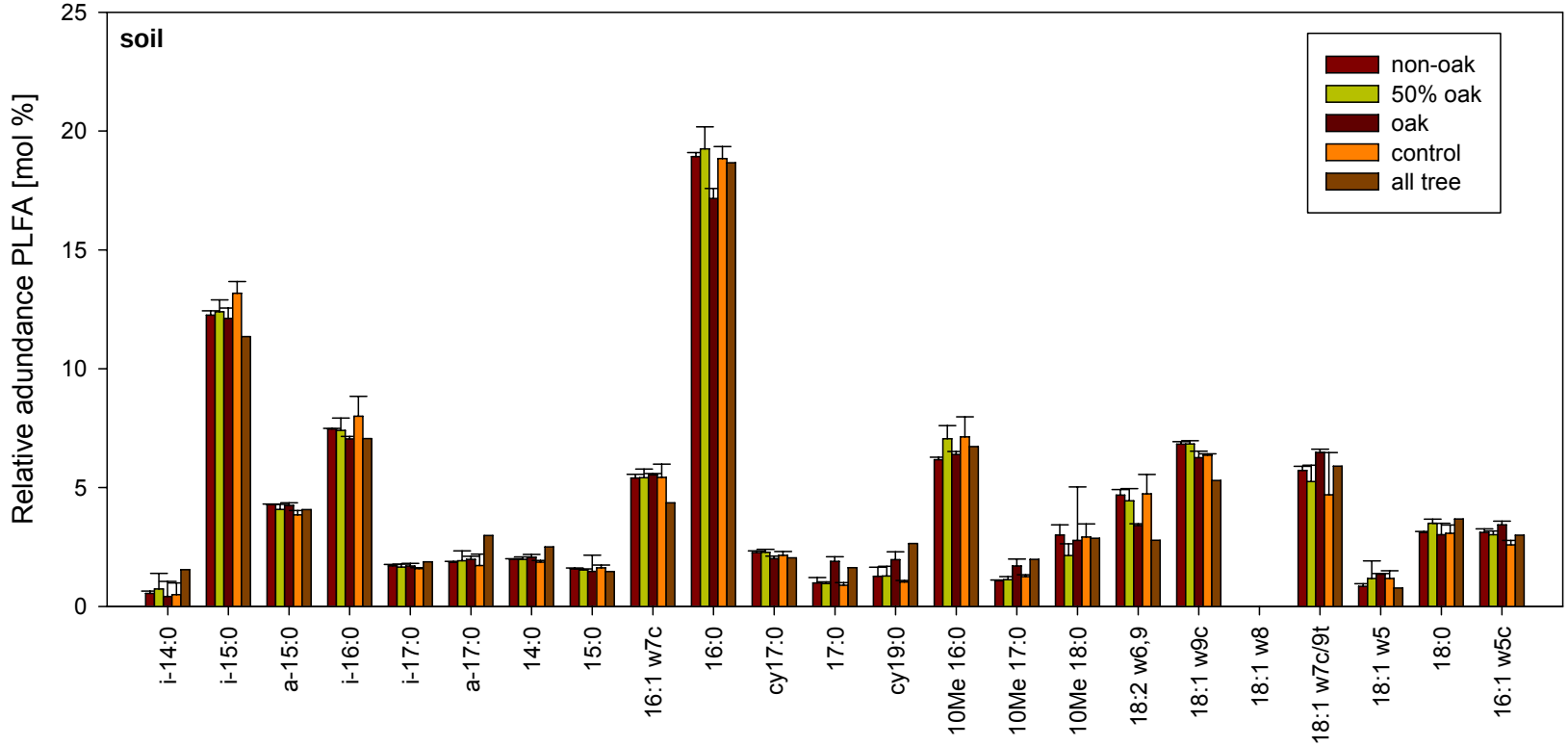
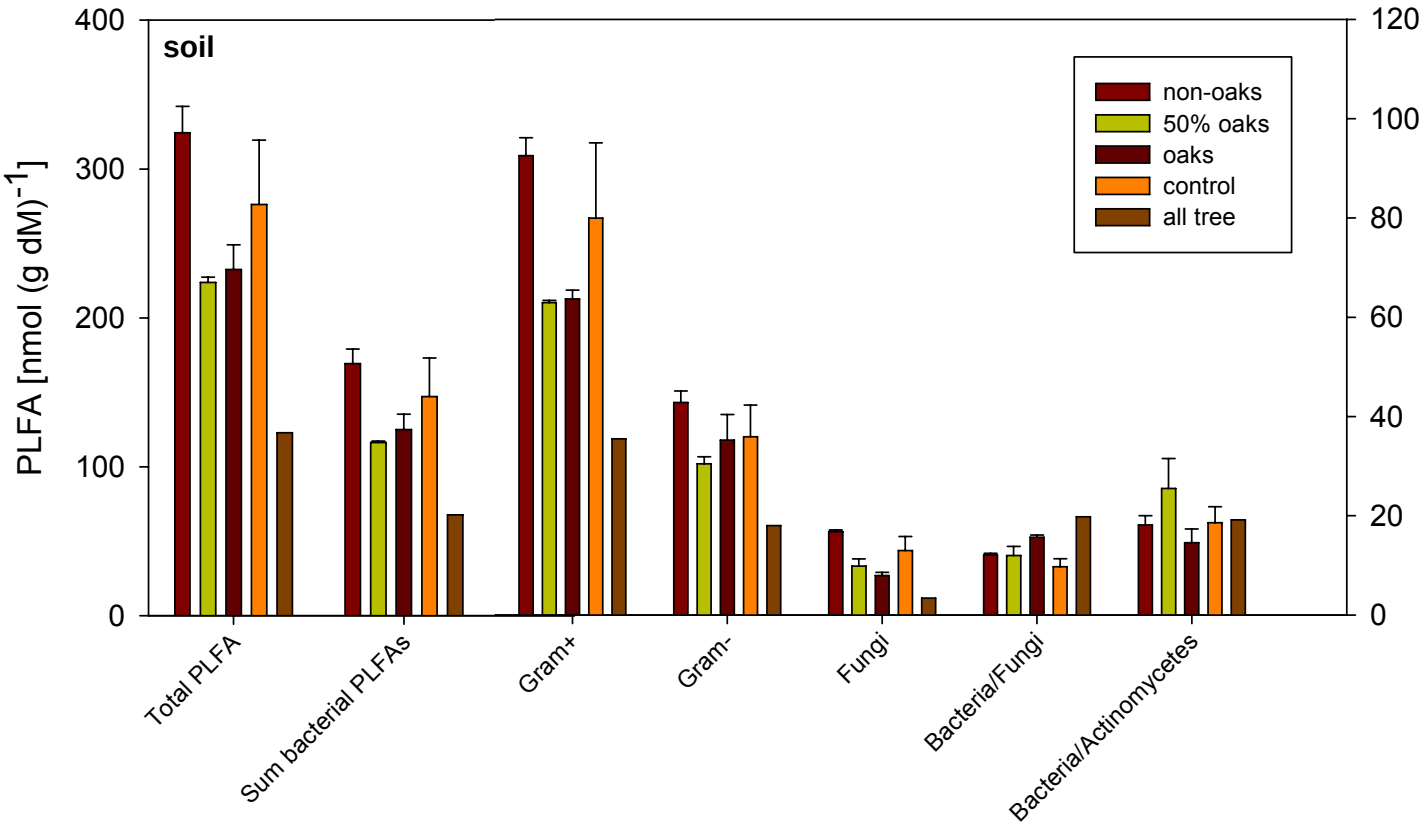


Fig. 6: Sums and ratios of phospholipids fatty acids (PLFAs) of various microbial groups in soil (0-10 cm depth) within the different tree treatments. Values are arithmetic means ± standard deviation (n = 3).



Tab. 5: Pearson correlation table between basic soil parameters and microbial community.

Korrelationen

		EC	pH	N	C	s161w5c	s182w69	s10Me180	TotPLFA	GramP	GramN	SumBact	GramPGramN	BactFung	BactActino
EC	Korrelation nach Pearson	1	-.729**	.821**	.796**	.408	.553	.568*	.421	.411	.333	.429	-.470	-.429	-.260
	Signifikanz (2-seitig)		.005	.001	.001	.167	.050	.043	.152	.163	.267	.143	.105	.143	.390
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
pH	Korrelation nach Pearson	-.729**	1	-.888**	-.897**	-.523	-.716**	-.590*	-.676*	-.715**	-.603*	-.702**	.698**	.682*	.000
	Signifikanz (2-seitig)	.005		.000	.000	.067	.006	.034	.011	.006	.029	.007	.008	.010	1.000
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
N	Korrelation nach Pearson	.821**	-.888**	1	.988**	.586*	.733**	.721**	.692**	.708**	.620*	.707**	-.619*	-.678*	-.147
	Signifikanz (2-seitig)	.001	.000		.000	.035	.004	.005	.009	.007	.024	.007	.024	.011	.631
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
C	Korrelation nach Pearson	.796**	-.897**	.988**	1	.561*	.737**	.717**	.692**	.722**	.614*	.713**	-.662*	-.631*	-.152
	Signifikanz (2-seitig)	.001	.000	.000		.046	.004	.006	.009	.005	.026	.006	.014	.021	.621
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
s161w5c	Korrelation nach Pearson	.408	-.523	.586*	.561*	1	.849**	.872**	.924**	.893**	.946**	.918**	-.184	-.514	-.180
	Signifikanz (2-seitig)	.167	.067	.035	.046		.000	.000	.000	.000	.000	.000	.547	.073	.556
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
s182w69	Korrelation nach Pearson	.553	-.716**	.733**	.737**	.849**	1	.842**	.960**	.954**	.881**	.945**	-.583*	-.725**	.098
	Signifikanz (2-seitig)	.050	.006	.004	.004	.000		.000	.000	.000	.000	.000	.037	.005	.750
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
s10Me180	Korrelation nach Pearson	.568*	-.590*	.721**	.717**	.872**	.842**	1	.886**	.863**	.878**	.893**	-.276	-.479	-.414
	Signifikanz (2-seitig)	.043	.034	.005	.006	.000	.000		.000	.000	.000	.000	.362	.098	.159
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
TotPLFA	Korrelation nach Pearson	.421	-.676*	.692**	.692**	.924**	.960**	.886**	1	.992**	.974**	.996**	-.438	-.671*	-.008
	Signifikanz (2-seitig)	.152	.011	.009	.009	.000	.000	.000		.000	.000	.000	.134	.012	.981
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
GramP	Korrelation nach Pearson	.411	-.715**	.708**	.722**	.893**	.954**	.863**	.992**	1	.957**	.994**	-.511	-.672*	.000
	Signifikanz (2-seitig)	.163	.006	.007	.005	.000	.000	.000	.000		.000	.000	.074	.012	.999
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
GramN	Korrelation nach Pearson	.333	-.603*	.620*	.614*	.946**	.881**	.878**	.974**	.957**	1	.978**	-.250	-.549	-.094
	Signifikanz (2-seitig)	.267	.029	.024	.026	.000	.000	.000	.000	.000		.000	.410	.052	.760
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
SumBact	Korrelation nach Pearson	.429	-.702**	.707**	.713**	.918**	.945**	.893**	.996**	.994**	.978**	1	-.437	-.637*	-.058
	Signifikanz (2-seitig)	.143	.007	.007	.006	.000	.000	.000	.000	.000	.000		.136	.019	.850
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
GramPGramN	Korrelation nach Pearson	-.470	.698**	-.619*	-.662*	-.184	-.583*	-.276	-.438	-.511	-.250	-.437	1	.687**	-.286
	Signifikanz (2-seitig)	.105	.008	.024	.014	.547	.037	.362	.134	.074	.410	.136		.009	.343
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
BactFung	Korrelation nach Pearson	-.429	.682*	-.678*	-.631*	-.514	-.725**	-.479	-.671*	-.672*	-.549	-.637*	.687**	1	-.356
	Signifikanz (2-seitig)	.143	.010	.011	.021	.073	.005	.098	.012	.012	.052	.019	.009		.232
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13
BactActino	Korrelation nach Pearson	-.260	.000	-.147	-.152	-.180	.098	-.414	-.008	.000	-.094	-.058	-.286	-.356	1
	Signifikanz (2-seitig)	.390	1.000	.631	.621	.556	.750	.159	.981	.999	.760	.850	.343	.232	
	N	13	13	13	13	13	13	13	13	13	13	13	13	13	13

**. Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

We detected 32 different fatty acids and 23 thereof were used for the further analysis (Fig. 5). The highest relative abundance was observed for the 16:0 and i15:0 fatty acids for the all treatments. The fatty acid 18:1ω8 usedas an indicator of type II methanotrophs, was not present in all plots.

Microbial biomass expressed as TotPLFA were significantly correlated (Tab.5) with soil pH and soil C and N contents. The highest microbial biomass was found on the control and non-oak girdled sites, whereas the lowest was on the all tree girdled sites (Fig. 6). Among the oak girdled sites we observed an increase from 50% oak girdled to all oak girdled plots. In the same way as microbial biomass the bacterial PLFAs expressed as SumBac was affected by the same soil parameter and showed the same size, since bacterial PLFAs make up a large portion of the total PLFAs.

The fungal PLFA 18:2ω6,9 was also higher on control and non-oak girdled plots but increase with increasing the girdling amount of oak trees (Fig. 5). The bacterial to fungal ratio was highest on the all tree and all oak girdled plots and lowest on the control sites.

The 10Me18:0 PLFA as an indicator of actinomycetes showed the highest values on the non-oaks girdled plots and the lowest on the 50% oak girdled sites (Fig. 5). Also, actinomycetes were significantly correlated with soil pH and soil C and N contents (Tab.5). Bacterial to actinomycetes ratio was highest on the non-oak girdled sites and lowest on the all oak girdled plots.

The relative abundance of arbuscular mycorrhizal (AM) fungal PLFA 16:1ω5c correlated positively with soil C and N content (Tab. 5) and was highest on the site with all oak girdled plots and lowest on the control sites (Fig. 5).

Fig. 7: Relative abundance of bacterial, actinomycetal and fungal phospholipid fatty acids (PLFAs) in litter within the different tree treatments. Values are arithmetic means ± standard deviation (n = 3). AM – arbuscular mycorrhiza.

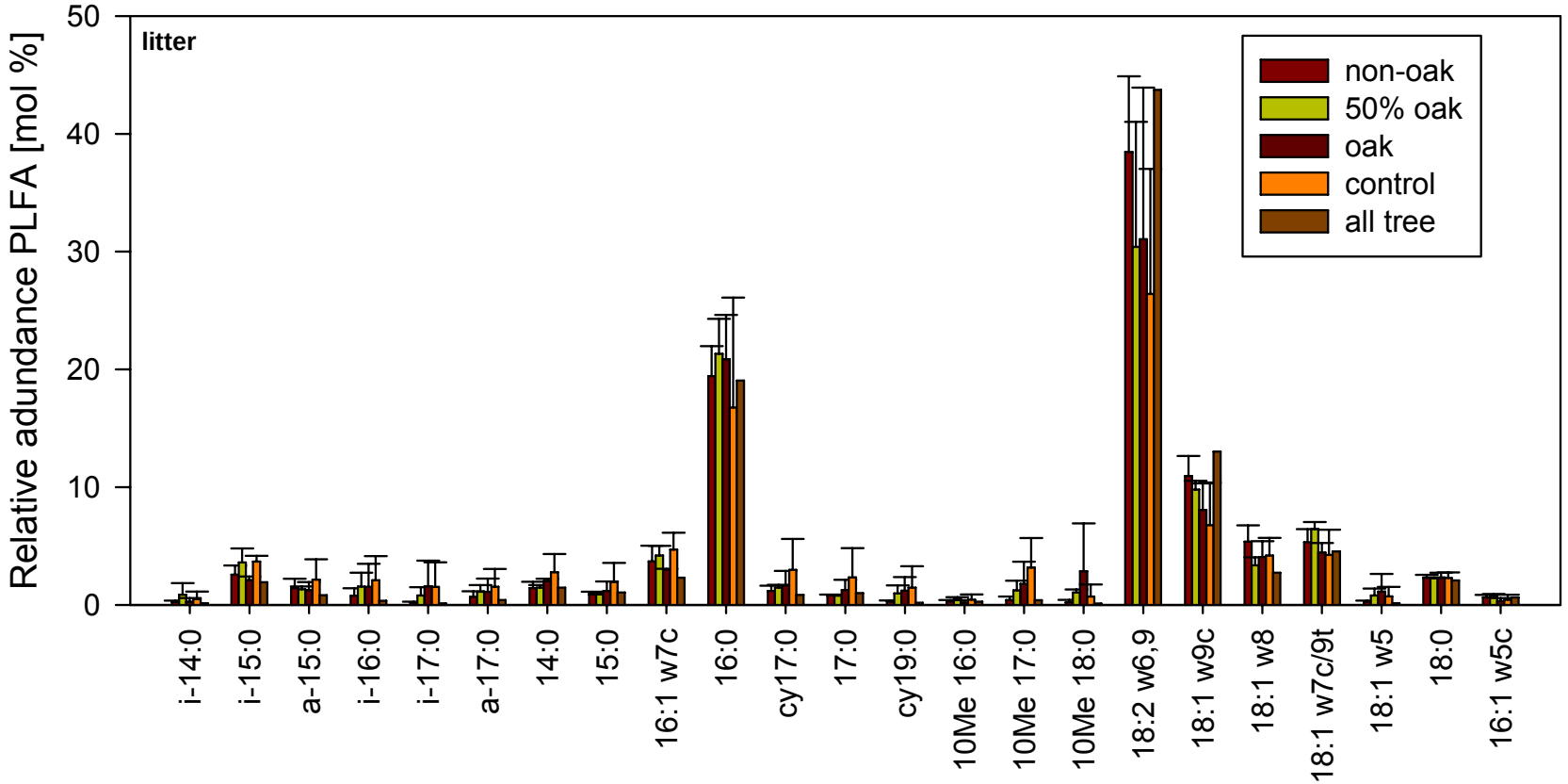
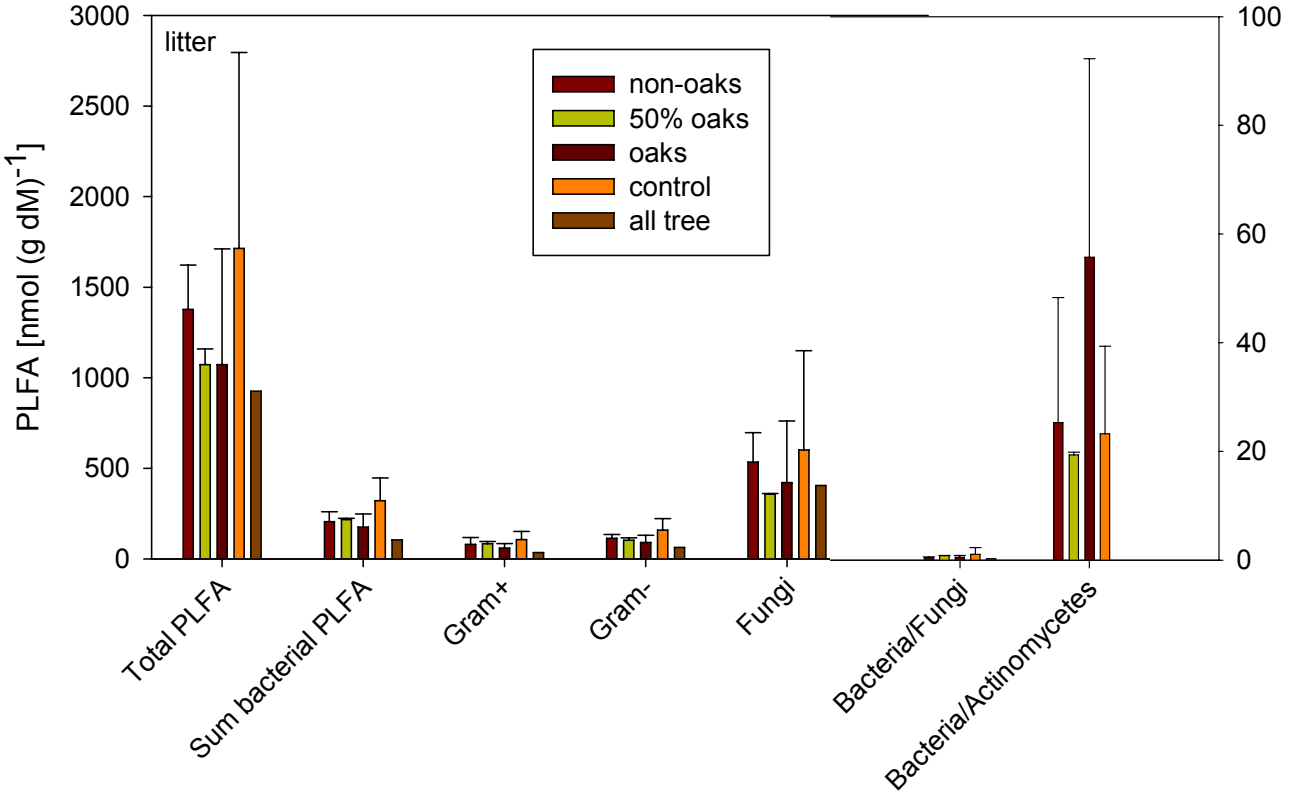


Fig. 8: Sums and ratios of phospholipids fatty acids (PLFAs) of various microbial groups in litter within the different tree treatments. Values are arithmetic means ± standard deviation (n = 3).



Tab. 6: Pearson correlation table between basic litter parameters and microbial community.

Korrelationen

		EC	pH	s161w5c	s182w69	s10Me180	TotPLFA	GramP	GramN	SumBact	GramPGramN	BactFung	BactActino
EC	Korrelation nach Pearson	1	-.349	-.172	-.263	-.124	-.302	-.246	-.349	-.310	.007	-.022	-.209
	Signifikanz (2-seitig)		.243	.574	.385	.685	.317	.418	.243	.303	.982	.944	.494
	N	13	13	13	13	13	13	13	13	13	13	13	13
pH	Korrelation nach Pearson	-.349	1	.544	.352	-.275	.373	.070	.303	.137	.093	-.350	.006
	Signifikanz (2-seitig)	.243		.055	.238	.363	.209	.820	.314	.654	.763	.241	.984
	N	13	13	13	13	13	13	13	13	13	13	13	13
s161w5c	Korrelation nach Pearson	-.172	.544	1	.596*	-.538	.622*	.258	.503	.226	.076	-.423	-.059
	Signifikanz (2-seitig)	.574	.055		.031	.058	.023	.396	.080	.458	.806	.150	.849
	N	13	13	13	13	13	13	13	13	13	13	13	13
s182w69	Korrelation nach Pearson	-.263	.352	.596*	1	-.285	.965**	.123	.750**	.336	.685**	-.553*	.268
	Signifikanz (2-seitig)	.385	.238	.031		.345	.000	.690	.003	.262	.010	.050	.376
	N	13	13	13	13	13	13	13	13	13	13	13	13
s10Me180	Korrelation nach Pearson	-.124	-.275	-.538	-.285	1	-.103	.625*	.278	.660*	-.432	.862**	-.333
	Signifikanz (2-seitig)	.685	.363	.058	.345		.738	.022	.358	.014	.141	.000	.266
	N	13	13	13	13	13	13	13	13	13	13	13	13
TotPLFA	Korrelation nach Pearson	-.302	.373	.622*	.965**	-.103	1	.368	.894**	.560*	.508	-.360	.145
	Signifikanz (2-seitig)	.317	.209	.023	.000	.738		.216	.000	.047	.076	.226	.636
	N	13	13	13	13	13	13	13	13	13	13	13	13
GramP	Korrelation nach Pearson	-.246	.070	.258	.123	.625*	.368	1	.727**	.944**	-.533	.606*	-.403
	Signifikanz (2-seitig)	.418	.820	.396	.690	.022	.216		.005	.000	.061	.028	.172
	N	13	13	13	13	13	13	13	13	13	13	13	13
GramN	Korrelation nach Pearson	-.349	.303	.503	.750**	.278	.894**	.727**	1	.866**	.153	.065	-.025
	Signifikanz (2-seitig)	.243	.314	.080	.003	.358	.000	.005		.000	.618	.832	.935
	N	13	13	13	13	13	13	13	13	13	13	13	13
SumBact	Korrelation nach Pearson	-.310	.137	.226	.336	.660*	.560*	.944**	.866**	1	-.285	.527	-.257
	Signifikanz (2-seitig)	.303	.654	.458	.262	.014	.047	.000	.000		.346	.064	.397
	N	13	13	13	13	13	13	13	13	13	13	13	13
GramPGramN	Korrelation nach Pearson	.007	.093	.076	.685**	-.432	.508	-.533	.153	-.285	1	-.703**	.513
	Signifikanz (2-seitig)	.982	.763	.806	.010	.141	.076	.061	.618	.346		.007	.073
	N	13	13	13	13	13	13	13	13	13	13	13	13
BactFung	Korrelation nach Pearson	-.022	-.350	-.423	-.553*	.862**	-.360	.606*	.065	.527	-.703**	1	-.320
	Signifikanz (2-seitig)	.944	.241	.150	.050	.000	.226	.028	.832	.064	.007		.287
	N	13	13	13	13	13	13	13	13	13	13	13	13
BactActino	Korrelation nach Pearson	-.209	.006	-.059	.268	-.333	.145	-.403	-.025	-.257	.513	-.320	1
	Signifikanz (2-seitig)	.494	.984	.849	.376	.266	.636	.172	.935	.397	.073	.287	
	N	13	13	13	13	13	13	13	13	13	13	13	13

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

**. Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

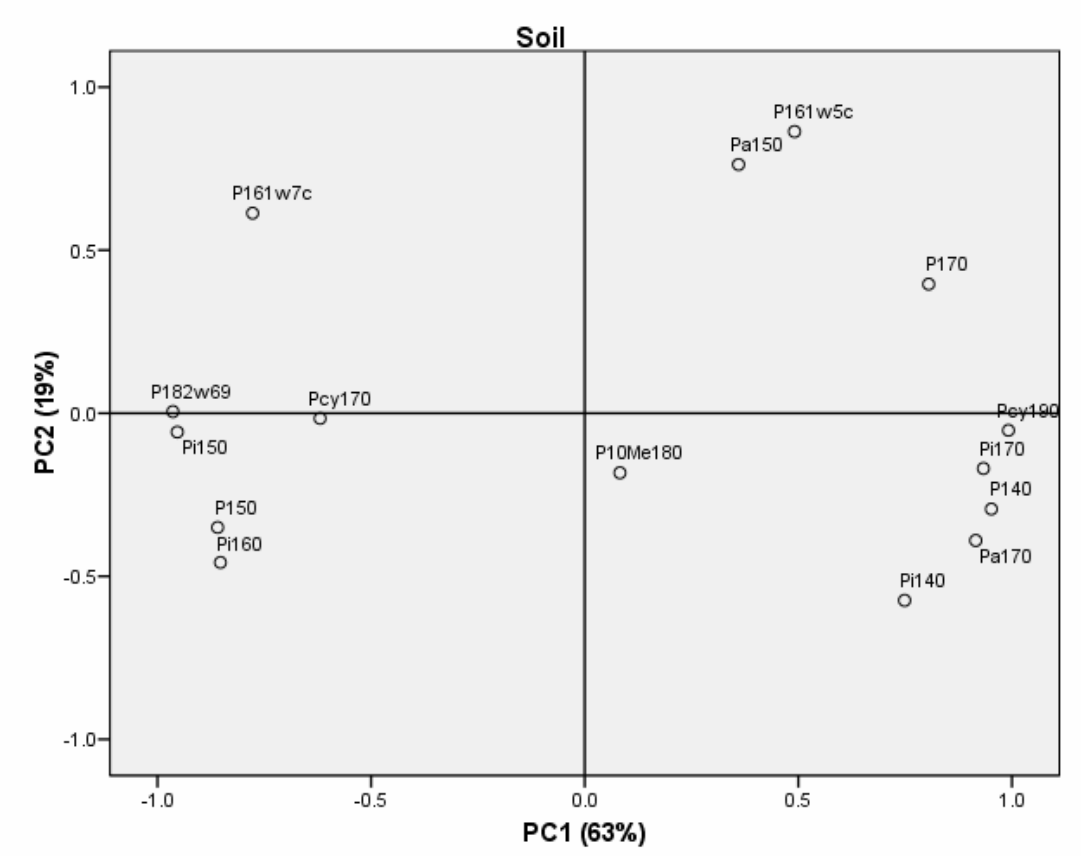
We detected 33 different fatty acids and 23 thereof were used for the further analysis (Fig. 7). The highest relative abundance was observed for the 18:2 ω6,9 and 16:0 fatty acids for the all treatments. In contrast to the soil samples, the fatty acid 18:1ω8 used as an indicator of type II methanotrophs, was present in all plots with the highest values at the plots with non-oak girdled trees (Fig.7)

No correlations were observed between microbial biomass expressed as TotPLFA and litter pH and eC (Tab.6). Similar patterns as for the soil samples but in much higher concentrations, we found the highest microbial biomass on the control and non-oak girdled sites, whereas the lowest was on the all tree girdled sites (Fig. 8). No differences in the microbial biomass were observed among the oak girdled sites (O50 and O). Bacterial PLFAs expressed as SumBac showed similar patterns as the TotPLFA but in much lower abundance.

In general, the relative abundance of litter fungal PLFA 18:2ω6,9 was much higher compared to the soil samples. The highest values were found on the site with all girdled trees and the lowest on the control site (Fig. 7). The bacterial to fungal ratio was highest on the control sites and lowest in the all tree girdled site, but in general, the values are much lower compared to the soil (Fig. 8).

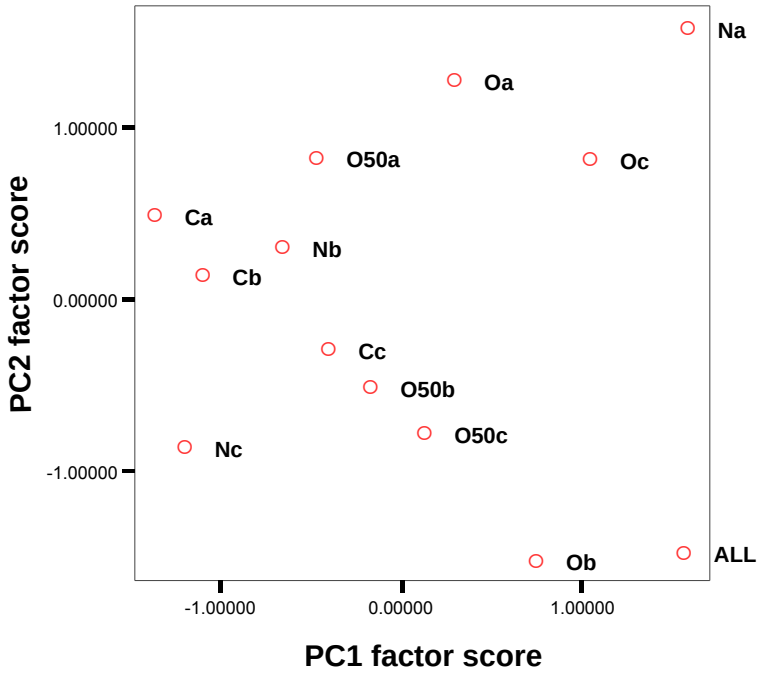
The 10Me18:0 PLFA as an indicator of actinomycetes showed the highest values on the oak girdled plots and the lowest on the non-oak and all tree girdled sites, respectively (Fig. 7). Accordingly, bacterial to actinomycetes ratio was highest on the oak girdled sites and lowest on the all 50% oak and all tree girdled plots, respectively. The relative abundance of arbuscular mycorrhizal (AM) fungal PLFA 16:1ω5c was lowest on the site with all oak girdled plots (Fig. 7).

Fig. 9: Loadings for the first two principal components (PC1 and PC2) of 15 microbial phospholipids fatty acids (mol%) extracted from the soil (0-10 cm depth; n=10).



The PLFAs ascribed to Gram-positive and Gram-negative bacteria, actinomycetes, fungi and AM fungi (Fig. 5) were subjected to PCA, where the first principal component (PC1) explained 63% and the second (PC2) 19% of the variance in the PLFAs. PC1 axis was primarily associated with positive relationships to pH (Tab. 5) and negative relationships to EC, OC and N and may as such be interpreted as a measure of the decomposition conditions, whereas the PC2 axis showed no correlations to the basic soil parameters.

Fig. 10: Score plot of principal component analysis showing the separation of five tree treatments along the studied hill slope.



We plotted the PC1 and PC2 score values for all analyzed samples, which resulted in clear separation of the five studied treatments and their position along the slop. The sites with high pH and lower C and N content were found on the right-hand site (positive PC1), while the sites with lower pH and higher C and N content were found on the left-hand site (negative PC1). Along the PC2 axis, the sites from the bottom of the hill are found at the top of Fig.10 (positive PC2), while the sites from the top of the hill are found at the bottom of Fig.10 (negative PC2).

Tab. 7: Correlation of main soil properties with PC1 and PC2 (n=3). EC = electrical conductivity; N = total nitrogen; C = organic carbon

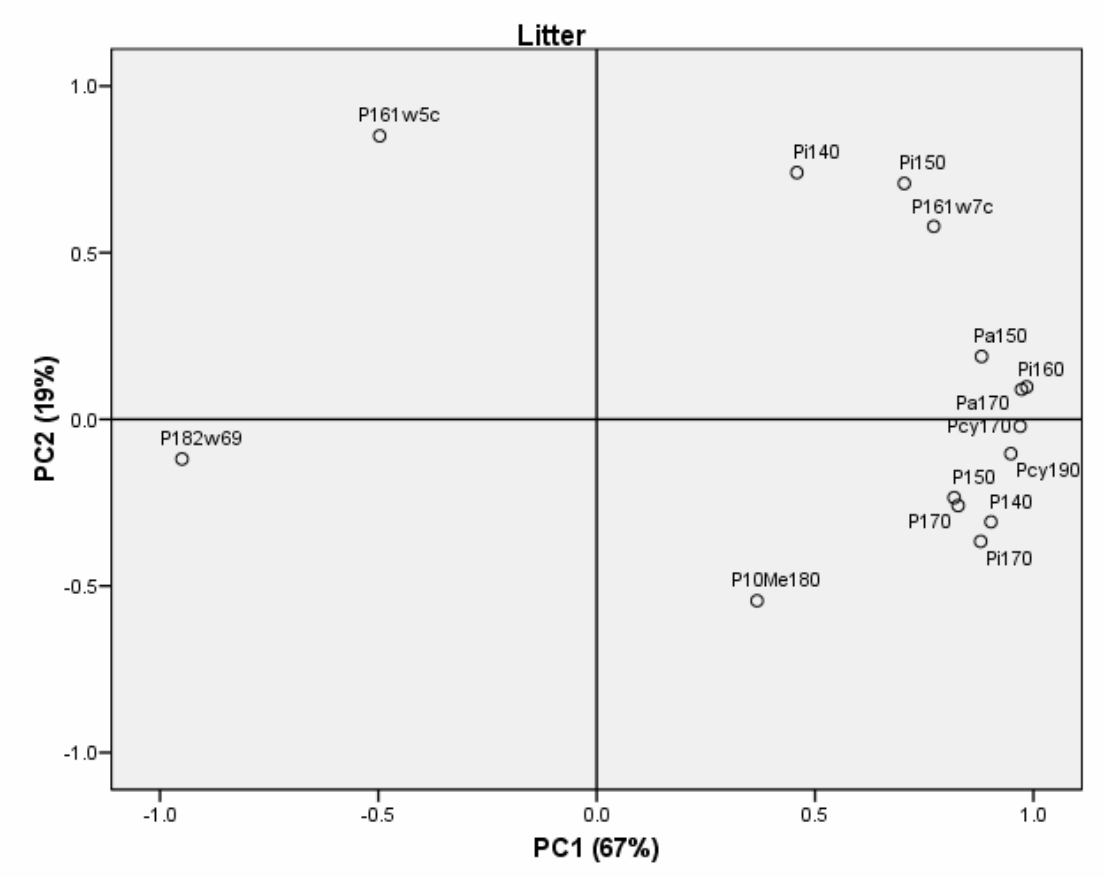
Korrelationen

		EC	pH	N	C	REGR factor score 1 for analysis 1	REGR factor score 2 for analysis 1
EC	Korrelation nach Pearson	1	-.948*	.991**	.976**	-.980**	.160
	Signifikanz (2-seitig)		.014	.001	.004	.003	.797
	N	5	5	5	5	5	5
pH	Korrelation nach Pearson	-.948*	1	-.922*	-.912*	.991**	.092
	Signifikanz (2-seitig)	.014		.026	.031	.001	.883
	N	5	5	5	5	5	5
N	Korrelation nach Pearson	.991**	-.922*	1	.990**	-.965**	.159
	Signifikanz (2-seitig)	.001	.026		.001	.008	.798
	N	5	5	5	5	5	5
C	Korrelation nach Pearson	.976**	-.912*	.990**	1	-.953*	.063
	Signifikanz (2-seitig)	.004	.031	.001		.012	.920
	N	5	5	5	5	5	5
REGR factor score 1 for analysis 1	Korrelation nach Pearson	-.980**	.991**	-.965**	-.953*	1	.000
	Signifikanz (2-seitig)	.003	.001	.008	.012		1.000
	N	5	5	5	5	5	5
REGR factor score 2 for analysis 1	Korrelation nach Pearson	.160	.092	.159	.063	.000	1
	Signifikanz (2-seitig)	.797	.883	.798	.920	1.000	
	N	5	5	5	5	5	5

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

**. Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

Fig. 11: Loadings fort the first two principal components (PC1 and PC2) of 15 microbial phospholipids fatty acids (mol %) extracted from the litter (n=10).



The PLFAs ascribed to Gram-positive and Gram-negative bacteria, actinomycetes, fungi and AM fungi (Fig. 6) were subjected to PCA, where the first principal component (PC1) explained 67% and the second (PC2) 19% of the variance in the PLFAs. PC1 axis was primarily associated with negative relationships to EC (Tab. 6) and may as such be interpreted as a measure of the leaching regime along the studied slope, whereas the PC2 axis showed no correlations to the basic soil parameters. PC1 was positively determined by bacteria PLFA and negatively by fungi and AM fungi.

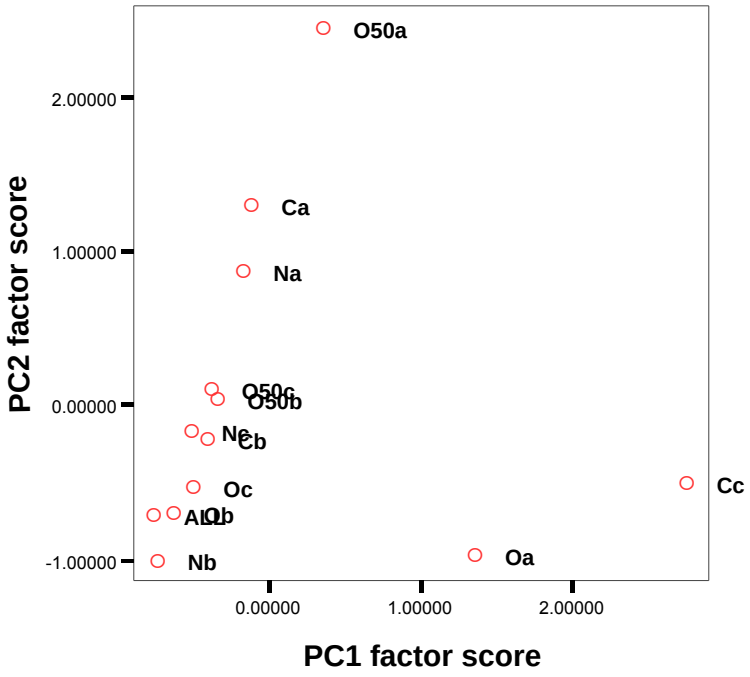
Tab. 8: Correlation of main soil properties with PC1 and PC2 (n=3). EC = electrical conductivity

Korrelationen

		EC	pH	REGR factor score 1 for analysis 1	REGR factor score 2 for analysis 1
EC	Korrelation nach Pearson	1	-.701	-.892*	-.308
	Signifikanz (2-seitig)		.187	.042	.615
	N	5	5	5	5
pH	Korrelation nach Pearson	-.701	1	.481	.252
	Signifikanz (2-seitig)	.187		.412	.683
	N	5	5	5	5
REGR factor score 1 for analysis 1	Korrelation nach Pearson	-.892*	.481	1	.000
	Signifikanz (2-seitig)	.042	.412		1.000
	N	5	5	5	5
REGR factor score 2 for analysis 1	Korrelation nach Pearson	-.308	.252	.000	1
	Signifikanz (2-seitig)	.615	.683	1.000	
	N	5	5	5	5

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

Fig. 12: Score plot of principal component analysis showing the separation of five tree treatments along the studied hill slope.



The PC1 and PC2 score values for all analyzed samples showed no clear separation of the five studied treatments. The sites with lower EC were found of the left-hand site (negative PC1), while the sites with higher EC were found on the right-hand site (positive PC1).

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