Final Report of the long-term exchange visit within the ESF-Network MOLTER

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Host

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Purpose of the visit

The aim of my long-term exchange visit to Dr. Cornelia Rumpel, and Dr. Marie-France Dignac, Laboratoire de Biogéochimie et Ecologie des millieux continentaux (BIOEMCO), Centre INRA Versailles-Grignon, France was to gain more insight into the mechanisms of soil organic carbon (SOC) sequestration along a land-use gradient in the tropical mountain rainforest region of Southern Ecuador. In the study area, natural forests have often been converted to pastures by slash-and-burn. With advanced pasture age the pasture grass (*Setaria sphacelata*, a C4 plant species) is replaced more and more by the invading tropical bracken fern (*Pteridium arachnoideum*, a C3 plant species) leading to the abandonment of this aged unproductive pasture sites (Hamer et al., 2009). Data on soil organic carbon stocks obtained in my running research project “Interactions of organic matter and microbial dynamics in pasture soils along management chronosequences” within the research unit 816 “Biodiversity and Sustainable Management of a Megadiverse Mountain Ecosystem in South Ecuador“ (www.tropicalmountainforest.org), funded by the German Foundation of Research (DFG), indicate significant increases of SOC stocks in the mineral soil of old pastures. However, until now the mechanisms behind are still uncertain. One important factor might be the observed high input of root derived carbon from the pasture grass (Potthast et al., submitted). Therefore, the objective was to unravel the contribution of plant root and plant shoot organic matter to the formation of soil organic matter (SOM) in mineral top soil (0-5 cm depth) along the land-use gradient: forest – 8 year old pasture – 17 year old pasture – 50 year old pasture and abandoned pasture using root versus shoot specific molecular biomarkers of *Setaria sphacelata, Pteridium arachnoideum* and forest organic layer combined with compound specific isotope analysis.

Work carried out during the visit

Extraction of free lipids

Free lipids were extracted from mineral soil (10g dw), organic layer and plant shoot as well as root residues (2g dw) with 20 ml dichlormethane:methanol (1:2, v:v). The suspensions were vortexed for 30s, agitated overhead for 2h and subsequently centrifuged at 2200 rpm for 10min. This extraction was repeated once. Thereafter the samples were rinsed with 5ml dichlormethane:methanol (1:2, v:v) by vortex and

centrifugation. The remaining samples were air-dried (24h) and used for saponification.

Saponification

To release biomarkers of cutins and suberins saponification was used as described in Mendez-Millan et al. (2010). Briefly, 500 mg of soil sample or 100 mg of plant or litter material was refluxed for 18h in an aqueous methanol solution (water:methanol, 1:9, V:V) containing 6% of KOH. Subsequently, the solution was filtered (GF/A Whatman glass microfibre filters), the residue was washed with water:methanol (1:9, V:V) and the pH of the filtrate was adjusted to 2 with 6 N HCl after the addition of 150 ml of distilled water. The acidified solution was extracted three times with 50 ml of dichlormethane. Then, the volume of the obtained extracts was reduced with a rotary evaporator and dried under N_2 completely. All dried extracts were redissolved in 400 µl of pyridine containing nonadecanoic acid (C19:0) as internal standard. Prior to analysis, an aliquot was sylilated at 70°C for 1h using BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane).

Analysis

The respective silylated monomers were identified according to their fragmentation pattern after analysis with GC/MS (Agilent HP5973) and comparison with published mass spectra and a mass spectra library (Wiley). Quantification was carried out with a gas chromatograph (GC) (Agilent HP6890) equipped with a flame ionisation detector (FID) by using the internal standard C19:0 and an external calibration with 16-hydroxyhexadecanoic acid. The δ^{13}C-signature of individual components was analysed using a GC HP5890 coupled with an Isochrom III isotopic mass spectrometer (Micromass-GVI Optima). The δ^{13}C values (expressed relative to Vienna PeeDee Belemnite in ‰) of the respective compounds were corrected for the C atoms introduced by the trimethylsilyl groups (δ^{13}C-value of BSTFA+TMCS as determined with EA-IRMS: -36.5‰).

All GCs were equipped with a SGE BPX-5 column (50m x 0.25m x 0.32m) and run with the same temperature program as described in Mendez-Millan et al. (2010) after injection of 1 µl of sample in the splitless mode.

Main results obtained

In total 27 different aliphatic compounds have been identified in the extracts of the four plant materials (Table 1) and further 9 compounds occurring in grass shoot or root or bracken frond or rhizome material might be of interest but have not been identified so far. In bracken fronds the highest concentrations of n-carboxylic acids, ω-hydroxy carboxylic acids and α,ω-alkanedioic acids were detected (Table 1). In contrast, α-hydroxy carboxylic acids and mid-chain hydroxyl acids were mainly present in grass samples. Thus, significant differences between grass and bracken organic matter exist regarding the chemical composition as well as the δ^{13}C-signature of the respective compounds (Table 1 and Table 2). The α,ω-alkanedioic acids $C_{16:0}$ and $C_{22:0}$ and the ω-hydroxy carboxylic acids $C_{18:0}$ and $C_{20:0}$ might be used as specific

markers for grass roots since they do not occur in grass shoots or only occur in very low amounts ($C_{16:0}$ diacid). Characteristic markers of grass shoots are the mid-chain hydroxy acids 9,10-epoxy 18-OH $C_{18:0}$ and x,18 diOH $C_{18:1}$ and the ω-hydroxy carboxylic acids $C_{22:1}$ and $C_{24:0}$ (Table1). Specific components of bracken frond organic matter are $\omega C_{14:0}$ and $C_{20:1}$ diacid, since they have not been detected in the other plant samples. In bracken rhizomes most compounds were below detection limit or only present in minor amounts except n-carboxylic acids. The δ^{13}C-signature of the aliphatic monomers identified in bracken frond extracts was always significantly lower than the signature obtained for the respective monomer extracted from bracken rhizome (Table 2). Such a clear trend was not detected between grass shoot and root derived monomers whereby 10 monomers had a similar δ^{13}C-value. The preparation and interpretation of data obtained for the soil samples along the land-use gradient: forest – 8year old pasture – 17year old pasture – 50year old pasture and abandoned pasture is in progress and will be included into the publication.

References

Hamer, U., Potthast, K., Makeschin, F., 2009. Urea fertilisation affected soil organic matter dynamics and microbial community structure in pasture soils of Southern Ecuador. Applied Soil Ecology 43, 226-233.

Mendez-Millan, M., Dignac, M.F., Rumpel, C., Rasse, D.P., Derenne, S., 2010. Molecular dynamics of shoot vs. root biomarkers in an agricultural soil estimated by natural abundance C-13 labelling. Soil Biology & Biochemistry 42, 169-177.

Future collaboration with host institution

In spring 2011 Marie-France Dignac is going to visit the Institute of Soil Science and Site Ecology at Dresden University of Technology for giving a guest lecture and discussing further steps. Some ^{13}C-analysis of PLFA extracts are in progress.

Projected publications

One publication is in progress and will be submitted in 2011 (ESF will be acknowledged). The proposed title is: Hamer, U., Rumpel, C, Dignac, M.-F.: Cutin and suberin biomarkers as tracer for organic matter dynamics in Ecuadorian pasture soils.

Table 1: Concentration (µg g^{-1} dw) of the identified aliphatic monomers present in the bound lipid fraction of grass shoot, grass root, bracken frond and bracken rhizome organic matter (n=3, SD in parenthesis, nd=not detectable).

		Grass		Bracken	
		Shoot	**Root**	**Frond**	**Rhizome**
n-Carboxylic acids					
Hexadecanoic acid	(n-$C_{16:0}$)	899 (193)	303 (65)	2029 (181)	500 (131)
Octadecanoic acid	(n-$C_{18:0}$)	193 (58)	142 (39)	345 (50)	138 (72)
Octadecenoic acid	(n-$C_{18:1}$)	108 (34)	145 (29)	204 (22)	129 (31)
9,12-Octadecadienoic acid	(n-$C_{18:2}$)	226 (51)	59 (15)	411 (36)	156 (36)
cis-9,12,15-octadecatrienoic acid	(n-$C_{18:3}$)	727 (186)	nd	437 (36)	58 (12)
Eicosanoic acid	(n-$C_{20:0}$)	45 (5)	50 (2)	178 (14)	nd
Total concentration		***2198 (519)***	***699 (162)***	***3604 (285)***	***981 (270)***
α-Hydroxy carboxylic acids					
2-Hydroxy eicosanoic acid	(α$C_{20:0}$)	92 (23)	53 (20)	nd	nd
2-Hydroxy docosanoic acid	(α$C_{22:0}$)	24 (8)	54 (12)	nd	32 (2)
2-Hydroxy tetracosanoic acid	(α$C_{24:0}$)	9 (1)	20 (2)	nd	nd
2-Hydroxy hexacosanoic acid	(α$C_{26:0}$)	14 (4)	14 (1)	28 (10)	nd
Total concentration		***139 (25)***	***141 (19)***	***28 (5)***	***32 (6)***
ω-Hydroxy carboxylic acids					
14-Hydroxy tetradecanoic acid	(ω$C_{14:0}$)	nd	nd	1995 (62)	nd
16-Hydroxy hexadecanoic acid	(ω$C_{16:0}$)	43 (4)	600 (65)	1263 (107)	27 (8)
18-Hydroxy octadecanoic acid	(ω$C_{18:0}$)	nd	81 (71)	56 (53)	nd
18-Hydroxy octadecenoic acid	(ω$C_{18:1}$)	194 (13)	219 (27)	68 (25)	nd
20-Hydroxy eicosanoic acid	(ω$C_{20:0}$)	nd	59 (9)	nd	nd
22-Hydroxy docosanoic acid	(ω$C_{22:0}$)	8 (2)	17 (4)	nd	nd
22-Hydroxy docosenoic acid	(ω$C_{22:1}$)	81 (36)	nd	368 (27)	nd
24-Hydroxy tetracosanoic acid	(ω$C_{24:0}$)	82 (95)	nd	89 (106)	nd
26-Hydroxy hexacosanoic acid	(ω$C_{26:0}$)	38 (33)	86 (19)	143 (13)	53 (22)
Total concentration		***446 (134)***	***1062 (166)***	***3982 (182)***	***80 (17)***
α,ω-Alkanedioic acids					
1,16-Hexadecadioic acid	($C_{16:0}$ diacid)	17 (6)	63 (40)	336 (14)	nd
1,18-Octadecenoic acid	($C_{18:1}$ diacid)	222 (148)	248 (171)	304 (170)	
1,20-Eicosenoic acid	($C_{20:1}$ diacid)	nd	nd	103 (32)	nd
1,22-Dodecosanoic acid	($C_{22:0}$ diacid)	nd	83 (43)	nd	nd
Total concentration		***240 (152)***	***394 (250)***	***743 (212)***	
Mid-chain hydroxy acids					
x,16-Dihydroxy hexadecanoic acids (x=8,9,19)	(x,16-diOH $C_{16:0}$)	274 (40)	114 (14)	197 (3)	nd
9,10,18-Trihydroxyoctadecanoic acid	(9,10,18-triOH $C_{18:0}$)	40 (3)	156 (53)	nd	nd
9,10-Epoxy, 18-hydroxyoctadecanoic acid	(9,10-epoxy 18-OH $C_{18:0}$)	44 (5)	nd	nd	nd
11,18-Dihydroxyoctadecenoic acid	(11,18-diOH $C_{18:1}$)	93 (18)	48 (9)	nd	nd
x,18-Dihydroxyoctadecenoic acids (x=9,10)	(x,18 diOH $C_{18:1}$)	54 (9)	nd	nd	nd
Total concentration		***533 (64)***	***317 (73)***	***197 (3)***	
Total		**3555 (750)**	**2613 (583)**	**8555 (652)**	**1093 (291)**

Table 2: δ^{13}C-signature (‰) of the identified aliphatic monomers present in the bound lipid fraction of grass shoot, grass root, bracken frond and bracken rhizome organic matter (n=3, SD in parenthesis, nd=not detectable).

		Grass		Bracken	
		Shoot	**Root**	**Frond**	**Rhizome**
n-Carboxylic acids					
Hexadecanoic acid	(n-$C_{16:0}$)	-16.4 (0.5)	-17.4 (0.7)	-31.6 (0.5)	-28.6 (0.7)
Octadecanoic acid	(n-$C_{18:0}$)	-22.6 (0.2)	-23.2 (0.7)	-29.4 (0.8)	-27.1 (0.9)
Octadecenoic acid	(n-$C_{18:1}$)	-18.5 (0.1)	-15.0 (0.9)	-32.8 (0.3)	-27.5 (1.2)
9,12-Octadecadienoic acid	(n-$C_{18:2}$)	-16.8 (1.4)	-12.2 (0.2)	-33.4 (0.5)	-27.5 (1.2)
cis-9,12,15-octadecatrienoic acid	(n-$C_{18:3}$)	-14.0 (0.4)	nd	-31.2 (1.5)	nd
Eicosanoic acid	(n-$C_{20:0}$)	-14.0 (1.6)	-17.9 (1.1)	-30.6 (0.1)	nd
α-Hydroxy carboxylic acids					
2-Hydroxy eicosanoic acid	(α$C_{20:0}$)	-17.0 (2.0)	-14.4 (1.0)	nd	nd
2-Hydroxy docosanoic acid	(α$C_{22:0}$)	-15.4 (0.2)	-20.8 (1.2)	nd	nd
2-Hydroxy tetracosanoic acid	(α$C_{24:0}$)	-18.3 (0.9)	-14.4 (0.8)	-29.5 (1.0)	nd
2-Hydroxy hexacosanoic acid	(α$C_{26:0}$)	-19.0 (1.3)	nd	nd	
ω-Hydroxy carboxylic acids					
14-Hydroxy tetradecanoic acid	(ω$C_{14:0}$)	nd	nd	-32.2 (0.2)	nd
16-Hydroxy hexadecanoic acid	(ω$C_{16:0}$)	-13.8 (1.3)	-15.0 (1.2)	-32.0 (0.2)	-26.0 (0.3)
18-Hydroxy octadecanoic acid	(ω$C_{18:0}$)	nd	-23.1 (0.9)	-27.1 (0.5)	nd
18-Hydroxy octadecenoic acid	(ω$C_{18:1}$)	-11.0 (0.7)	-12.0 (0.5)	nd	nd
20-Hydroxy eicosanoic acid	(ω$C_{20:0}$)	nd	-18.0 (0.2)	nd	nd
22-Hydroxy docosanoic acid	(ω$C_{22:0}$)	nd	-10.9 (1.3)	nd	nd
22-Hydroxy docosenoic acid	(ω$C_{22:1}$)	-15.7 (2.8)	nd	-29.3 (1.3)	nd
24-Hydroxy tetracosanoic acid	(ω$C_{24:0}$)	-16.6 (2.2)	-16.3 (0.8)	-28.7 (3.2)	-25.3
26-Hydroxy hexacosanoic acid	(ω$C_{26:0}$)	-16.3 (1.4)	nd	-26.0 (0.8)	-25.4 (2.2)
α,ω-Alkanedioic acids					
1,16-Hexadecadioic acid	($C_{16:0}$ diacid)	-11.8 (0.9)	-12.6 (0.0)	-32.3 (0.4)	nd
1,18-Octadecenoic acid	($C_{18:1}$ diacid)	-21.4 (2.3)	-13.2 (0.8)	-29.0 (1.1)	nd
1,20-Eicosenoic acid	($C_{20:1}$ diacid)	nd	nd	-31.1 (0.1)	nd
1,22-Dodecosanoic acid	($C_{22:0}$ diacid)	nd	-13.9 (0.1)	nd	nd
Mid-chain hydroxy acids					
x,16-Dihydroxy hexadecanoic acids (x=8,9,19)	(x,16-diOH $C_{16:0}$)	-14.8 (0.8)	-12.6 (3.8)	-30.5 (1.9)	-24.9 (0.9)
9,10,18-Trihydroxyoctadecanoic acid	(9,10,18-triOH $C_{18:0}$)	-13.8 (1.4)	-14.5 (0.5)	nd	nd
9,10-Epoxy, 18-hydroxyoctadecanoic acid	(9,10-epoxy 18-OH $C_{18:0}$)	-14.7 (0.7)	-23.3 (1.0)	nd	nd
11,18-Dihydroxyoctadecenoic acid	(11,18-diOH $C_{18:1}$)	-13.3 (0.9)	-12.2 (0.8)	nd	nd
x,18-Dihydroxyoctadecenoic acids (x=9,10)	(x,18 diOH $C_{18:1}$)	-13.4 (0.7)	-12.3 (1.2)	nd	nd