Report of a ESF Long visit grant within the MOLTER program.

1. Purpose of the visit

Just starting with my PhD research, *The impact of termites on soil organic matter dynamics in the tropics*, I wanted to obtain in-depth knowledge on laboratory organic matter characterization. The Department of Soil Science at the München Technical University was chosen as the host institution for a 4-week exchange visit, because it has one of the most extensive laboratories dedicated to the study of soil organic matter (SOM) in Europe. My host, prof. Dr. Ingrid Kögel-Knabner, is one of the leading authorities in the field of SOM characterization and therefore very well-suited to introduce me to the field.

Furthermore, a test set of termite mound soil samples was analyzed as outlined further in this report. This allowed me to familiarize myself firsthand with the specific laboratory techniques. Knowledge was gathered concerning the appropriate sample gathering, pretreatment and processing. Additionally, this allowed to consider the constraints inherent to tropical soils (low SOM, high Fe-oxides content), and how to address them. Future sample gathering and analysis can therefore be executed more efficiently.

2. Description of the work carried out during the visit

Soil organic matter characterization was outlined by senior researcher Dr. Sandra Spielvogel. The SOM components were described, as well as the laboratory procedures to analyze them. A program was drawn up to get to know all of these different techniques during the stay.

The first week focused on lignin analysis of the brought termite mound samples. In this procedure, complex lignin molecules are broken down into monomers by alkaline oxidative degradation using CuO. After filtration and acidification to remove humic acids, the monomers were purified over a C_{18} column and sililated. The resulting phenolic components were separated using gas chromatography and detected in a coupled mass spectrometer (GC/MS). The resulting chromatogram was compared with those of standard solutions of the 15 most common lignin monomers, to identify and quantify these components.

The second week I got the possibility to assist in a cutin/suberin analysis of another PhD student. After alkaline hydrolysis (in MeOH) of the soil samples, filtration, and acidification, the aliphatic monomers are sylilated and quantified similar to lignin, using GC/MS. As cutin and suberin occur in such small quantities, this analysis was not deemed feasible for the termite mound samples, containing 1% OC or less. However, the procedure, interpretation and applications are now clear to me and might be used in the future.

During the third week, the neutral sugars in the termite mound samples were analyzed. Present polysaccharides were hydrolysed by trifluoracetic acid and reduced using $NaBH_4$. The resulting monosaccharides are then acetylated to increase their adsorption affinity in the gas chromatograph's column. After GC separation, the components are quantified by a coupled flame ionization detector.

The final week offered 3 remaining days to consult with the present experts on the interpretation of the obtained results. Additionally, an extensive introduction was given to two state of the art techniques: 13C-nuclear magnetic resonance analysis (13C-NMR) and Nanoscale secondary ion mass spectrometry (NanoSIMS). Future research may very well harness these promising methods.

3. Description of the main results obtained

Besides insight into the procedures of all of the analyses listed above, a first set of data from termite mound samples was also obtained. The results of the sugar analysis are summarized in Table 1.

Table 1. Results of sugar analysis

Sample Nr.	Description	weight [mg]	OC [%]	OC [mg]	Total sugar [mg]	Total sugar / soil [Mass%]	Total sugar / OC [Mass%]	Rhamnose [mg]	Fucose [mg]	Ribose [mg]	Arabinose [mg]	Xylose [mg]	Mannose [mg]	Galactose [mg]	Glucose [mg]	GM/AX
1	Outer crust (5,5 m)	1124,2	1,02	11,5	2,48	0,22	**21,66**	0,15	0,00	0,00	0,45	0,16	0,45	0,57	0,70	**1,67**
2	Inner section (4,8 m)	1132,5	0,84	9,51	1,54	0,14	**16,16**	0,00	0,00	0,00	0,40	0,00	0,27	0,44	0,43	**1,79**
3	Central hive (3,5 m)	1356,7	0,60	8,14	1,29	0,10	**15,84**	0,00	0,00	0,00	0,34	0,00	0,21	0,39	0,35	**1,78**
4	Inner section (2,2 m)	1142,5	0,71	8,11	0,76	0,07	**9,42**	0,00	0,00	0,00	0,27	0,00	0,00	0,25	0,25	**0,92**
5	Mound foot (0,9 m)	1032,6	1,31	13,5	1,33	0,13	**9,80**	0,00	0,00	0,00	0,34	0,00	0,24	0,33	0,41	**1,71**
6	Mound foot (-0,4 m)	2110,0	0,39	8,23	0,90	0,04	**10,88**	0,00	0,00	0,00	0,33	0,00	0,00	0,31	0,26	**0,94**
7	Fresh chamber wall	1057,7	1,11	11,7	2,47	0,23	**21,01**	0,19	0,00	0,00	0,51	0,30	0,33	0,47	0,66	**1,00**
8	Old chamber wall	1003,0	1,00	10,03	2,03	0,20	**20,28**	0,13	0,00	0,00	0,50	0,13	0,29	0,45	0,54	**1,18**
9	Fungus comb	122,4	37,9	46,4	14,6	11,9	**31,46**	0,46	0,04	0,00	1,34	**7,26**	1,58	0,99	2,94	**0,30**

OC: organic carbon content. GM/AX : (Galactose + Mannose) / (Arabinose + Xylose)

From this first data set, it is hard to make generalizations yet, but it appears that the sugar content, both absolute and as % of the OC, is highest in the outer crust and in termite chambers.

The GM/AX ratio bears clues to the origin of the SOM, as galactose and mannose monomers originate mainly from bacterial metabolism, as where arabinose and xylose usually result from plant residue. The very low value for the fungus comb sample is explained by the fungal decomposition of the foraged litter and wood, yielding much xylose.

Table 2. Results of Lignin analysis

Sample	Description	OC [%]	Total phenolic content [mg]	Total phenolic content [mg/mg Corg]
1	Outer crust (5,5 m)	1,02	0,04081	0,00200
2	Inner section (4,8 m)	0,84	0,10790	0,00642
3	Central hive (3,5 m)	0,60	0,02972	0,00248
4	Inner section (2,2 m)	0,71	0,08148	0,00573
5	Mound foot (0,9 m)	1,31	u.q.	u.q.
6	Mound foot (-0,4 m)	0,39	u.q.	u.q.
7	Fresh chamber wall	1,11	0,19249	0,00868
8	Old chamber wall	1,00	0,19813	0,00992
9	Fungus comb	37,9	-	-

u.q. : unquantifiable trace quantities - : unquantifiable due to water interference

Table 2 shows only the total phenolic content, due to very low quantities of the individual components. Because of water interference during the sililation of the fungus comb sample, the lignin content could not be quantified. However, the chromatogram showed large amounts of phenolic compounds present, unlike in the other mound samples. It appears very little of this lignin in the foraged plant material remains after the combined digestion of the *Termitomyces* fungus and the termites.

Lack of a reference soil sample in this dataset limits the conclusions that can be drawn from these analyses. However, this exploratory research suggests a difference in the SOM characteristics of termite mound chambers as compared to the other mound samples. Future sampling campaigns, planned in may 2011, will yield a more exhaustive dataset to quantify these differences using the techniques mastered during this exchange visit.

Another promising topic to explore, is the OM characterization of multiple fungus comb samples of varying age and from various location. These structures at the heart of Macrotermitinae mounds are responsible for the very efficient decomposition of lignocellulosic matter. Elucidating these breakdown processes is key to understanding the impact of mound building termites on SOM dynamics. Besides a Lignin analysis, a cutin/suberin analysis and solid state 13C-NMR seem appropriate techniques.

4. Future collaboration with host institution

No formal plans for future collaboration are in place at the moment, but it is certainly possible. In any case, the made contacts are available for consult concerning future results.