1. Purpose of the visit

I applied compound-specific gas chromatography-combustion-isotope-ratio mass spectrometry (GC/C-IRMS) in combination with alkaline CuO oxidation to determine the stable isotope composition of lignin-derived phenols in heavy organo-mineral fractions from topsoils under Araucaria forest (C_3) and grassland (C_4) as well as from the respective above- and belowground plant materials at the Laboratoire de Biogéochimie et Ecologie des Milieux Continentaux (BioEMCo), INRA-CNRS-Univ. in Thiverval-Grignon (France). The soil and plant materials were sampled in the southern Brazilian highlands, where C_4 grassland has been replaced by Araucaria forest (C_3). A former study detected ^{13}C depletion in C_4 grasslands from plant to heavy organo-mineral fractions from the upper mineral soil and with increasing soil depth (Dümig et al., 2008). This phenomenon was also found for other C_4 grasslands, whereas enrichment of ^{13}C generally prevails in C_3 forests. It was suggested that the isotopic depletion in C_4 grasslands originates from the relative accumulation of ^{13}C depleted lignin and/or lipids owing to a lower decomposition rate compared to the isotopically heavier cellulose, at least in the early stages of biodegradation (e. g. Martin et al., 1990; Henderson et al., 2004). However, the extent to which lignin is degraded or stabilised in soils is still a matter of debate (e.g. Dignac et al., 2005).

The objectives were to characterise lignin sources and to evaluate if the prevailing ^{13}C depletion in grassland soils could be related to lignin preservation in the organo-mineral fraction.

2. Laboratory analysis

The quantification of lignin-derived phenols by cupric oxide (CuO) oxidation (Hedges & Ertel, 1982) was carried out for 23 plant, organic surface layer and organo-mineral fraction samples according to the modified method of Kögel & Bochter (1985). Briefly, the samples (50 mg for plants and organic surface layers, 500 mg for organo-mineral fractions) were oxidized with 250 mg CuO and 2 M NaOH at 172° C under N_2 for 2h. Afterwards, a standard containing ethylvanillin was added to assess the recovery of lignin products, which was usually in the range of 60 – 80 %. The solution was adjusted to pH 1.8 – 2.2 and left over night for humic acid precipitation. Thereafter, the lignin-derived phenols were purified by eluting through a C18 column and derivatized by adding BSTFA (N, O-Bis (trimethylsilyl) trifluoro-acetamide). The silylated lignin monomers were separated on a HP 6890 gas chromatograph equipped with a SGE BPX-5 column (50 m length, 0.25 mm inner diameter,

0.32 μm coating) and detected by flame ionization detector (GC/FID). The GC oven temperature was kept at 100° C for 2 min, then heated to 172° C at 8° C/min, to 184° C at 4°C/min and to 300° C at a rate of 10° C/min. The samples were injected in split mode (1:10). Phenol concentrations were calculated with the internal standard phenylacetic acid. The results are given as mean values obtained from at least two replications. CuO oxidation products are composed of vanillyl (V)-units (vanillin, acetovanillone, vanillic acids), syringyl (S)-units (syringaldehyde, acetosyringone, syringic acid) and cinnamyl (C)-units (ferulic and p-coumaric acids). The sum of V-, S- and C-type phenols (VSC) expressed as mg VSC g^{-1} SOC or mg VSC g^{-1} plant OC is used to estimate the amount of lignin.

The $^{13}C/^{12}C$ isotopic signature of the lignin-derived CuO oxidation products was determined by compound specific isotope analysis (Goñi and Eglinton, 1996). Samples were measured on a GC HP5890 coupled via a combustion interface to an Isochrom III isotope ratio mass spectrometer (IRMS) (Micromass-GVI Optima). A volume of 0.3 μl was injected in splitless mode. The same temperature program was used as for the quantification of lignins (GC/FID) and the CuO procedure was repeated 2-3 times as described above. The respective C18 column eluates were combined in order to obtain a concentration of each phenol in the extract higher than 2 nmol C/μl, which represents the detection limit of the GC/C-IRMS. Each composite sample was analyzed three times by GC/C-IRMS. The ^{13}C content of silylated phenol monomers were corrected for carbon addition during derivatization using BSTFA. In dependence of the phenol, derivatization introduced three to six carbon atoms with their distinct ^{13}C contents influencing the ^{13}C content of the phenol. Therefore, the $\delta\ ^{13}C$ values were corrected using the ^{13}C content of the derivatizing agent as described in Dignac et al. (2005). The mean $\delta\ ^{13}C$ values for V-, S-, and C-type lignin were calculated by weighting the ^{13}C contents of each monomer with its relative contribution to the corresponding lignin type. These values were used in combination with the concentrations of V, S and C to calculate the $\delta\ ^{13}C$ values of the total analyzable lignin VSC.

3. Results

3.1 Isotopic signatures of plant tissues and organic surface layers

Lignin content and $^{13}C/^{12}C$ isotopic signatures of lignin-derived phenols and total organic carbon (OC_{tot}) from plants and organic surface layers are presented in Table 1. The needles of the conifer *Araucaria angustifolia* and the grass *Andropogon lateralis* have OC_{tot} δ ^{13}C values typical for C_3 and C_4 plants of -26.5 ‰ and -12.3 ‰, respectively. The organic surface layers in Araucaria forest (C_3), composed of *A. angustifolia* and deciduous trees, have tree-derived isotopic signatures (-27.4 ‰ and -29.0 ‰).

All plant tissues and organic surface layers yield lignin phenols expressed as VSC lignins with $^{13}C/^{12}C$ isotopic signatures (weighted by the contents of the single lignin phenols) that are lighter (3.7 - 6.2 ‰) than their respective total organic carbon (OC_{tot}) δ ^{13}C values (Figure 1). These findings are consistent with the heavier isotopic composition of most carbohydrates in plant tissues relative to lignin (4 - 7 ‰) or to whole-plant material (1 - 2 ‰) (Deines, 1980; Benner et al., 1987). Other studies have reported similar values (2.0 - 7.0 ‰) for ^{13}C depletion of lignin compared to OC_{tot} of C_3 and C_4 plant tissue (e.g. Benner et al., 1987; Goñi and Eglinton, 1996). Organic surface layers in Araucaria forest show higher degrees of ^{13}C depletion than plant samples, possibly as a result of lignin contributions from deciduous trees with a greater extent of ^{13}C depletion. The isotopic difference between VSC lignins of the C_4 grass *A. lateralis* and the C_3 conifer *A. angustifolia* (or organic surface layers) amounts to 13.5 ‰ (or 17.7 ‰), which is in agreement with the difference between OC_{tot} δ ^{13}C values of 14.2 ‰ (or 15.9 ‰). The distinct ^{13}C isotopic signatures of C_3 vs. C_4 plants enables to relate soil organic matter to plant sources for VSC lignins in accordance with OC_{tot}.

3.2 Isotopic patterns in heavy organo-mineral fractions

Heavy fractions have VSC lignin contents which are about 14 times lower on average compared to plants and organic surface layers. The differences in ^{13}C abundance of VSC lignins between C_4- and C_3-derived heavy fractions (12.3 ‰) are similar to those of OC_{tot} (10.4 ‰). Consequently, VSC lignins and OC_{tot} of heavy fractions show similar trends in $\delta^{13}C$ values with increasing soil depth (Figure 1) and the isotopic signatures of both mineral-associated lignins and OC_{tot} of heavy fractions consistently indicate vegetation origin.

In all plant tissues, organic surface layers and C_3-derived heavy fractions the highest δ ^{13}C values were found for cinnamic phenols (p-coumaric and ferulic acids). $^{13}C/^{12}C$ isotopic signatures of lignin-derived phenols in natural soils have only been reported for maize (C_4)

and wheat (C_3) lignin from an agricultural field experiment (Dignac et al., 2005; Bahri et al., 2006). These studies and those of marine sediments (Goñi and Eglinton, 1996) also revealed that cinnamic phenols are ^{13}C enriched vs. vanillyl (V) or syringyl (S) units and sometimes OC_{tot}. The heavier isotopic composition of C-type units is attributed to a different biosynthetic origin from that of the other phenols or to their more direct pathway of incorporation into the lignin polymer of the growing plant tissue as the degree of ^{13}C depletion generally increases with the number of enzymatic steps required for biosynthesis (O`Leary, 1981; Goñi and Eglinton, 1996).

In contrast, C_4-derived heavy fractions show another pattern of ^{13}C contribution to V, S and C units (V > C > S). V units, partly also C units, have higher $\delta\ ^{13}C$ values relative to OC_{tot} (Figure 2).

In most C_3-derived heavy fractions the structural units of lignin are ^{13}C depleted relative to OC_{tot}. Only the humus-rich Oa horizon from soil FP shows homogeneous isotopic signatures for V-, S- and C-type phenols as well as OC_{tot}, indicating a C_3 origin (Figure. 2). In this soil, sampled in a patch of Araucaria forest, the forest was established more recently on grassland compared to other Araucaria forest topsoils with dominating C_3 derived SOC stocks (Dümig et al., 2008). The Ah1 horizon, which is about 20 cm deep, shows a C_3 origin for lignin and C_4-derived $OC_{tot,}$ indicating non-lignin components of the heavy fraction derived from under grassland. Thus, grass lignin must be largely decomposed and replaced by lignin from trees, which may point to higher turnover rates for lignin than total SOC. Similar results have been obtained for agricultural soils (Dignac et al., 2005).

3.3 From plant litter to soil organic matter: shifts in isotopic signatures in grassland and forest soils

Grassland soils (C_4) show ^{13}C depletion in the decay continuum from plant litter to heavy fractions of the upper mineral soil and with soil depth (Dümig et al., 2008). For samples which were analysed for isotopic signatures of lignin, the OC_{tot} $\delta\ ^{13}C$ values show ^{13}C enrichment (3.6 ‰) of C_3- and depletion (2.4 ‰) of C_4-derived heavy fractions relative to aboveground plant litter (Table 2). There are several mechanisms for isotopic shifts which may induce the prevailing ^{13}C enrichment for C_3 forest, whereas depletion of ^{13}C dominates in C_4 grasslands.

VSC lignin of heavy fractions shows higher $\delta\ ^{13}C$ values than those of plants or organic surface layers and the ^{13}C enrichment is greater for C_3- (5.2 ‰) than C_4- (1.3 ‰) derived

heavy fractions (Table 2). Bahri et al. (2008) detected depletion in ^{13}C for maize lignin phenols during decomposition during laboratory incubation. This unusual observation was explained by the preferential degradation of root vs. shoot derived lignin. Another study detected ^{13}C enrichment (about 2 - 5 ‰) in maize and wheat lignin from roots relative to lignin from leaves (Bahri et al., 2006). For OC_{tot} it was found that roots are enriched in ^{13}C relative to leaves and C_3 trees also show also greater enrichment (mean 3.6 ‰) than C_4 grasses (mean 1.1 ‰). Thus, lignin of heavy fractions may be isotopically heavier, either as a result of differences in ^{13}C content between lignin of above- and belowground plant inputs or isotopic fractionation during microbial decomposition.

Heavy fractions with a C_3 signature and tree roots show similar OC_{tot} ^{13}C enrichments relative to leaves, suggesting that the isotopic difference between above- and belowground plant inputs is the primary driver of ^{13}C enrichment between plant litter and the upper mineral forest soil. It also implies that lignin of heavy fractions is mainly derived from root material and lignin of aboveground plant litter is largely decomposed in the decay continuum from organic surface layer to the upper mineral soil. This is in agreement with the observations of higher decomposition rates for leaves than roots in soils (Bertrand et al., 2006) and that plant lignin content of roots is about two times greater than that of shoots (Rasse et al., 2005).

In subtropical highland soils, the VSC lignin contents of heavy fractions are low compared to temperate agricultural (Guggenberger et al., 1994; Kiem and Kögel-Knabner, 2003) or forest soils (Guggenberger et al., 1994; Rumpel and Kögel-Knabner, 2002) as well as temperate and semiarid grassland soils (Heim and Schmidt, 2007; Ganjegunte et al., 2005). This is may be due to favourable climatic conditions for degradation of SOM.

Lignin is a high molecular weight, three dimensional macromolecule and plant-residue lignin inputs to soils are large as lignin is the second most abundant component of vascular plant tiussue after cellulose (Crawford, 1981). However, it is not extractable as an intact polymer from soils. CuO oxidation or any other method based on the release of lignin monomers does not completely depolymerize lignin, and only phenolic monomers and dimers are released (Hedges and Ertel, 1982). Thus, CuO oxidation most probably underestimates the lignin content of heavy fractions, so lignin could contribute to ^{13}C depletion in grassland soils as they are ^{13}C depleted relative to OC_{tot}.

4. Tables and Figures

Table 1: δ ^{13}C values (‰) of bulk materials and lignin-derived phenols and VSC contents (mg VSC g^{-1} OC) from plants, organic surface layers and heavy organo-mineral fractions of soils in grassland (G), Araucaria forest (FP, F1, F2) and shrubland (S).

	C_3 conifer	C_4 grass	organic surface layers				heavy	organo-	mineral	fractions[c]			
	A. angustifolia	*Andropogon*	in Araucaria forest		G		FP		F1			F2	S
δ ^{13}C values (‰)	needle	*lateralis*	I	II	Ah1	Ah3	Oa	Ah1	Ah1	Ah2	2Ah2	Ah1	Ah1
bulk sample	-26.5	-12.3	-27.4	-29.0	-14.3	-15.1	-25.0	-17.3	-24.9	-24.4	-23.6	-27.7	-25.1
lignin-derived phenols													
vanillin	-31.3	-16.3	-32.2	-33.0	-14.8	-12.5	-26.0	-24.7	-27.0	-28.6	-27.8	-32.4	-30.6
acetovanillone	-31.5	-13.1	-32.5	–	–	–	–	–	-30.1	–	–	–	-29.7
syringaldehyde	-34.2	-20.3	-39.5	-35.5	-16.8	-19.8	-27.4	-27.2	–	-27.7	-26.9	-32.1	-31.3
vanillic acid	-31.2	-17.5	-36.2	-35.5	-11.8	-11.4	-26.4	-23.1	-31.8	-25.5	-24.7	-33.4	-34.0
acetosyringone	-31.9	-23.2	-30.1	-37.6	-20.3	-19.7	-25.1	-26.4	-30.6	-26.2	-27.0	-35.7	-31.8
syringic acid	-24.9	-20.8	-30.6	-35.0	-22.2	-13.8	-26.2	-26.0	-28.3	-26.0	-26.3	-33.7	-33.6
p-coumaric acid	-28.0	-14.3	-29.6	-29.2	-15.8	-15.3	-23.8	-20.4	-27.5	-24.1	-23.0	-28.5	-27.4
ferulic acid	-27.1	-13.8	–	-30.5	-13.6	-12.9	-25.6	-22.3	-28.0	-26.0	-24.5	-29.2	-28.2
means[a]													
V units	-31.3	-16.0	-32.9	-34.2	-13.0	-12.0	-26.2	-23.6	-30.4	-26.5	-25.7	-32.9	-30.4
S units	-28.5	-21.1	-34.8	-35.6	-20.0	-17.9	-26.2	-26.5	-30.0	-26.6	-26.4	-32.9	-32.5
C units	-27.7	-14.1	-29.6	-30.3	-14.3	-13.9	-24.8	-21.6	-27.9	-25.3	-23.9	-29.0	-27.8
VSC	-30.2	-16.7	-33.6	-35.1	-16.0	-14.8	-25.8	-24.1	-29.3	-26.1	-25.9	-31.7	-31.2
lignin contents (mg VSC g^{-1} OC)													
V units	15.7	15.1	16.9	7.7	0.5	0.7	0.4	0.6	2.5	1.3	1	0.3	1.5
S units	5.4	20.3	13.1	10.1	0.6	0.5	0.3	0.3	1.1	0.5	0.2	0.5	2.6
C units	3.3	30.8	3.1	3.7	0.5	0.2	0.2	0.2	2.1	0.8	0.2	0.3	0.6
VSC	24.3	66.2	33.0	21.5	1.6	1.4	0.9	1.1	5.8	2.6	1.5	1.0	4.7
C/V	0.2	2.0	0.2	0.5	0.9	0.3	0.7	0.5	0.9	0.6	0.2	1.0	0.5
S/V	0.3	1.3	0.8	1.3	1.0	0.7	0.8	0.6	0.5	0.4	0.2	1.7	1.9
$(ac/al)_v$[b]	0.16	0.14	0.26	0.85	1.53	0.77	0.99	2.69	2.68	2.37	1.19	1.09	1.33
$(ac/al)_s$[b]	3.44	0.38	0.70	0.27	1.12	1.46	1.08	1.48	1.42	1.45	2.43	0.21	1.56

[a] weighted by the contents of lignin-derived phenols
V = vanillyl units, S = syringyl units, C = cinnamyl units

[b] (ac/al)v = acid to aldehyde ratio of vanillyl units, (ac/al)s = acid to aldehyde ratio of syringyl units

[c] G: grassland; FP: patch of Araucaria forest; F: Araucaria forest; S: shrubland

Table 2: Mean δ ^{13}C values (‰) of vanillyl (V), syringyl (S) and cinnamyl (C) units of lignin and total organic carbon from plant sources and heavy organo-mineral fractions.

		mean	$^{13}C/^{12}C$	isotopic	signature*
C_4 signature	V	S	C	VSC	total OC
plant source[a]	-16.0	-21.1	-14.1	-16.7	-12.3
heavy fraction	-12.5	-18.9	-14.1	-15.4	-14.7
difference[b]	3.5	2.2	0.0	1.3	-2.4
C_3 signature					
plant source[a]	-32.8	-33.0	-29.2	-33.0	-27.6
heavy fraction	-28.0	-28.7	-25.7	-27.7	-24.0
difference[b]	4.9	4.2	3.4	5.2	3.6

* weighted by the contents of the individual lignin-derived phenols
V = vanillyl units, S = syringyl units, C = cinnamyl units

[a] C_4: A. lateralis, C_3: mean of A. angustifolia and both organic surface layers

[b] ^{13}C enrichment (+) or depletion (-) from plant to soil organic matter

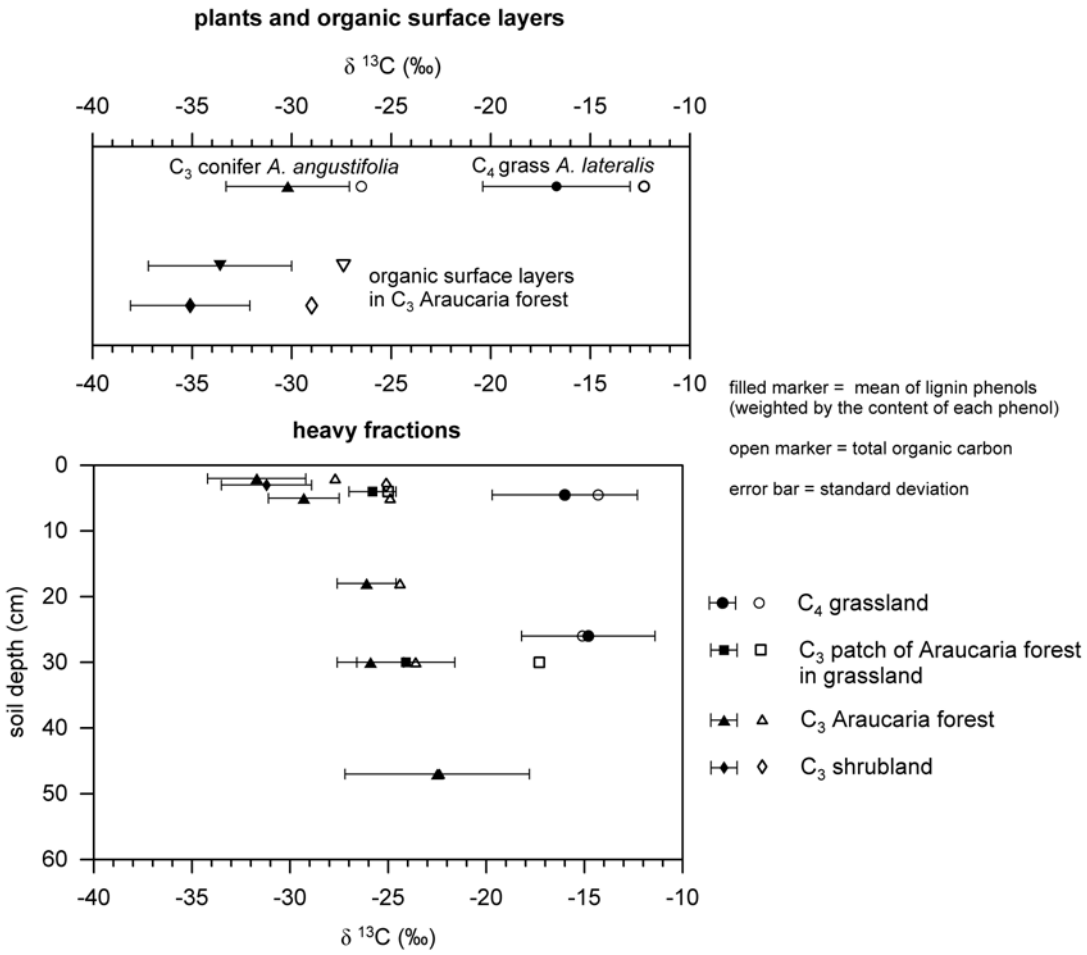

Figure 1: $\delta\ ^{13}C$ values (‰) of VSC lignins (weighted average of isotopic values is calculated by using the concentration of each phenol; standard deviation as error bar) and total organic carbon for C_3 *Araucaria angustifolia*, C_4 grass *Andropogon lateralis*, organic surface layers in C_3 Araucaria forest and heavy organo-mineral fractions.

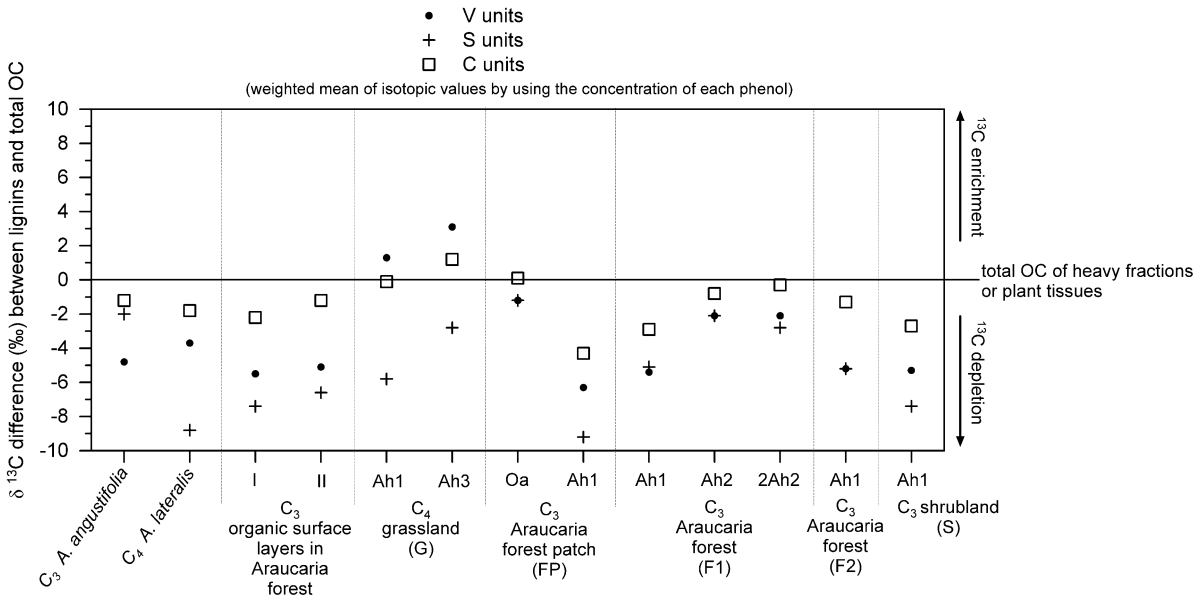

Figure 2: $^{13}C/^{12}C$ isotopic difference (‰) between total organic carbon and vanillyl (V), syringyl (S) and cinnamyl (C) lignin units (weighted average is calculated by using the concentration of each phenol) for *Araucaria angustifolia* (C_3 conifer), *Andropogon lateralis* (C_4 grass), organic surface layers in Araucaria forest and heavy organo-mineral fractions with C_4- and C_3-signature.

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6. Publications

One article is under progress and will be submitted in 2009 (Soil Biology and Biochemistry or Organic Geochemistry). The title of the article may be

"Evidence of lignin preservation in the organomineral fraction of soils with andic properties from compound-specific $\delta\ ^{13}C$ analysis"

or

"Lignins contribute to ^{13}C depletion in C_4 grassland soils as shown by compound-specific $\delta\ ^{13}C$ analysis"

In addition, I intend to combine the results from lignin analyses with those from ^{13}C NMR spectroscopy and sugar analysis (analysis still to be performed) for another publication.

8. Comments

The duration of the stay was not three weeks according to schedule, but only from 15.6.2009 to 25.6.2009 at the Laboratoire de Biogéochimie et Ecologie des Milieux Continentaux (BioEMCo). I reduced the stay because of technical difficulties with the GC/C-IRMS. Therefore, I could not measure 10 samples with the GC/C-IRMS. However, Dr. Cornelia Rumpel and the technician of the GC/C-IRMS measure and evaluate these 10 samples. I will obtain the results by the end of October 2009.