Final Report of a long-term exchange visits within the ESF MOLTER

1 Purpose of the visit

The aim of the scientific visit was to develop a new isotopic pool dilution technique that would enable us to study the dynamics of free amino sugars in litter and soils. The visit was also to have an exchange of expertise between the applicant's and the host's institutions. The host institute has a state-of-the-art knowledge in the field of pool dilution experiments with bio-available organic compounds (mainly neutral sugars and amino acids); the applicant has recently developed a new methodology for the precise measurement of the $\delta^{13}C$ of amino sugars in soils using LC-c-IRMS. Combining both expertise's would offer new research opportunities to study the dynamics of specific molecular biomarkers in soils and sediments.

2 Description of the work carried out during the visit

The first two weeks were dedicated to familiarize myself with the analytical instruments of the host lab, gathering different soil and forest liter samples and the development of an adequate sample purification and pre-concentration procedure using cation exchange resin. The development of a good purification and pre-concentration procedure was of capital important since free amino sugar concentrations in soils an litter (however only little literature data was available, the only literature handling the subject that was found reported the concentration of amino sugars in soil solution to be lower than there limit of detection (0.01 mg $N \cdot L^{-1}$)were assumed to be low.

The tested conditions were:

Cation exchange resin in H^+ elution using 3M NH_3, 1M HCl and 50mM NH_3, Na^+ form with elution using 50mM NH_3 and NH_4^+ form elution using 50mM NH_3. Reference solutions were applied on the cation exchanged resin pact on a plastic column, dissolved in both 30mL milliQ water and 30mL 10mM $CaSO_4$. The recovery of a wash step using a anion exchange resin in Cl^- were also tested.

During the next two weeks the extraction procedure itself was developed by evaluating the recovery of spiked soil and litter samples at different spike concentrations additionally the free amino sugar concentration of the different soil and litter types were determined. The tested samples were a soil sample of a beech forest, one year old litter of the same beach forest and soil of a mixed deciduous riparian forest at the border of the Danube.

Unfortunately we were not able to measure the isotopic concentration of the free amino sugars in the soil and litter samples due to technical problems with the LC-IRMS equipment at the moment these analyses were planed. Because of this the pool dilution experiment itself could not be performed. (These experiments will be further performed at the applicant lab.)

3 Description of the main results obtained

1) For the development of the purification procedure following results was obtained:

Ref Samples				
Form	**Elution**		**Recovery**	**Comment**
H+	HCl 3M	H_2O	Good recovery (between 70 and 100)	
		$CaCl_2$	Recovery ok but peak shap bad	Problem with salts (bad resolution, variable RT)
H+	NH_3 3M	H_2O	Good recovery (between 80 and 100)	Compounds are not totally stable in these condition, better results when 100µL 100mM HCl is added before second evaporation
		$CaCl_2$	Good recovery (between 80 and 100)	
H+	NH_3 50mM	H_2O	0	Probably not eluted (pH doesn't change enough in the vicinity of the binding sites)
		$CaCl_2$	0	
Na+	NH_3 50mM pH 3 and 4	H_2O	0	Too much salt
		$CaCl_2$	0	
NH4+	NH_3 50mM pH 4	H_2O	<50% (30ml)	Not totally eluted or not totally retained
		$CaCl_2$	<50% (30ml)	
Cl^-	Water	H_2O	between 90 and 100% recovery	

H^+ form with 3M HCl or 1M NH_3 seem to be the methods of choice to for sample clean up, additional purification could be done by passing the samples over an anion exchange column in Cl^- form. The down side of the 1M HCl method is that it elutes also the strongly bounded cations of the column, resulting in a pour chromatographic separation. The downside of the 3M NH_3 method is that the amino sugars have a limited stability in basic conditions.

2) The soil extraction experiments gave following results.

Sample	Solvent	Recovery (spiked samples)(%)		Soil concentration (ng/g (field wet soil))	
		GalN	GluN	GalN	GluN
Riparian soil	H2O	116 (± 14)	107 (± 24)	1.4(± 0.8)	3.2(± 2.2)
	10mM $CaCl_2$	105 (± 11)	95 (± 20)	0.3(± 0.1)	0.8(± 0.1)
Beech soil	H2O	113% (± 12)	96%(± 14)	0.9 (± 0.2)	1.4 (± 0.7)
	10mM $CaCl_2$	119%(± 4)	103%(± 14)	0.7 (± 0.1)	1.5 (± 0.2)
Beech Litter	H2O	-	83% (± 16)	-	82.3 (± 14.1)
	10mM $CaCl_2$	-	95% (± 10)	-	76.8 (± 13.7)

- Could not be determined due to a major interfering peak

i) The measured concentrations are the first successful attempt to measure amino sugar concentrations in soil and litter to our knowledge

ii) The use of an anion exchange purification step didn't improve the apparent purity of the resulting chromatograms significantly.

iii) Due to the much higher concentration and the relative apparent peak purity of the chromatographically peak for Glucosamine (GluN) compared to the soil samples. Pool dilution experiments will be most likely to succeed using the liter samples.

4 Future collaboration with host institution

No formal further collaboration with the host institute is agreed but it makes no doubt that both formal as well as informal collaboration with the host institute will take place in the future.

5 Projected publications/articles resulting or to result from your grant

The planed pool dilution experiment will definitely lead to a publication about the method development and the obtained results.