Activity Report

Olivier Duboc
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Analysis of soil lignin contents and its stable carbon isotope signature
in samples from three experimental sites and from translocated soils,
on an altitudinal transect in the Austrian Limestone Alps.

Supervisors: M.H. Gerzabek and F. Zehetner, Institute of Soil Research, University of Natural Resources and Applied Life Sciences, Vienna, Austria (BOKU)
Hosts: C. Rumpel and M-F. Dignac, Biogeochemistry and Ecology (BioEMCo), Centre INRA Versailles-Grignon, Thiverval Grignon, France

1 Purpose of the visit

The purpose of the visit was to provide additional information on organic matter decomposition in an experiment carried out in the Austrian Limestone Alps (EU-funded project CLISO (2006-2009)), which consisted in inducing soil warming by downslope translocation of soil cores, and in conducting in-situ decomposition experiments in three climate zones (1900, 1300 and 900 m above sea level (asl.)).

The objective was to investigate lignin decomposition on selected samples, using the alcaline CuO method (Hedges and Ertel, (1982), modified by Kögel and Bochter, (1985)) for quantification of the lignin derived phenols, and the characterization of their ^{13}C isotope signature.

2 Work carried out

The vanillyl (V), syringyl (S) and cinnamyl (C) phenolic compounds deriving from lignin monomers have been extracted from **34** Samples. Samples were:

- 3 soils[1] + 1 maize litter for substrate characterization at t=0. Measurements of samples at 1900 and 1300 m asl. as well as maize litter have been replicated once. Due to lack of time, the sample at 900 m asl. has not been replicated. These data were used to calculate the amount and composition of lignin in the mixed substrates, with the mixing ratio soil + maize = 0,73 + 0,27.
- 30 incubated samples corresponding to the **5 groups** of soil translocations (i.e.: 2 altitudinal translocations from 1900>1300 and from 1900>900 + 3 *in-situ* translocations) *** 3 replicates * 2 incubation years** (i.e. t=1yr and t=2yrs)

Quantification was made with a Gas Chromatograph coupled to a Flame Ionization Detector (GC-FID). The sum of VSC units has been quantified and served as an estimate of intact lignin structural units, and the ratios of acidic / aldehydic forms of V and S units (i.e. $(Ac/Al)_V$ and $(Ac/Al)_S$) was used as an indicator of lignin alteration, as these ratios have been shown to increase with lignin degradation (Kögel, 1986). C/V and S/V ratios were calculated as well, their decrease being related to lignin degradation.

A further question arose concerning the effect of sample drying temperature prior to analysis on monomer extraction: samples have been dried at 105°C, whereas OM characterization studies

[1] Soils at the study sites had high C content (Leptic Histosols) and thus should be referred to as „litter“. In order to differentiate them in the text from the incubated maize litter, they will be referred to as „soil“.

typically don't dry samples at more than 60°C. Therefore, the effect of drying temperature on the 3 soils and on the maize litter was also investigated. As for the other substrates dried at 105°C, the 60°C sample measurements were also **replicated once**, except at 900 m asl.

The planned analysis of ^{13}C signature of lignin derived phenols in the GC/C-IRMS could not be performed due to some technical problems, and because of limited time left for it (the phenol extraction took most of my time). Non-derivatized extracts have been conserved in the freezer for this purpose.

3 Results

No statistical analysis has been performed yet on the data. Therefore, results will be presented without referring to the significance of differences between the different treatments (i.e., altitude, time, etc...). Results are expressed here in mg g^{-1}, and still have to be converted to mg gOC^{-1} for further data interpretation.

3.1 Quantification of V, S, and C units in the *in-situ* translocated soils

The lignin in maize litter had a markedly different composition compared to that in soil substrates, with much higher S and C contents. Accordingly, the quantity of extracted phenols was higher in maize litter than in soils (Fig. 1). The determination of the phenol composition allows for a first characterization of lignin decomposition prior to isotope measurement.

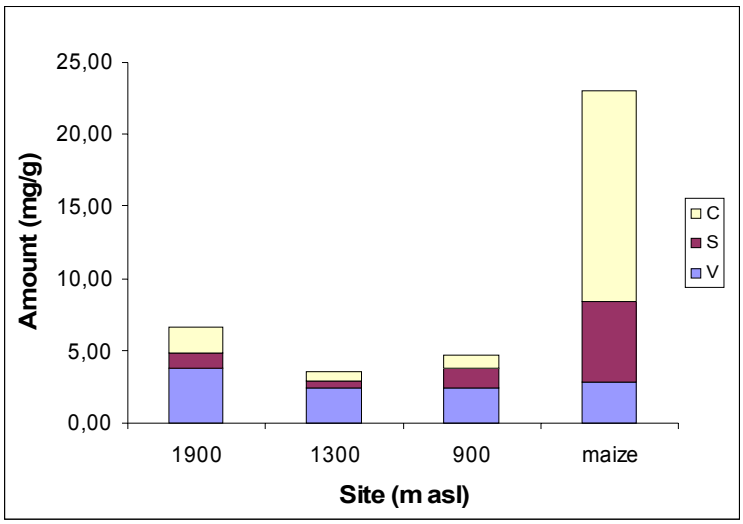

Fig. 1.: Amount of V, S, C units in the soil lignin at the three study sites and in the maize litter at t=0.

After two years of incubation, lignin content was lower than in the soil substrates. C and S phenols decreased more than V phenols (Fig. 2).

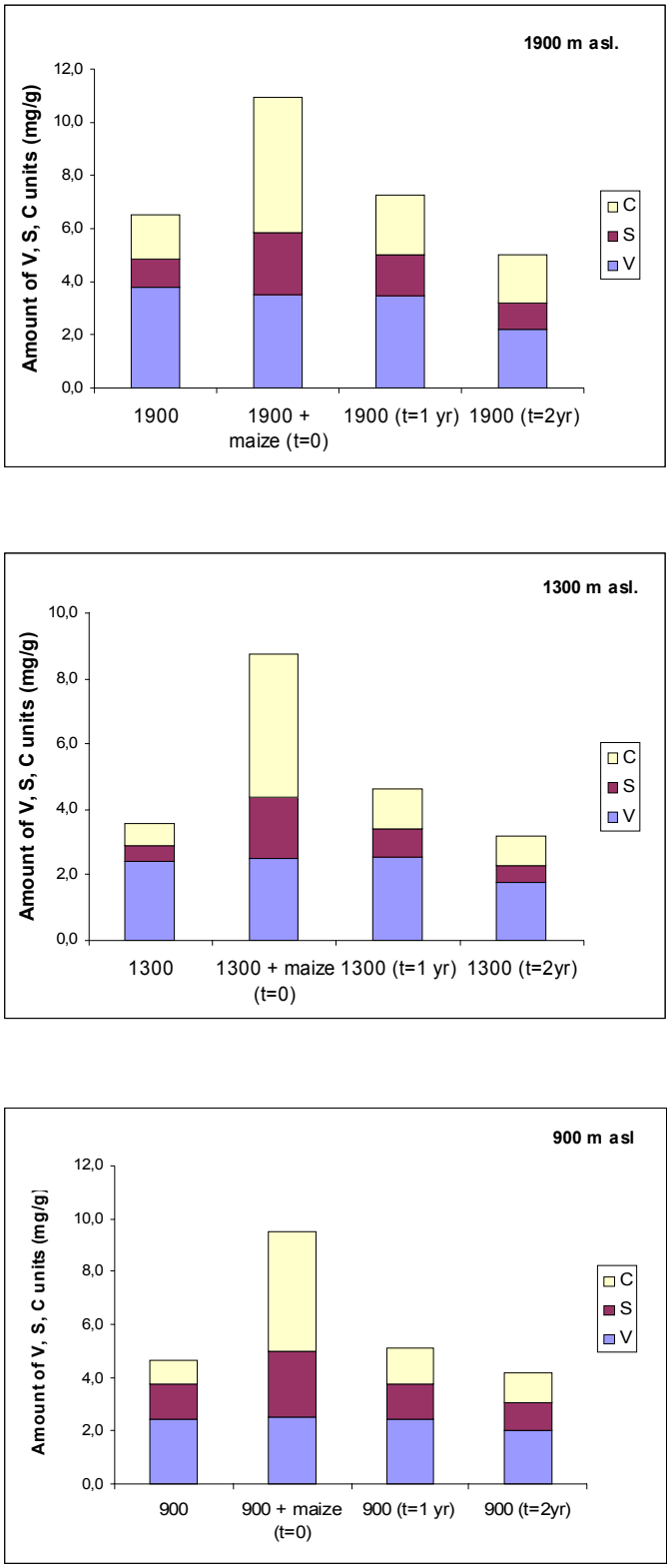

Fig. 2: lignin content and composition at the 1900, 1300 and 900 m asl sites: before maize application, and in mixed substrates at t=0, 1, and 2 years of incubation.

3.2 Lignin content and degradation indicators in altitudinal translocations

In the case of altitudinal translocations, substrate characteristics at t=0 were identical for the three incubation sites at 1900, 1300 and 900 m asl.

Lignin content in translocated soils decreased by about the half during incubation. After two years, the site at 1900 m asl showed the strongest lignin decrease, and the site at 1300 m asl showed the lowest (Fig. 3)

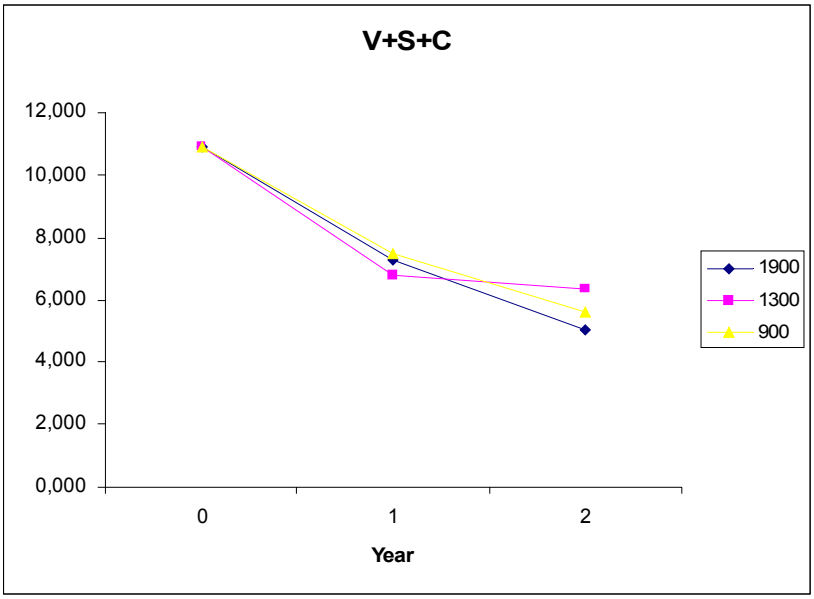

Fig. 3: Sum of V, S and C phenols in altitudinally translocated soils, during 2 years of decomposition.

Both $(Ac/Al)_V$ and $(Ac/Al)_S$ increased during the two years of decomposition, but with contrasting trends: while the $(Ac/Al)_V$ increased mainly during the second year at 1900 and 900 m asl, $(Ac/Al)_S$ increased more linearly at these two sites (Fig. 4). The 1300 m asl site showed different trends with more changes during the first year.

The C/V ratio decreased sharply during the first year and didn't change from year 1 to year 2, and S/V ratio followed a similar pattern but decreased more slightly than C/V (Fig. 4).

The increase of the first two ratios (i.e.: $(Ac/Al)_V$ and $(Ac/Al)_S$) indicated a stronger degradation of lignin at 900 m asl compared to higher elevation sites. Contrastingly, the decrease pattern of S/V ratio indicated a stronger degradation at 1900 m asl compared to lower elevation sites, and the decrease of the C/V ratio showed no clear trend.

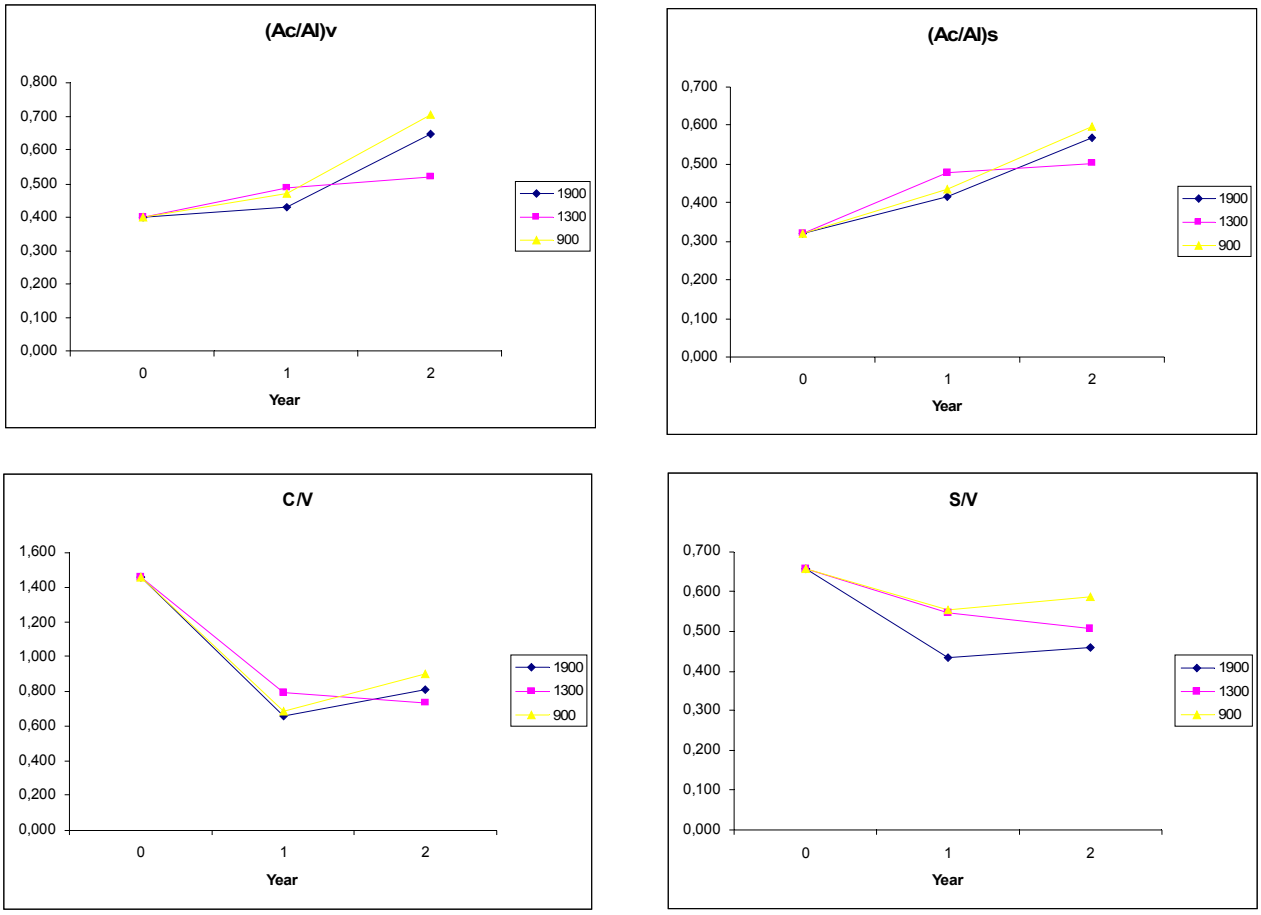

Fig. 4: Lignin degradation indicators in translocated soils during two years of incubation at the original elevation site (i.e.: 1900 m asl.) and at the translocation sites (i.e.: 1300 and 900 m asl.)

3.3 Effect of sample drying temperature on phenol extraction

The effect of drying temperature was limited within the range of 0 to -5% for the four analyzed samples (i.e. 1900, 1300, 900 m asl. soil + maize litter; Fig. 5). The strong standard deviations illustrate the high variability of the measured effect. The average results remain however within the precision range of the analytical method itself, indeed, there was a standard deviation of 10% (for the sum of VSC phenols, data not shown) between the standard soils from the 6 measurement series performed during the exchange visit.

Similarly, the difference of degradation indicators between samples dried at 60ºC and at 105ºC remained below 10% on average, and showed a high variability (Fig. 6).

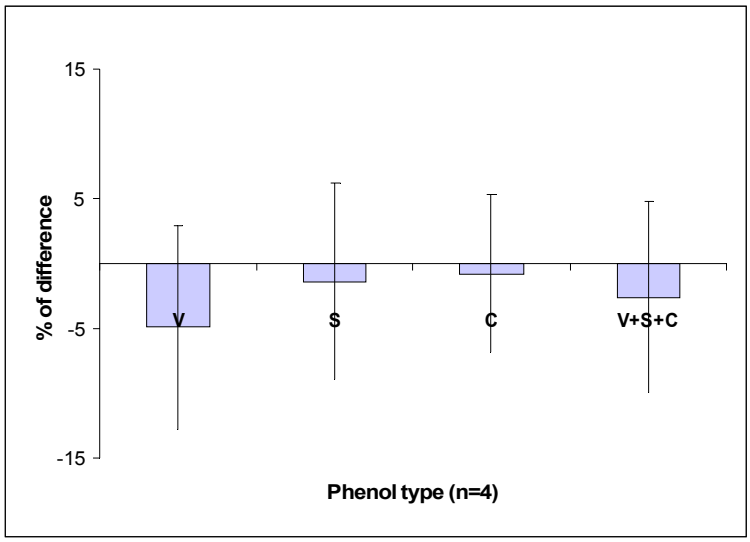

Fig. 5: Effect of drying temperature on phenol extraction (% of the difference between the value at 105°C compared to 60°C)

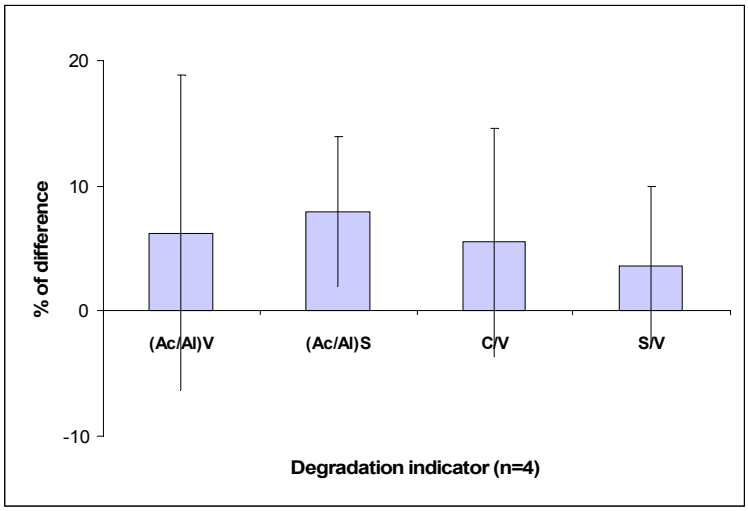

Fig 6: Effect of drying temperature on lignin degradation indicators (% of the difference between the value at 105°C compared to 60°C)

4 Planned publication(s)

The publication of the main results on lignin decomposition along the transect will be published together with the results obtained earlier in this study (i.e. ^{13}C and ^{15}N in bulk SOM and maize litter).

Furthermore, a short communication on the effect of drying temperature has been planned.

5 Other comments

Many thanks to the ESF and the Molter reviewer-team for making this enriching experience possible. I recently graduated from a MSc and my interests are manifold, so that such exchange visits are best suited in order to help me choosing the direction I want to give to my (scientific) career. Besides from this, the host-team was very nice and welcoming, and I had the occasion to share experiences with PhD / Post-Doc students working at the INRA.

Literature:

Hedges, J.I., Ertel, J.R., 1982. Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Analytical Chemistry 54, 174–178.

Kögel, I., 1986. Estimation and decomposition pattern of the lignin component in forest humus layers. Soil Biology & Biochemistry 18, 589–594.

Kögel, I., Bochter, R., 1985. Characterization of lignin in forest humus layers by high-performance liquid chromatography of cupric oxide oxidation products. Soil Biology & Biochemistry 17, 637–640.