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PhD Student: Anna Andreetta

Host Institution

UMR Bioemco
Batiment EGER
Campus AgroParisTech
78850 Thiverval Grignon
France

Marie-France Dignac

SOURCES AND DISTRIBUTION OF ESTER-BOUND LIPIDS IN MEDITERRANEAN FOREST SOILS

Introduction

The characterisation of topsoils has recently received enhanced attention particularly for environmental monitoring. Humus forms seen as morphological expression of biotic and abiotic soil component interaction, should be used as a cheep and reliable tools for the assessment of top soil properties (Wilson et al., 2001; Ponge et al., 2002). Several studies (Ponge et al., 1997; Ponge 2006) have demonstrated that morphological features of humus profile were connected with their physicochemical as well as biological properties.

Profile morphology, particularly with regard to the organic horizons, should be seen as feedback to trees and represent specific nutrient and carbon storage strategies. Litter action control and modulate resources (van Breemen et al., 1998; Northup et al., 1998).

Each plant species has a unique chemical composition, and also within a given plant the various tissues differ from one another in their chemistry. Since plant molecular structures are considered as a driver of C stabilisation in soil, tracing plant tissues and soil organic matter (SOM) can clarify SOM accumulation mechanisms. Molecular characterization of soil lipids often provides valuable bio-geochemical information about the impact of vegetation, microorganisms, and abiotic factors on the soil C sequestration processes. In addition to solvent-extractable lipid fraction, recent investigations have addressed macromolecules such as cutins and suberins as effective markers for above and belowground plant tissues (Mendez-Millan et al., 2010).

Cutin and suberin are bio-macromolecules common in vascular plants which primarily function as barriers to prevent water loss. Several studies reveal that plant tissue structure of Mediterranean sclerophyllous shrubs and trees is strictly related to the strategies they enact to cope with the stress

conditions typically of those environments. For this reason, the choice of these biomarkers may be particularly indicated for Mediterranean climate and environment.

Cutin is a major component of leaf cuticle and is mainly composed of short chain (C_{14}-C_{18}) hydroxy- and epoxy acids (Halloway, 1982). Suberin occurs in the periderm of roots and barks and is composed predominantly of long chain (C_{20}-C_{32}) aliphatic acids, diacids and ω-hydroxy acids and minor amounts of phenolic constituents (Kollatukudy and Espilie, 1989).

Incorporation of molecular techniques to chemical phenomena (surface chemistry) is very important because many biogeochemical processes and ecosystem functions are determined at this scale (Dahlgren, 2006). Research across multiple spatial scales is necessary to fully understand the large-scale response (e.g., global change) and the underlying processes regulating the response. Understanding plant-soil feedback in Mediterranean ecotype forest is a major challenge because of the fragility of such ecosystem characterised by strong ecological constraints, as long summer drought.

AIMS OF THE WORK AND VISIT

The purpose of the research stay at Bioemco (France) was to analyse cutin and suberin biomarkers in different soils, plant tissues and litter samples. The aim of this work was to evaluate the incorporation of cutin and suberin biomarkers specific for different litter components (leaves, acorns, woody component, flowers) and for root biomass in two different forest humus forms and the corresponding soils.

Sub-goals were to 1) identify litter components and root specific biomarkers through the quantification of plant inputs and soil contents of lipids and aliphatic monomers specific for cutins and suberins; 2) compare biomarker differences in terms of contents and composition between plant tissues and soils in the two different types of soils; 3) assess the degree of bioturbation and mixing of above-ground plant litter with the mineral soil (Nierop et al., 2004).

One purpose of the present study were also to check the hypothesis that since differences were found between *Quercus ilex* leaves and branchlets properties in the two study sites (Bussotti et al., 2002), then differences should be detected in lipid composition and content in the litter of the same sites.

MATERIAL AND METHODS

Study Sites

The study areas are two mixed Mediterranean forests of *Quercus ilex* (holm oak) in costal stands in Tuscany (central Italy) differing for their ecological features: a mesic site (Colognole, CL) and xeric

one (Cala Violina, CV). Previous studies (Bussotti et al., 2003; Bussotti et al., 2002) have found differences between the two sites in litter production, phonological behaviour and chemical composition of leaf litter because of different edaphic conditions (water availability).
The most represented matrix in litter are the leaves: 65%(CL) and 48% (CV) of the total litter production. Wood represented an important fraction, 19% (CL) and 29% (CV), followed by acorns, 7.3% (CL) and 13%(CV).

Sampling and sample preparation

Triplicates of each horizon were taken separately from the soil profiles. In addition samples of the litter and roots were collected. Litter separated in its major components: *Quercus ilex* leaves, acorns, woods, flowers and leaves from other plant species. Successively soil samples were ground at 200μm and plant tissues at 100μm.

Analysis of the biomarkers

Free lipids

Samples of soils (5 g), plant tissues and organic horizons (2 g) were extracted successively (three times) using dichloromethane/methanol (DCM/MeOH). The residues were air-dried and used for cutin and suberin depolymerization.

Cutin-suberin characterization (Mendez-Millan et al., 2010)

Saponification of the residues was used to release cutin and suberin biomarkers. Lipid-free samples (100 mg for plants and organic horizons and 1 g for soils) were refluxed for 18 h in an aqueous solution of potassium hydroxide in methanol. The solution was filtered and the residue washed with methanol/water. After conversion in their acidic form, lipids were extracted with dichloromethane. Depolymerisation extracts were dissolved in pyridine. Silylation with BSTFA containing 1% of trimethylchlorosilane was performed in order to transform hydroxy and carboxylic acid functions in their trimethylsilyl ether and ester derivatives (TMS ether/TMS ester).
Silylated saponification products were separated with a gas chromatograph (GC). The compounds of interest were chromatographically well resolved for plant tissues and soil samples in both sites.
The chromatograph of roots (Cala Violina) was reported in Fig.1 as example. Compounds were identified with an Agilent HP5973 Electron Impact (70 eV, scan range m/z 40-700) mass spectrometer (MS) according to their fragmentation pattern supported by comparison with published mass spectra (Eglinton el al. 1968; Hunneman and Eglinton 1971; Holloway and Deas 1973) and with mass spectra library (Wiley).

They were quantified by flame ionisation detection (FID) by using nonadecanoic acid standard, which was added prior to derivatisation.

RESULTS AND DISCUSSION

In this report I presented some of the results because the large amount of data needs to be further analysed and detected.

Compounds specific for cutin and suberin were identified according to their fragmentation patterns in tissue samples (*Q.ilex* leaves, roots, wood, acorns and flowers) and in soil samples for both sites, Cala Violina (CV) and Colognole (CL).

The different compounds are presented according to their chemical structures, displayed into chemical classes.

n-Carboxylic acids

The n-Carboxylic acids ranged from *n*-C_{16} to *n*-C_{28}. In all the plant tissues, the hexadecanoic acid and the unsaturated octadecanoic acids (*n*-$C_{18:1}$, *n*-$C_{18:2}$) dominated in the *n*-Carboxylic class.

n-alcohols

The *n*-alcohols were identified in very low concentration and were characterised by the predominance of *n*-alcohol $C_{26:0}$, *n*-$C_{28:0}$ and *n*-$C_{30:0}$ compounds.

ω-hydroxy carboxylic acids

The contribution of ω$C_{18:0}$ was dominating in roots and was higher than Q.ilex leaves in both sites.

Suberins are characterised by monomers with more than 20 carbon atoms with a predominance of ω$C_{24:0}$ and ω$C_{26:0}$ monomers. The detected contribution of the ω-hydroxy carboxylic acids with more than 20 carbon atoms to total ω-hydroxy carboxylic acids was lower in leaves than in roots. However the presence of these long chain compounds indicated that aboveground tissues are suberized.

α,ω-alkanedioic acids

The α,ω- alkanedioic acids were mostly realised from roots. The major monomers were $C_{16:0}$ diacid and $C_{18:1}$ diacid.

Mid-chain hydroxy acids

The class named “mid-chain hydroxy acids” comprise seven n-carboxylic mono or diacids bearing 2 or 3 hydroxyl groups 0 or 1 unsaturation. As suggested by Goñi and Hedges (1990a), the 9,10,18-trihydroxyoctadecanoic acid (9,10,18-triOH $C_{18:0}$) can be found as such in the biopolymer or be formed from the conversion of a part of 9,10-epoxy, 18-hydroxyoctadecanoic acid (9,10-epoxy, 18-OH $C_{18:0}$), since the epoxy function react upon basic hydrolysis to be converted into vicinal diols and vicinal methoxy-alcohol groups. The isomeric mixture of 9-methoxy, 10,18-dihydroxyoctadecanoic acid and 9-hydroxy, 10 methoxy, 18-hydroxyoctadecanoic acids (9-MeOH 10,18-diOH $C_{18:0}$ and 10-MeOH 9,18-diOH $C_{18:0}$) can derive from the 9,10-epoxy, 18-OH $C_{18:0}$ as well, and was used to quantify the epoxy compounds.

The 9-hydroxyhexadecan 1,16 dioic acid (9-OH $C_{16:0}$ diacid) was observed in all tissues, with higher contribution in leaves than in roots.

Peaks that were previously attributed to the 11,18-dihydroxy octadecenoic (11,18-diOH $C_{18:1}$) and to a mixture of 9,18- and 10,18- dihydroxy octadecenoic acids (x,18-diOH- $C_{18:1}$) (Mendez-Millan, 2010), were also identified in both, leaves and roots.

The isomeric mixture of (x,16)-dihydroxy hexadecanoic acids (x=8,9,10) (x,16-diOH $C_{16:0}$) were the most common monomers identify in *Q.ilex* leaves, while they were less abundant in roots if compared with other compounds. The 9,10,18-triOH $C_{18:0}$ was identified in all tissues and it was, together with $\omega C_{18:1}$, the major compound in roots.

Short-chain (C_{14}-C_{18}) acids with hydroxy and epoxy groups in the mid chain position are typical constituents of cutin and suberin (Holloway 1982; Kolattukudy and Espelie 1989; Kogel-Knabner, 2002). The cutin composition is similar to suberin, but suberin contains only the C_{16} and C_{18} hydroxy and epoxy acids whereas wider range of C_{14} to C_{18} were observed in cutin (Holloway 1982; Kolattukudy and Espelie 1989). The identification of the C_{14} to C_{18} mid-chain-substituted acids in Q.ilex Mediterranean forest soils indicate that cutin and suberin are important plant inputs in the samples studied.

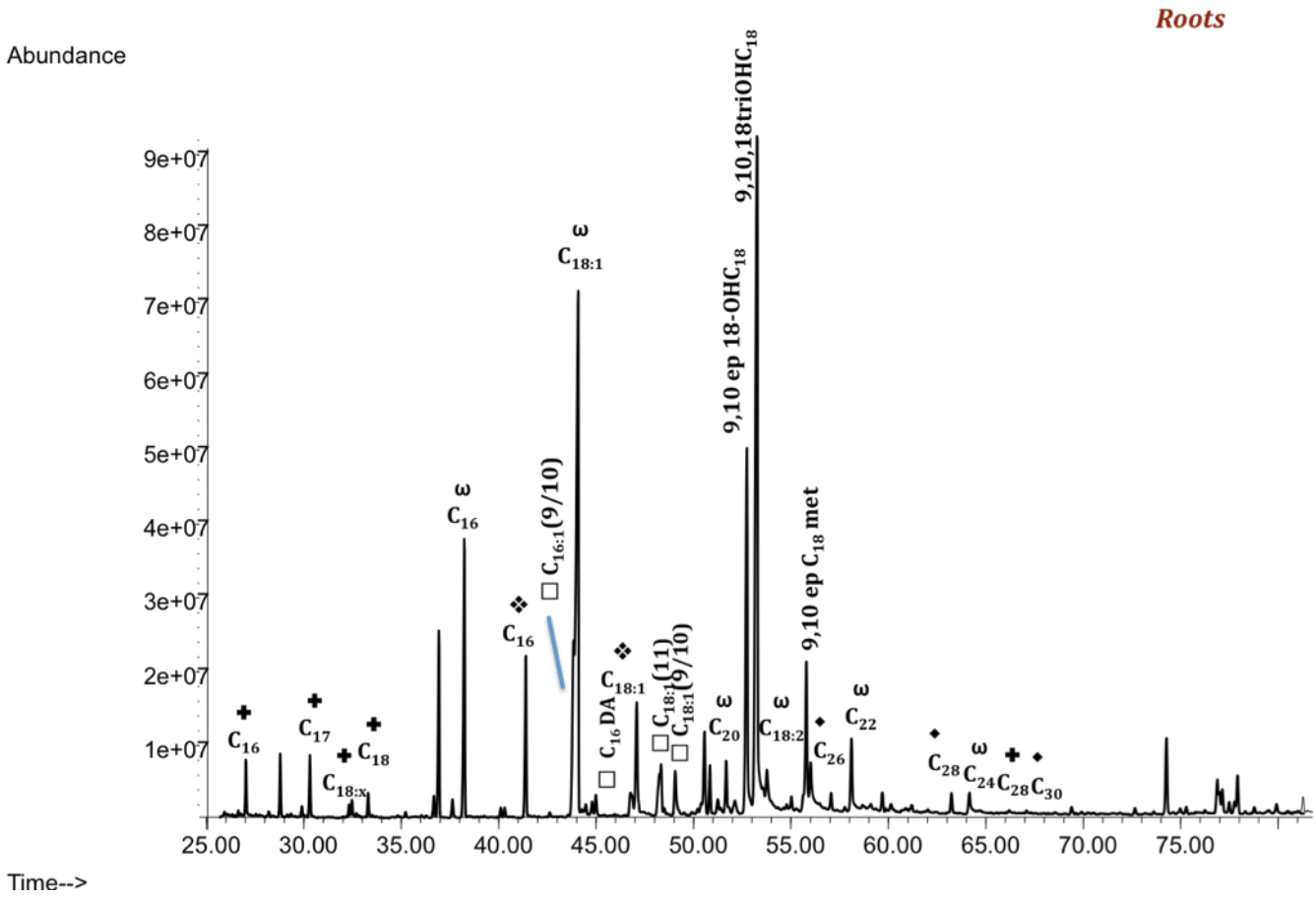

Fig. 1. Chromatographic separation of the aliphatic compounds with GC/MS. Total Ion current traces obtained from the saponification of roots. Legend: ◆ n-alcohol; ✚ n-carboxylic acids; **ω** ω-hydroxy carboxylic acids; □ mid-chain hydroxy acids; ❖ α,ω-alkanedioc acids. $C_{n:1:}$ indicates chain length and the number of double bonds. $C_{n(9/10/11):}$ indicates chain length and the position of the mid-chain hydroxy group.

Source specific composition of cutin and suberin in forest soils

The compositions of hydrolysates from Mediterranean *Quercus ilex* forest soils are comparable to previously reported base hydrolysis products of forest soils and confirm the conclusion of the other studies, namely that suberin and cutin are major sources of hydrolysable aliphatic constituents in soils.

Colognole

Colognole soil profile is characterised by an Amphi mull humus form with the first mineral horizon (A) macro-structured. The organic layers (OF and OH) presented about the same compounds, except for the absence of the $\omega C_{18:1}$ in OF. Probably because the contribution of root tissues in OF is negligible. The importance of 9,10,18-triOH $C_{18:0}$ and Epoxy $C_{18:0}$ was diminished compared with

plant tissues in the correspondent litter layer. The isomeric mixture of x,16-diOH $C_{16:0}$, $\omega C_{18:1}$ and *n*-$C_{16:0}$ were predominant in both mineral horizons (A and AB) analysed.

Cala Violina

The humus form of Cala Violina is a Mull with the first mineral horizon (AE) meso-structured. All the main compounds were identified. The eluvial E horizon was characterised by a decreasing of all the peaks; the predominant monomer was 9,10,18-triOH $C_{18:0}$ followed by x,16-diOH $C_{16:0}$, $\omega C_{18:1}$. In the below horizon EB, a major number of monomers were distinguished, with higher peaks than in E horizon.

The composition of cutin and suberin monomers in the soils is similar to the patterns observed in the corresponding vegetation reflecting the preservation of plant biomolecules such as cutin and suberin.

Plant tissues CV vs. CL

Q.ilex leaves

The isomeric mixture x,16-diOH $C_{16:0}$ was the predominant peak in both sites, with a major extension in Cala Violina (CV), followed by Epoxy $C_{18:0}$ and 9,10,18-triOH $C_{18:0}$ in CV, while in CL epoxy and trihydroxy monomers were not abundant. Differences founded between Q.ilex leaves aliphatic compounds could be related to different condition of water availability between the two studied sites.

Roots

The chromatographs for root samples were very similar in both sites. Without a precise quantification of monomers was not possible to highlight any similarity or dissimilarity.

After the first identification of monomers specific for above and belowground plant tissues and soil samples, I will quantify the main compounds to better compare the properties of plant tissues from different plant organs and between the two studied sites. This allows to deepening differences that have been already found, as the aliphatic monomer compositions in Q. ilex leaves that seem to be influenced by water stress conditions. Furthermore consideration about sources and degradation of organic matter in two different types of soil should be possible, since monomers specific for cutin and suberin were found also in soil samples.

FORTHCOMING ACTIONS

- 19/23 September 2010. Participation at the "SOM 2010-Organic Matter Stabilisation and Ecosystem Function" conference organised by BIOEMCO with a poster, which will show some of the results based on my work on suberin and cutin biomarkers, granted by European Science Foundation, within the Molter program.
- A publication in collaboration between Di.P.S.A. (University of Firenze) and BIOEMCO.

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