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Impact of agricultural practices on the quality of soil organic matter

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To my parents,
my grandparents
and "ma petite sœur".

Summary

The work quantifies the losses of organic carbon from no tillage soils (NT) compared to those under tillage (T) and characterizes the soil organic matter. Soil samples collected at different depths were taken in Potshini site (South Africa). Three analyzes were done:
Elemental analysis - isotope ratio mass spectrometry (EA-IRMS), to quantify carbon stocks in the different soil types and to evaluate the changes in isotopic ratios ($^{13}C/^{12}C$).
Curie point pyrolysis - gas chromatography - mass spectrometry (Py-GC-MS) to determine semi-quantitatively the soil chemical components.
Nuclear magnetic resonance spectroscopy (NMR) used as a semi-quantitative technique to understand the state of degradation of soil organic matter.
The main results show that NT samples have a greater stock of soil organic carbon and a higher lignin content that T samples; especially NT crust layer is characterized by a greater abundance and diversity of molecules, with a lower extent of degradation of soil organic matter.

Riassunto

La tesi sperimentale si propone di quantificare e confrontare le perdite di carbonio organico dai terreni sottoposti a semina diretta (NT, senza lavorazione) e dai terreni sottoposti ad aratura tradizionale (T). Campioni di suolo sono stati prelevati a differenti profondità nel sito di Potshini (Sud Africa) e analizzati tramite:
Analisi elementare - spettrometria di massa isotopica (EA-IRMS), usata per determinare lo stock di carbonio organico e per valutare i cambiamenti dei rapporti isotopici ($^{13}C/^{12}C$) nei diversi terreni.
Pirolisi a punto di Curie - gascromatografia - spettrometria di massa (Py-GC-MS), per caratterizzare e determinare in maniera semi-quantitativa i composti chimici presenti nel suolo.
Spettroscopia di risonanza magnetica nucleare (NMR), tecnica semi-quantitativa che permette di stabilire il grado di decomposizione della sostanza organica.
I risultati principali mostrano come i campioni prelevati dal terreno NT contengano una maggiore quantità di carbonio e un alto contenuto di lignina rispetto a quelli prelevati dal terreno T; in particolar modo, la crosta del terreno NT presenta una maggiore abbondanza e diversità di composti chimici, caratterizzati da un minor grado di decomposizione.

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Chapter 1

Introduction

1.1 Context of the research project

The drastic build-up of greenhouse gases (GHGs) such as carbon dioxide (CO_2) and methane (CH_4) in the atmosphere inexorably affects ecosystems. It is because soils exchange carbon (C) with the atmosphere through photosynthesis and decomposition of organic matter, and that the soil pool, the biggest of terrestrial ecosystems has been drastically reduced by human activities (Lal, 2003a), that the current rise in atmospheric GHGs concentration is thought to be potentially mitigated by C sequestration in soils (Batjes, 1998; Lal, 2003b). In this context, understanding the balance between soil C inputs and outputs and their mechanisms and factors of control is key to improve C sequestration in soils while supporting important ecosystem functions such as the storage of nutrients, the production of food along with a filter function and a buffering capacity. Furthermore C is the main energy source for microorganisms.

SOC stocks. The soil organic carbon (SOC) stock is the largest C stock of terrestrial ecosystems, with a total amount of 2500 gigatonnes (Gt), of which 1550 Gt of organic carbon (OC) and 950 Gt of inorganic C (IPCC 2011). This reserve has decreased considerably (50-60 %) due to changes of use of soil, such as deforestation, soil tillage (T) and drainage.
These practices affect SOC, stabilized initially through chemical, physical or biochemical processes (Christensen, 1996; Stevenson, 1994). They have dramatically changed SOC contents (Edwards and Bremmer, 1967; Elliot, 1986; Tisdall and Oades, 1982, Six et al., 2000). T disrupts soil structure and soil aggregates, which increases the rate of soil organic matter decomposition due to exposure to microbial oxidation (Dick, 1983; Elliott, 1986;

Powlson et al., 1987; Reicosky et al., 1995).
In contrast, when agricultural land is no longer used for cultivation and allowed to revert to natural vegetation, SOC can accumulate by processes that essentially reverse some of the effects responsible for SOC losses from when the land was converted from perennial vegetation: Post and Kwon (2000) studied the factors that determine the direction and rate of change in SOC content when soil management practices changed, and showed an increase of the proportion of light SOC fractions (highly decomposable) in particular placing organic matter deeper in the soil (directly by increasing belowground inputs or indirectly by enhancing surface mixing by soil organisms) and ingreasing physical protection through either intra-aggregate or organomineral complexes.
Deforestation changes SOC stocks and soil organic matter (SOM) quality.
To clarify, in the text the term SOC is used to talk about stocks; SOM to talk about quality.
CO_2 emission through deforestation and conversion to cultivated fields, as reported by studies carried out in Brazil (Tivet et al., 2013), reduce both labile (hot water extractable organic carbon - HWEOC, carbon in acid-extracted polysaccharides - CTPS, particulate organic carbon - POC) and stable fractions (mineral-associated organic matter, $< 50\mu m$ - MAOM). In particular, at Ponta Grossa site, it was measured an average reduction, in the first 5 cm of soil, of respectively 56 and 45 % for HWEOC and CTPS. Similar results were found in a study conducted in Spain under rainfed Mediterranean conditions, with reduction of SOC of up to 40 % (Blanco-Moure et al., 2013).
The suppression of T through the use of no-tillage (NT) practices (where crop residues or other organic amenities are retained on the soil surface and sowing/fertilizing is done with minimal soil disturbance) entails larger C and N stocks than in soils under T (Neto et al., 2010; Paustian et al., 1997; Sá et al., 2001). This was shown to be due to a better stabilization and protection of SOC (Six et al., 2002).
"Intensive" NT systems (e.g. diversity of cover/relay crops and high annual biomass input) at the tropical Lucas do Rio Verde site, increased HWEOC, CTPS, and enhanced the short-term process of SOC stabilization in the mineral-associated fraction. In addition, relatively higher resilience indices of POC and MAOC fractions were observed under NT systems with high biomass input (Tivet et al., 2013).
Arshad et al. (1990) have made a study on a Donnelly silty loam soil in Canada, under NT and T. The C (and N) contents of the NT soil are 26 % higher than those of the CT soil, a result observed also by Blevins et al. (1977, 1983); the pH of NT soil was significantly lower than the pH of T soil: increased amounts of organic C under NT may have contributed to the acidification (Dalal, 1989). Also the soil structure (clearly better in no-plowed soil) may be important for SOC storage, thanks to the physical protection of

SOC against mineralisation (due to its inaccessibility to microbial attack); a well-structured soil also reduces the detachment of fine particles that contribute to losses of SOC by erosion, particularly in semi-arid and subhumid tropical soils (Feller and Beare, 1997).
However, a great number of investigation with contradictory results were done to assess the C storage of soil under NT and T practices and some articles do not see NT practices as a possible way to improve SOC stock (Baker et al., 2006).

SOM quality. Not only do shifts in land management affect SOC stocks, but also SOM quality. There is thus a need to also evaluate this: several studies were conducted on the quality of SOM.
The solid-state ^{13}C NMR is a technique used to characterize qualitatively the SOM by analyzing the 4 main chemical families identified in the spectra: alkyl, O-alkyl, aromatic and carboxylic regions (Kögel-Knabner, 2000 and 2001).
Study by Baldock et al. (1997) shows lower alkyl C/O-alkyl C ratios (A/O-A, useful index of extent of decomposition) for NT compared to T; it was observed that alkyl C increased and O-alkyl C decreased with the advancement of extent of SOM decomposition (increase of A/O-A ratio). Besides, the decrease of A/O-A ratio may be due to the input of fresh soil organic matter.
The impact on soil aggregates status of NT and T pratices applied on a Leptic Typic Xerorthent soil in Jerez de la Frontera was studied by Panettieri et al. (2013). They confirm with solid-state ^{13}C NMR that T pratices caused changes in the characteristics of SOM (higher A/O-A ratio). The article also assesses the effect of T for parameters that have a fast reponse to soil management, such as total organic carbon (TOC), permanganate oxidizable C, and microbial biomass C. All these parameters show a clear reduction with T; for these reasons, the authors conclude that T techniques should be completely avoided.
The study demonstrates that also the β-glucosidase activity (enzymatic activity that hydrolyzes the polysaccharides to release glucose) is lower under T.
Extracellular soil enzymes hold a cardinal position in nutrient dynamics by regulating bioavailability of elements, and hence are linked with soil health: they play crucial role in transformations of C, nitrogen (N), phosphorous and sulphur at fundamental level of nutrient cycling; these parameters were proposed as sensitive indicators for soil quality, health, and physico-chemical properties (Dick, 1997; Sinsabaugh et al., 2008; Hartmann et al., 2009; Finkenbein et al., 2012). These indicators become even more significant for assessing the performance of different management practices in culti-

vated soils aiming to improve soil fertility. Soil enzymes were analyzed by Pandey et al. (2013) at Agriculture farm of Banaras Hindu University (India): the study revealed that activities of β-glucosidase, cellobiohydrolase, glycine-amino peptidase and alkalyne phosphatase were the highest under NT, while low SOC content and regular disturbance under the conventional T practice led to lower enzymatic activities.
The disruption of macroaggregate, the high degradation status of SOM together with the reduced input of fresh plant residues left on soil surface dramatically worsened the soil quality under T.

Soil crusting. NT, T and cropping management systems are critical components for reducing raindrop impact on soil particles due to the avaliability of crop residue to protect the soil surface. Excessive T can damage soil structure, leading to increased soil erosion and soil crusting. Crusts are thin soil surface layers more compact and hard, when dry, than the material directly beneath. The surface crust is caused by a breakdown of soil aggregates due to raindrop impact: the raindrop splash detaches particles that fill soil pores. Then, when rapid drying occurs, a hard crust layer can form, in the top of the soil. Generally, two main types of physical crust are distinguished by their mode of formation (Chen et al., 1980): structural crust (developed in situ) and depositional crust (formed of particles which were transported from their original location). Studies in Africa have led to a more detailed classification (Casenave and Valentin and Casenave, 1989; Bresson and Valentin, 1990; Valentin, 1991).
Soil crusting has many effects on soil properties. Crust formation markedly reduces the macroporosity of the soil surface layer: the first study of the effects of crust formation on infiltration was presented by Duley (1939). Infiltration can be reduced even in very sandy soil due to the formation of a crust as observed in Niger by Valentin (1981) and in Mali by Hoogmoed and Stroosnijder (1984), and this can cause runoff and floods (Valentin, 1981; Albergel, 1987; Casenave and Valentin, 1992).
In contrast, soil crusting can have some beneficial effects: in the northern fringe of the Sahel, even where rainfall water infiltrates totally, it is still insufficient to grow any crop; here; runoff from crusted soils is collected naturally or artificially and concentrated over a surface where conditions are more favourable to infiltration.
Crusts influence water erosion: on the other hand, as mentioned, they reduce infiltration and encourage runoff and erosion; on the other hand, they limit detachment because of their strength (Kowal, 1974; Roose, 1973; Remley and Bradford; 1989) and further impoverishment in clay particles (Valentin, 1981 and 1991). To distinguish this, one must consider the spatial scales of observations: at the plot scale, crusts can protect soil from water erosion; at

larger scale, heavy runoff produced by crusts upslope generally fosters more severe rill and gully erosion downslope (Planchon et al. 1987).
Soil crusts influence also the eroding impact of wind which sorts particles, removes and deposits them. Loose particles of the sandy crusts can be readily removed by wind leaving an erosion crust which is more resistant to wind erosion.
Because of the reduction of the water infiltration into soil, crusts deplete the possible water storage and the change of germination; moreover, they form a mechanical obstacle for seedling emergence.
The soil structure of NT system stands up better than T system against raindrops and this property is even improved if it includes high amounts of crop residue (Al-Kaisi, 2012); also T properly executed, appropriate to the conditions (e.g. soil texture, soil moisture, energy and time) can destroy the existing crusts but at the same time it offers the best conditions for the formation of the new ones.
The crust, in certain cases, may have biological origin and not physical. This crust is characterized by a high C content, since it is composed by communities of living organisms, most often fungi, lichens, cyanobacteria, bryophytes, and algae in varying proportions. The formation of the biological layer typically occurs in arid- and semi-arid ecosystems. They perform important ecological roles including carbon fixation, nitrogen fixation, soil stabilization, alter soil albedo and water relations, and affect germination and nutrient levels in vascular plants (Belnap et al., 2003).

Isotopic ratio. Another way to characterize the soil is to evaluate C isotopic abundance (^{13}C and ^{12}C). A useful index used to describe this is $\delta^{13}C$. The $\delta^{13}C$ method is increasingly used to follow C dynamics in the soil (Balesdent and Mariotti, 1996; Collins et al., 1999): the presence of C_3 or C_4 plants (that have a different photosynthetic pathway) and carbonates (that raise values) affect the soil $\delta^{13}C$.
For examples, as mean values (Ghashghaie 2001):

$\delta^{13}C_{CO_2\,air} = -8\,‰$
$\delta^{13}C_{C_3\,plant} = -27\,‰$
$\delta^{13}C_{C_4\,plant} = -11\,‰$

Balesdent et al. (1990) have done a detailed study on the effect of T and NT on SOM mineralization estimated from ^{13}C abundance in wheat and maize fields (at Boigneville, France). SOM from C_3 vegetation (wheat) is heterogeneous: the measured $\delta^{13}C$ values varying by as much as 3 to 4 ‰. In T plot it can be seen that topsoil OC ($\delta^{13}C = -25,2\,‰$) was enriched in ^{13}C compared with plant litter ($\delta^{13}C = -26,3\,‰$), and that deeper horizons (more than 30 cm) were richer in ^{13}C (up to $\delta^{13}C = -23,7\,‰$) than the

topsoil. NT plot also showed such enrichment. This enhancement of ^{13}C with depth may be due to partial mineralization and humification processes. In maize (C_4 plant) field, in NT plot, the enrichment in OC at the topsoil ($\delta^{13}C = -12,5\,‰$) is clearly visible, as well as its distribution with depth; instead there is a perfect homogenization, caused by ploughing, in T plot. The $\delta^{13}C$ is always correlated with the age of SOM: the enrichment can be explained by (i) isotopic fractionation associated with microbial respiration or fermentation (Macko and Estep, 1984), (ii) faster mineralization of ^{13}C-poored components (Nissenbaum and Schallinger, 1974; Benner et al., 1987) and (iii) decrease in the $\delta^{13}C$ of CO_2 with time in the past, as result of fossil combustion (Suess effect) over the last 150 years.

1.2 Presentation of Project DESTOC

PROJECT DESTOC: Understand the loss of organic carbon from soils in order to improve the modeling and to develop engineering methods suitable to the long-term sequestration of carbon.

1.2.1 Objectives

The research project DESTOC, in which I am involved, seeks to identify and understand the losses of OC from soil under NT compared to those under T and characterize qualitatively the SOM. The soil samples were collected in South Africa (Potshini site of KwaZulu-Natal) from parcels under T, NT and overgrazing without plowing (OV) condition (explained in subsection 2.1.1).

The project focuses on 4 main points:

- Impact of agricultural practices on soil physical properties.
- Impact of agricultural practices on soil chemical properties (in which I work).
- Impact of agricultural practices on soil biological properties.
- Relationship between soil properties and OC losses in dissolved, particulate and gaseous form.
- Impacts of pedo-climatic conditions on cultivated soils and OC losses.

1.2.2 Methods

The sampling campaign was carried out in June 2013, 19 months after crop (*Zea mays* culture), and includes:

1. the collection of samples, for each soil type, every 2 cm, up to 10 cm deep. The objective is to follow the effect of the different agricultural practices on the dissolved organic carbon (DOC) and the distribution and quality of OC in the surface horizon;
2. the collection of soil clods to measure the structural stability and the granulometry of the aggregates present in the 3 soil types;
3. the collection of about 50 soil cylinders 5 cm deep (only for T and NT), first to analyze and compare the CO_2 release (during an incubation of 15 days in laboratory) and after to evaluate the OC quality with different analysis.

For the CO_2 measures, soil was treated in different ways: the analysis was carried out on cylinders not disturbed and at different degrees of compaction. The project also includes a new sampling of the same African soil parcels, in order to observe the time evolution of the physical, chemical and biological. In addition, the losses of OC due to the erosion of agricultural parcels are evaluated through rain simulation experiments on the soil of INRA (Institut National de la Recherche agronomique) at Orléans.

1.2.3 Preliminary results (2013)

The first results obtained indicate that the soil plots under NT release less CO_2 than those under T (figure 1.1).
For both parcels, the removal of the surface crust leads to a decrease of CO_2 release (figure 1.2), suggesting that this layer is biologically the most active. Instead, the response to the compaction is different for the two types of soil: this latter in soil under NT tends to slightly increase the CO_2 release, but in soil under T it decreases significantly the CO_2 release. This might suggest that overgrazing and compaction associated with it may partially compensate for the destocking of C induced by plowing.

The results obtained from the first analyzes on the DOC quantification show that:

- Soil under T present a certain homogeneity of the DOC contents in the first 10 cm (about 0,2 mg DOC/g soil). There are no particular differences between the crust and the lower layers.

- In soil under NT, DOC appears to increase with depth.
- DOC content of soil under OV seems particularly variable, perhaps due to a greater quantity of maize residues in the soil compared to the other soil parcels.

However, DOC contents do not seem to be significantly different between the soils under T and NT.
Instead, microbial activity and microbial biomass C are significantly lower in NT parcel.

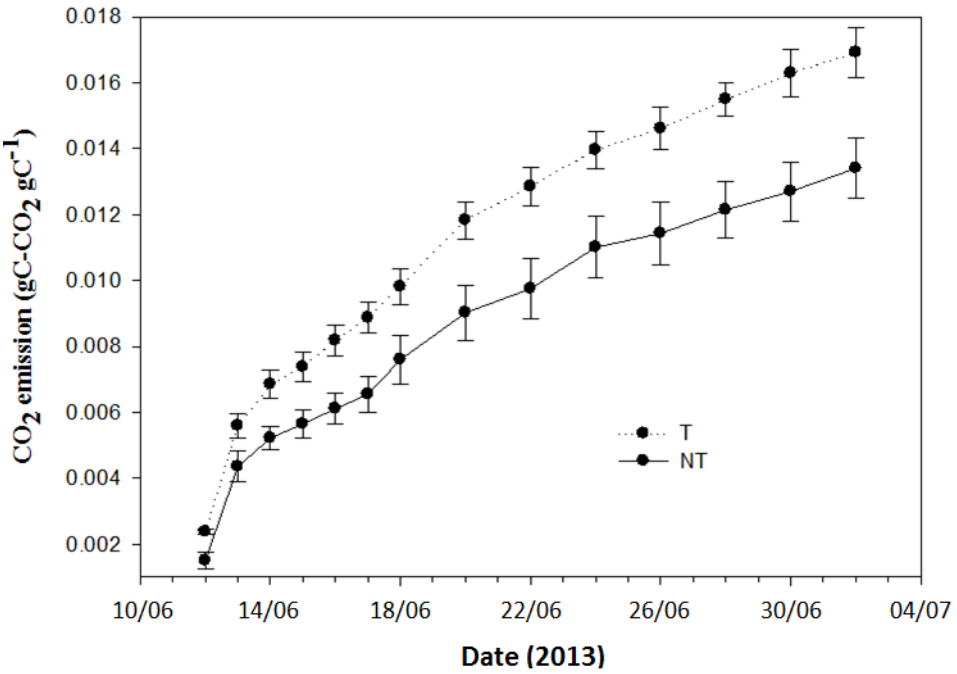


Figure 1.1: CO_2 emissions from T and NT soils.

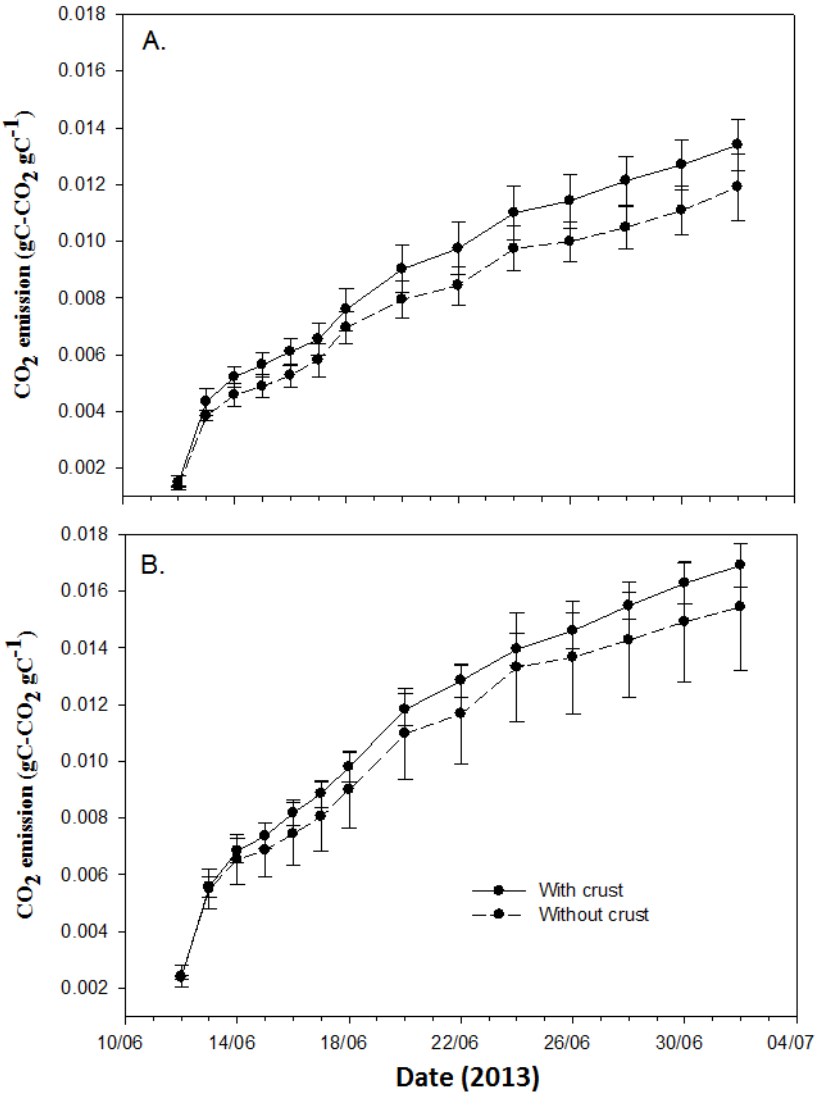


Figure 1.2: Impact of soil surface crusting on soil CO_2 emissions for both NT (A) and T (B) soils.

1.2.4 Project 2014 and objectives of internship

The soil samples collected in 2013 are analyzed in order to compare SOC stocks and SOM quality under the different practices (focus of my internship).
With my work I aim to investigate the impact of agricultural practices on the quality of SOM by studying the chemical properties of the different soil types. In particular, I have done three types of analyzes:

- Elemental analysis (EA) coupled to isotope ratio mass spectrometry with magnetic sector analyzer (IRMS), aimed to quantify the stock of SOC (and secondly N) present in the 3 soil types and to evaluate the changes in $\delta^{13}C$.
- Pyrolysis (Py) and gas chromatography (GC) coupled to mass spectrometry with quadrupole analyzer (MS) to determine semi-quantitatively the molecular composition of the soil in order to compare the parcels.
- Nuclear magnetic resonance spectroscopy (NMR) used as a qualitative technique to understand the state of degradation of SOM and determine the principal chemical functional group in soil.

Chapter 2

Materials and methods

2.1 Soil samples

2.1.1 Sample taking

The soil samples were collected in June 2013 by M. Alexis from Potshini site of KwaZulu-Natal in South Africa. The soils are acidic Acrisols (W.R.B., 1998) with gentle slope and Maize culture (Mediterranean climate). The samples come from three different parcels: soil under T condition, NT condition and OV condition (not plowed as NT plot, but subjected to an overgrazing of 6 cows/50 m^2 for 4 days). It is worth pointing out that this last practice is not a real overgrazing (which consists of an exposure to intensive grazing for extended periods of time) since it is carried out for a short time. The OV practice was applied to assess whether there is any positive effect on the quality of SOM and soil restoration.
NT and T parcels are subjected to free grazing.
The sampling was carried out at different depths and for almost all samples have been collected three replicas, to follow potential change in C distribution at thin scale and to allow bulk SOM characterization. When existing, crust was collected separately.

	EA-IRMS			**Py-GC-MS**			**^{13}C NMR**		
	NT	*T*	*OV*	*NT*	*T*	*OV*	*NT*	*T*	*OV*
Crust	1	3	-	1	1	-	1	1	-
0-2 cm	3	3	3	-	-	1	-	-	1
2-4 cm	3	3	3	-	-	1	-	-	1
4-6 cm	3	3	3	-	-	-	-	-	-
6-8 cm	3	3	3	-	-	-	-	-	-
8-10 cm	3	3	3	-	-	-	-	-	-
0-5 cm	3	3	-	1	1	-	1	1	-
5-10 cm	1	1	-	-	-	-	1	1	-
10-15 cm	1	1	-	-	-	-	1	1	-

Table 2.1: Soil samples analyzed with EA-IRMS, Py-GC-MS and ^{13}C NMR. The numbers indicate the number of replicas analyzed for each soil sample.

2.1.2 Sample grinding

The samples were dried at room temperature.
To proceed with the soil analysis it is first necessary to homogenize the samples pulverizing to a particle diameter lower than 0,2 mm, in order to have a greater representativeness of the soil components.
At first, each sample was passed through a 2 mm sieve, to eliminate the larger plant residues; after, to reduce the particle diameter, samples were put into a grinder (planetary ball mill). The instrument consists of two Agate grinding jars which are arranged eccentrically on a so-called sun wheel. Inside each jar, a soil sample is poured (1/3 of the height) with seven grinding Agate balls. After sealing the jars, we can operate the instrument. The direction of movement of the sun wheel is opposite to that of the jars: the balls are subjected to rotational movements and the interplay between frictional and impact forces produces a high degree of soil size reduction. 15 minutes grinding, a speed of 450 rpm and a change in the direction of rotation every 4 minutes allow to reduce the particle size $<$ 0,2 mm.
After each sample preparation, the jars and the balls are cleaned with *milliQ* H_2O and Ethanol 96 % to avoid contamination. For some samples it was not possible using the planetary ball mill because soil compacted on jar's borders (or it was not present in sufficient quantity), therefore the grinding of the samples was performed manually, using mortar and pestle.

2.1.3 Sample demineralization

NMR spectroscopy and Py-GC-MS analysis requires an adequate amount of C in order to obtain a good signal from the detector.
It is therefore necessary to concentrate the SOM of the samples through demineralization with hydrochloric acid (HCl) 10% and hydrofluoric acid (HF) 6M, which dissolve respectively carbonate and silicate minerals (protocol adapted from Alexis et al., 2012).
Sample aliquots from crust and 0-5, 5-10, 10-15 cm (0-2, 2-4 cm for OV), were weighed with analytical balance (about 7 g for T and NT and 4 g for OV, in 50 ml plastic tubes) and demineralized.

Reagent solution. To treat the soil samples it was necessary to prepare in a plastic bottle (HF dissolve glass) 2150 ml of solution starting from HF 40% and HCl 37 %.
Quantity of H_2O milliQ = 534 ml (before adding the acids).
Quantity of HF 40 % = 538 ml.
Quantity of HCl 37 % (density 1,18 g/ml, molar mass 36,46 g/mol) = 1078 ml, obtained from the following calculation:

$$M_1 = \frac{\% \cdot d \cdot 1000}{100 \cdot MM} \rightarrow M_1 = \frac{37 \cdot 1,18 \cdot 1000}{100 \cdot 36,46} \rightarrow M_1 = 11,97 \ \frac{mol}{l}$$

$$M_1 \cdot V_1 = M_2 \cdot V_2 \rightarrow 11,97 \cdot V_1 = 6 \cdot 2150 \rightarrow V_1 = 1078 \ ml$$

Demineralization. Reagent was added to obtain a soil/solution ratio of 1/6 (w/v). Tubes were put before in tumbler shaker, in order to make effective demineralization, and then centrifuged. The supernatant was then pipetted and discarded. Centrifuge revolution set to 1000 rpm for 20 minutes.
Protocol was repeated 5 times: 2 times carried out 15 hours of tumbler shaker, 3 times carried out 2 hours.

Neutralization of pH. To remove the acids, 6 rinsing with $milliQ\ H_2O$ were done until pH of supernatant (checked with litmus paper) reached the value of $milliQ\ H_2O$ (about pH 6).
Samples so stored in the freezer for a night.

Lyophilization. Lyophilizer (*Cryogen Cosmos*) works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. Before the process, caps were removed and above each tube was placed an inert tissue (nylon, 20 μm pores) to allow water to exit from the tubes during the sublimation.
Tubes remained a night in the lyophilizer until the stabilization of T and P parameters, respectively -82,5 ℃ and 3,7 · 10 mTorr.

After finishing the process, the samples were re-weighed and re-analyzed for C content in order to make a mass balance, therefore to quantify the loss of OC. The mass loss was obtained from the calculations:

$$Mass\ loss = \frac{(Soil\ mass\ before\ dem) - (Soil\ mass\ after\ dem)}{Soil\ mass\ before\ dem}$$

The carbon loss was calculated as follows:

$$Carbon\ loss = \frac{(Carbon\ mass\ before\ dem) - (Carbon\ mass\ after\ dem)}{(Soil\ mass\ before\ dem)}$$

2.2 Elemental Analysis - Isotope Ratio Mass Spectrometry

EA-IRMS is a bulk measurement technique which provides representative data for the average elemental composition and isotopic signature of the entire sample.

2.2.1 EA principles

In order to measure the element percentages of the different soils, the bulk sample has to be weighed and placed in a tin capsule. The sample capsule is lowered into the elemental analyzer (*Elementar Vario Pyro Cube*) combustion furnace through an auto-sampler carousel, at which time the sample is combusted at elevated temperatures under a flow of oxygen into NO_x, CO_2 and H_2O residual. The combusted sample is carried by a helium gas stream into a reduction chamber where nitrous oxides are converted into N_2 and excess O_2 is removed; the analyte is next carried through a chemical trap to remove water that was produced from combustion, and then into a zeolite molecular sieve where separation of CO_2 and N_2 is performed.
The isotope ratios for samples are reported relative to the reference standard gases (CO_2 and N_2).

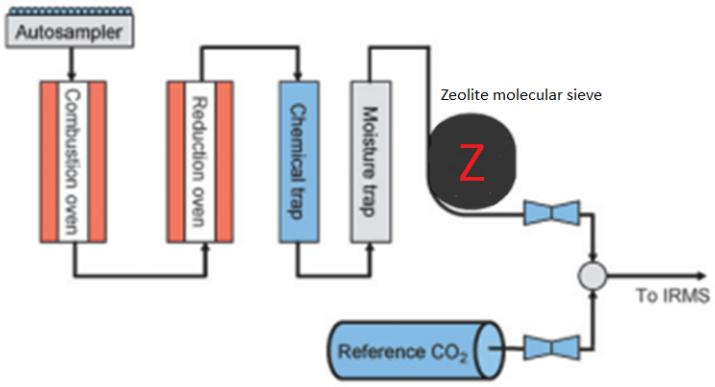


Figure 2.1: Elemental analyzer scheme (modified from Muccio and Jackson, 2009).

2.2.2 IRMS principles

The Isotope Ratio Mass Spectrometer (*Micromass Isoprime*) measures the relative abundance of C (^{12}C, ^{13}C) isotopes.
The instrument can be schematically divided into four parts:

- Ionizer
- Ion accelerator
- Mass analyzer
- Detector

The instrument uses a hard ionization technique (Electron Ionization), producing electrons through thermionic emission by heating a wire filament that has electric current running through it, to induce ionization and fragmentation in the neutral molecules of the sample effluent. The ions produced are then accelerated by an electric field and sent to the mass analyzer. This instrument is a magnetic sector that employs a magnet to deflect the ion beam along a circular trajectory of 90°. The trajectory described inside the sector by the ions is given by the balance between the magnetic force $F_n = Bzev$ and the centrifugal force $F_c = \frac{mv^2}{r}$, from which we get the mass/charge ratio:

$$\frac{m}{z} = \frac{B^2 r^2 e}{2V}$$

The detector is a transducer (for ions) that converts the ion beam into an electrical signal (measured in nano-Amperes) processed by the computer.

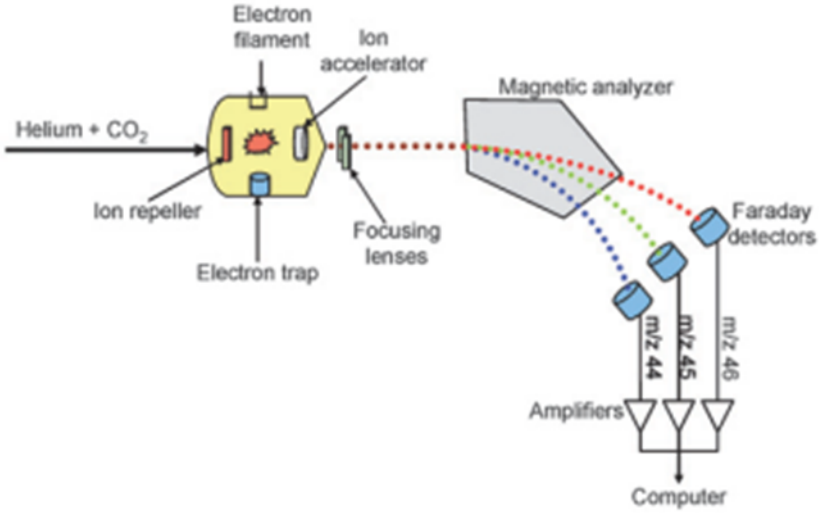


Figure 2.2: Isotope ratio mass spectrometer scheme (Muccio and Jackson, 2009).

For the isotopic analysis is used a Multicollector detector, which consists of several detectors in series: the instrument detects the ions scattered from the analyzer simultaneously and not individually, in our case the $\frac{m}{z}$ ratio of CO_2 (signal 44 and 45) and N_2 (signal 28, 29 and 30).
The final results are expressed as C and N percentages and, for C isotopic ratio, $\delta^{13}C$.
$\delta^{13}C$ is a relative value of deviation from an international standard, expressed as a permille (‰). The δ value of C is calculated as:

$$\delta^{13}C = \left(\frac{\left[\frac{^{13}C}{^{12}C}\right]_{sample} - \left[\frac{^{13}C}{^{12}C}\right]_{standard}}{\left[\frac{^{13}C}{^{12}C}\right]_{standard}} \right) \cdot 1000\,(‰)$$

The standard reference established for ^{13}C is the Pee Dee Belemnite carbonate (PDB): this material has a high $^{13}C/^{12}C$ ratio (0.011237), and was established as $\delta^{13}C$ value of zero. Use of this standard gives most natural material a negative $\delta^{13}C$.

2.2.3 Sample weighing

The samples were weighed on an analytical balance (*Sartorius*) in tin capsules (8x5 cm, pressed, ultraclean) using tweezees and spatulas cleaned with

Ethanol 96 % for each different sample. To analyze soil samples it is necessary weighing also a standard compound in order to obtain a calibration line required for the quantification of C and N present in the samples. The standard used in the laboratory is the tyrosine, of which are well known C, N percentages and $\delta^{13}C$:
$C = 59,66\,\%$
$N = 7,73\,\%$
$\delta^{13}C = -23,2\,‰$ (vs PDB)
The tyrosine was weighed with increasing concentrations, from 0,2 to 2 mg, to calibrate and assess the measure trueness (figure 2.3), so correct the soil $\delta^{13}C$; at the start, at the end and interposed between soil samples, a weighing (1,2-1,5 mg) was done in order to condition and verify the stability of the machine.

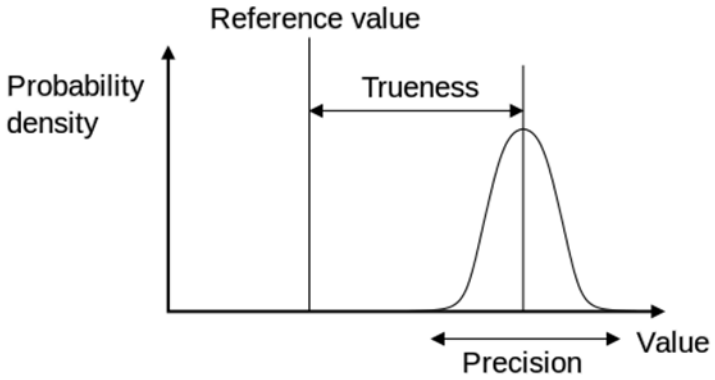


Figure 2.3: Accuracy consists of Trueness (proximity of measurement results to the true value) and Precision (reproducibility of the measurement). Taken from the ISO 5725 series of standards (http://en.wikipedia.org/wiki/Accuracy_and_precision).

For each soil sample was weighed approximately 25 mg; this quantity allows to obtain a strong C (and N) signal.
EA-IRMS analysis was also performed on demineralized samples in order to estimate the loss of C and the change in $\delta^{13}C$ values. For demineralized samples were weighed about 4 g, since the C is more concentrate.
For the superficial layers was made a rough estimate of C and N amounts (knowing the density).

2.2.4 Statistical analysis

The statistical analyzes were carried out on samples 2 cm deep, because only for each of these we dispose of 3 replicas. However, given the small number of samples (they cannot be assumed to be normally distributed), non-parametric tests were conducted to evaluate variability and compare the C, N percentage and $\delta^{13}C$ for the different soil types.
All the computations were performed using **R** software; the statistical significance was expressed in terms of *p-value*, with 0,05 as a significance level.
`Kruskal-Wallis test` was performed for each cultural practice to assess the variation along the 10 cm profiles.
`Wilcoxon signed-rank test` (one-tailed, assuming that NT-T, OV-T, OV-NT >0) was used to compare two by two all the soil layers (NT vs T, OV vs T, OV vs NT). It is not a very indicative test in this case, since it does not take account of the C and N variations in the different layers of the same parcel.
`Wilcoxon-Mann-Whitney test` (one-tailed) could be more indicative in order to determine possible differences between the soil types, comparing each soil layer: unfortunately samples have only 3 replicas so this non-parametric test cannot achieve the significance level; in this case it is more useful to observe the differences evaluating the trend of the graphs.
For these reasons, in section 3.2 only the results of the Kruskal-Wallis test are taken into account and discussed.

2.2.5 Graphical representation

EA-IRMS results are expressed in Excel plots that relate $\%C$, $\%N$ and $\delta^{13}C$ with the corresponding depth of soil samples (section 3.2). The points of the graphics correspond to the mean values of the 3 replicas.
The standard deviations (σ) were calculated in two ways:

- Std from the mean value of the 3 replicas (σ intra-plot) of each sample.
- Std from the mean value of 5 analysis repetitions of a random replica (analytical σ). We obtain a single data that also represents the homogeneity of the samples.

In graphs, for every point, was selected the largest value between σ intra-replicas and internal σ.

2.3 Pyrolysis - Gas Chromatography - Mass Spectrometry

2.3.1 Py-GC-MS principles

Py-GC-MS is a method in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry.
Each soil sample, after weighing, is introduced in a Fe-Ni capsule; tetramethylammonium hydroxide was added (TMAH, 25% w/v in methanol): it allows detection of carboxylic and phenolic hydroxyl groups as methyl derivatives (more volatile). Soil is pyrolysed (with a *Pilodist Curie Point Pyrolyzer*) for 9,9 s in the ferromagnetic tube with a Curie temperature of 650 ℃.
The products are injected into the gas cromatograph (*GC Trace*) at 280 ℃ under a He flow of 1 ml/min: they are separated using a 30 m Rxi-5Sil MS capillary column (i.d. 25 mm, apolar film thickness 0,5 μm). The oven temperature is maintained at 50 ℃ for 10 min and then programmed from 50 to 310 ℃ at a rate of 2 ℃/min.
The GC is coupled to a quadrupole mass spectrometer (*DSQ*), operating at 70 eV in the electron ionization (EI) mode.
Ions produced by EI are separated in a quadrupole based on the stability of their trajectories in the oscillating electric fields that are applied to the rods. Results are displayed as spectra of the relative abundance of detected ions (the total ion current chromatogram, TIC, which represents the summed intensity across the entire range of masses being detected at every point in the analysis) as a function of the mass-to-charge (m/z) ratio (for each detected ion). The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern. The identification of chemical compounds was performed with *Xcalibur* software and *NIST* database.

2.3.2 Samples analyzed

Demineralized samples (subsection 2.1.3) provide a better signal than untreated samples. However, the protocol for the removal of minerals may also lead to a loss of organic molecules: it was therefore necessary to analyze also the corresponding original samples.
The Py-GC-MS was so performed on the samples indicated in table 2.1, before and after demineralization.
In order to obtain a good signal from the untreated samples it was necessary to weigh about 10 mg of soil, whereas for demineralized samples only about 1 mg.

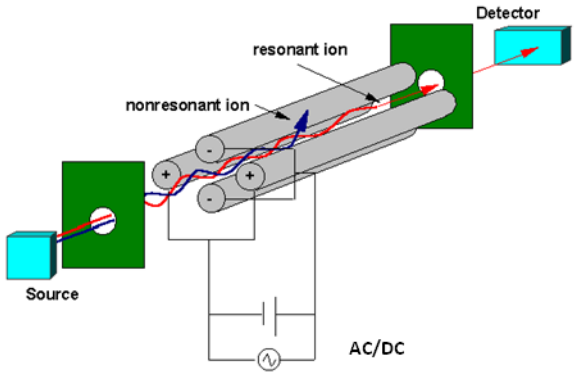


Figure 2.4: Quadrupole scheme (modified from http://chromservis.cz/category/gc-ms-tof).

At the end of the runs a sample (NT 0-5 cm non-demineralized) was re-analyzed to verify that there were not differences from the first, so the success of all runs. The comparison of the two (theoretically) identical chromatograms indeed did not reveal any particular difference.

2.3.3 Graphical representation

In section 3.3 are reported the mass chromatograms: for the first, the number and the symbol that distinguish the main peaks identify respectively the molecule (reported in a table) and the chemical family (explained in the legend); for the others only a symbol characterizes the peaks (the names of the main compounds that not appear in the first chromatogram are written directly on the figures).

2.4 Nuclear magnetic resonance spectroscopy

2.4.1 NMR principles

Nuclear magnetic resonance (NMR) spectroscopy is a method for atomic and molecular level structure elucidation. During a NMR experiment, the sample of interest is placed into an external static magnetic field that forces the nuclei spins to distribute themselves among different energy levels. The energy difference (ΔE) between those levels is dependent upon the magnetic properties and the strength of the surrounding magnetic field of the affected nuclei. Consequently, ΔE is different for nuclei in different chemical and

physical environments.
Spin transitions between those levels can be induced if an additional electromagnetic field with a frequency corresponding exactly to ΔE is applied. In this case, the induced transitions can be detected as a resonance signal, Free Induction Decay (FID), which is the sum of the radiation of all the nuclei in function of time. FID information is converted through the Fourier transform into a spectrum readable, in function of frequency.
ΔE and the resonance frequency are also dependent upon the strength of the external magnetic field which makes it difficult to compare results obtained with two different NMR spectrometers. In order to circumvent this problem, the resonance frequency is given relative to a reference as chemical shift in ppm in relation to a standard, the tetramethylsilan (TMS).
The NMR used in laboratory is a CP-MAS ^{13}C NMR. This instrument allows to analyze the samples in solid-state, after accurate preparation. Cross polarization (CP) is used to enhance the signal of nuclei with a low gyromagnetic ratio (^{13}C) by magnetization transfer from nuclei with a high gyromagnetic ratio (^{1}H). By combining cross polarization with magic-angle spinning (MAS), high resolution spectra can be obtained for rigid solids.
A spectrum can be divided in various chemical shift regions, that can be attributed to different functional groups. The chemical shift regions 0-45, 45-110, 110-160 and 160-220 ppm are assigned to alkyl C, O-alkyl C, aromatic C and carboxylic C, respectively (Wilson, 1987).
The figure 2.5 shows in more detail the assignments of the main peaks of a spectrum.

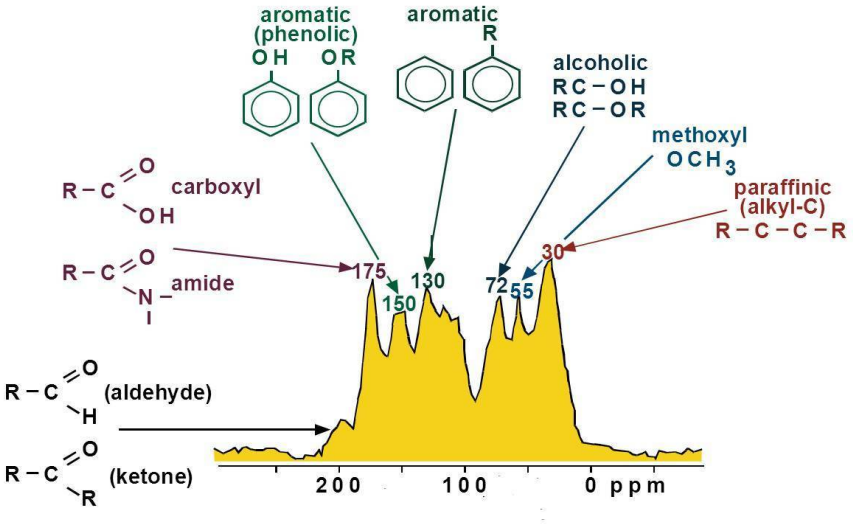


Figure 2.5: Classification of the signals in solid ^{13}C NMR spectrum (modified from Bortyatinski et al., 1996).

2.4.2 Samples analyzed

For the NMR analysis, the samples (indicated in table 2.1) were demineralized and checked with a magnet to ensure that there were no magnetic properties (otherwise caused for example by the presence of iron oxides) that would damage NMR instrument. It was hence possible proceeding with the analysis. The instrument used was a *Bruker Ultrashield 500 WB Plus*. The integration of the chemical shift regions of the spectra obtained was performed with *Dmfit* program. So A/O-A ratios were simply calculed by dividing Alkyl C area by O-Alkyl C area of each spectrum. Instead it is not possible to compare the individual chemical shift regions between the different spectra due to the different amount of matter analyzed and to the various acquisition times, characteristic for each analysis.

2.4.3 Graphical representation

As first, in section 3.4, a NMR spectrum shows the main chemical compounds and their origins present in the different regions. All the original spectra are placed in a summary figure. Alkyl/O-Alkyl C ratios are reported in an Excel plot that relates the values with the corresponding depth of soil samples. The percentages of the principal chemical shift regions for each type of soil are represented on Excel bar graphs.

Chapter 3

Results and discussion

3.1 Sample demineralization

Table 3.1 shows mass loss for each sample after demineralization (dem). Each treated sample lost more than 90 % of the initial mass, indicating that the acid attack was effective. The amount dissolved and removed from the soil samples was principally the mineral fraction. However, it is necessary to assess the amount of carbon lost during the process.

Soil layer	Soil mass before dem (g)	Soil mass after dem (g)	**Mass loss**
NT Crust	7,024	0,462	0,93
NT 0-5 cm	7,009	0,427	0,94
NT 5-10 cm	7,015	0,502	0,93
NT 10-15 cm	7,018	0,417	0,94
T Crust	7,000	0,356	0,95
T 0-5 cm	7,016	0,502	0,93
T 5-10 cm	7,014	0,435	0,94
T 10-15 cm	7,022	0,524	0,93
OV 0-2 cm	4,072	0,322	0,92
OV 2-4 cm	4,008	0,294	0,93

Table 3.1: Mass loss.

Table 3.2 provides C percentage values before and after the acid treatment and C loss.
The C masses are the products of soil mass and C fraction (%C/100) before and after demineralization. The loss of organic carbon is not negligible: averagely for each sample was lost about 50 % of the initial value. This

soluble C was probably associated to minerals dissolved in the supernatant (Rumpel et al., 2006). Probably fulvic acids are the main compounds lost with acid treatment, since they are soluble at low pH. The discussion on humic acids is taken up in sections 3.4 (NMR results). In particular, in Py-GC-MS section was made a qualitative comparison between samples before and after demineralization to evaluate the compounds that may have been lost due to the treatment.

Soil layer	C before dem (%)	C after dem (%)	**C loss**
NT Crust	5,34	46,23	0,43
NT 0-5 cm	1,77	13,99	0,52
NT 5-10 cm	1,93	16,20	0,40
NT 10-15 cm	1,60	11,66	0,57
T Crust	1,95	21,47	0,44
T 0-5 cm	1,75	9,54	0,61
T 5-10 cm	1,89	13,96	0,54
T 10-15 cm	1,67	11,00	0,51
OV 0-2 cm	3,91	33,59	0,32
OV 2-4 cm	1,86	11,55	0,54

Table 3.2: Carbon loss.

The difference in $\delta^{13}C$ values before and after demineralization is well visible in chart 3.1.
The good parallelism between the values before and after the treatment shows that demineralization influence uniformly the samples: the change of $\delta^{13}C$ values in fact is always between 1,27 and 1,48 permille points.

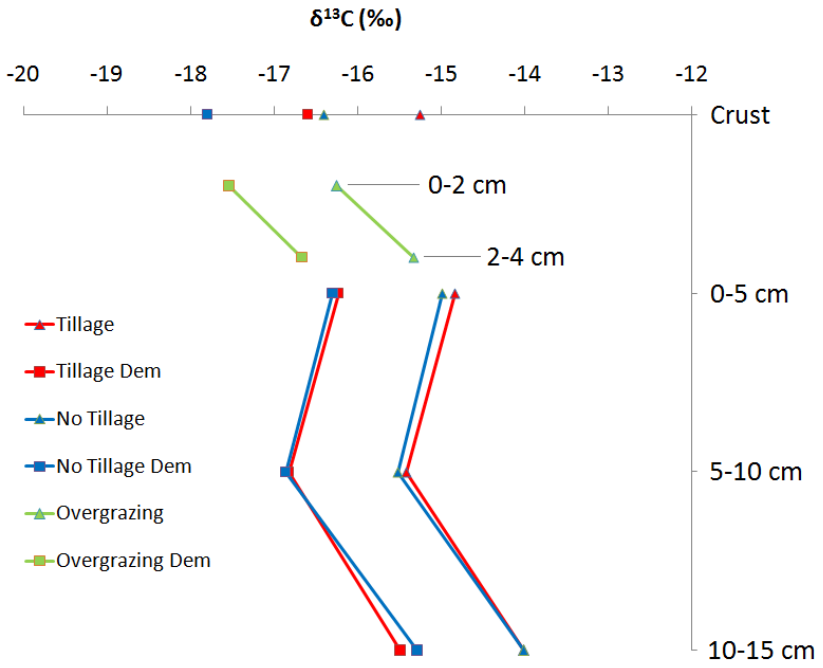


Figure 3.1: Changes in $\delta^{13}C$ values after demineralization.

3.2 EA-IRMS

3.2.1 Soil carbon content

The graph 3.2 displays the percentage values of C found with elemental analysis at different depths.
Kruskal-Wallis test is significant for NT ($p = 0,04286$ with crust and $p = 0,04053$ without crust) and OV ($p = 0.01945$). This is clearly visible observing the trends of the lines in the graph 3.2.
This result illustrates that in a tilled soil the organic matter is well mixed, so homogenized along the profile due the mechanical working.
Despite this trend seems obvious for T soil, it is noteworthy that this is already well visible in the first 10 cm of soil (at the small-scale). The C percentages in the first cm in OV (3,57) and NT (2,84 without taking into account the crust) are much higher than T ones (whose values vary from 1,78 to 1,82 %), although in the deeper layers the trend reverses slightly.
Regarding the crust layers, the simple fact that we have 3 replicas for T and only one for NT soil may indicate that the former is more prone to physical crusting. But a great difference is noticed between C percentage of crust taken from T (1,76) and NT (5,34) parcels. To understand the reason of this

large difference it is necessary to analyze the compounds present in the different types of soil with Py-GC-MS. The high value found in NT crust may suggest that its origin could be not physical, but biological. Crust layers are discussed in detail in section 3.3.

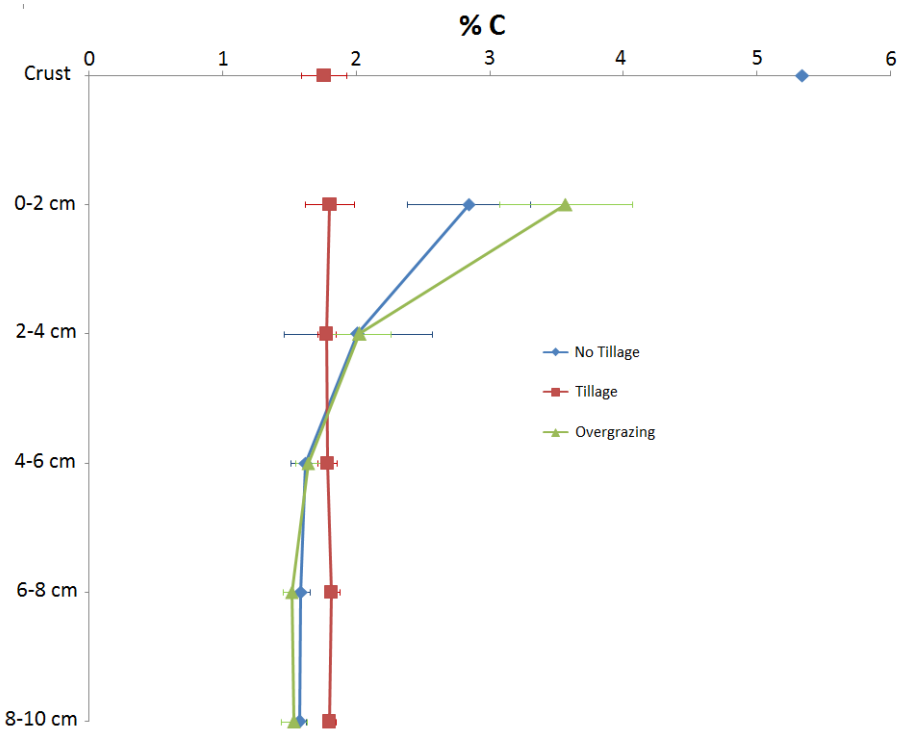


Figure 3.2: Comparison between NT, T and OV carbon percentages. Each point represents the mean value of the 3 replicas, with related σ (explanation in subsection 2.2.5).

3.2.2 Soil nitrogen content

The graph 3.3 shows approximately the same trend of the previous graph (3.2), with a larger σ for the first two layers (as in C percentage analysis). Kruskal-Wallis test is significant only for OV ($p = 0.01892$), but visibly (although not confirmed by the test) NT line has the same trend of OV. As well as for the C percentage, also for N the same discussion applies: in T soil there is a greater homogeneization of the profile and apparently a lower amount of total N, definable with the bulk density of the soil in the different layers. Similarly, NT crust has a N percentage (0,43) clearly higher than the mean value of T crust (0,14).

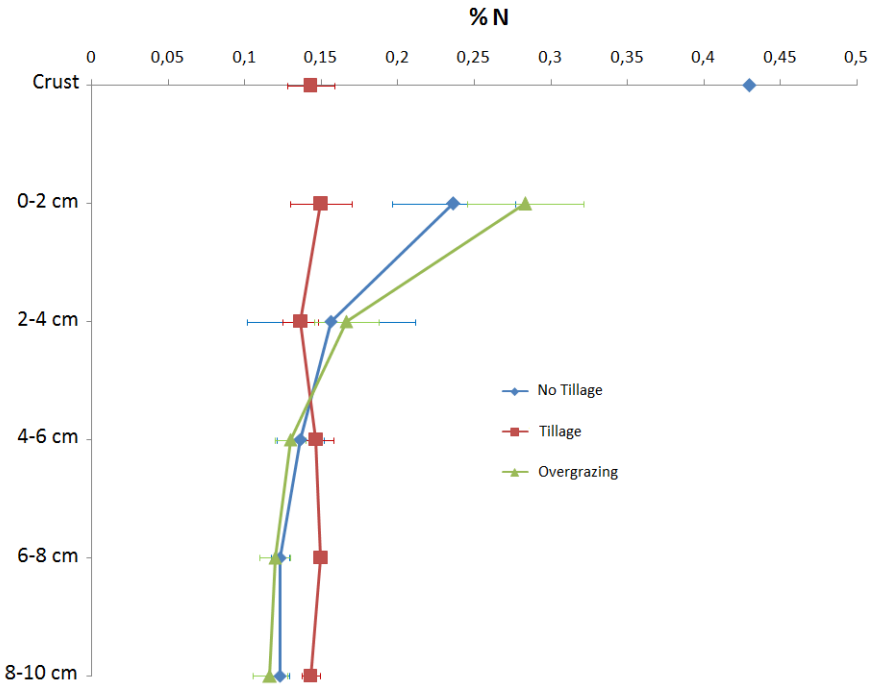


Figure 3.3: Comparison between NT, T and OV nitrogen percentages.

3.2.3 Soil $\delta^{13}C$

The graph 3.4 compares the $\delta^{13}C$ values of the 3 different parcels (abscissa: $\delta^{13}C$ values expressed as a permille; ordinate: depth).
Kruskal-Wallis test is significant only for OV ($p = 0.01945$), in which it can be seen an increasing trend with depth (from -16,15 to -13,93 ‰). This is consistent with what was already described by several authors (Balesdent et al., 1990; O'Brien and Stout, 1978; Volkoff and Cerri, 1987; Natelhoffer and Fry, 1988): the C remaining after partial mineralization can differ from the initial carbon, generally being richer in ^{13}C. This is in accord with the increase of ^{13}C with depth, feature well known in areated soils that are assumed to have been under the same vegetation; it is usually attributed to humification processes.
NT trend is similar (from -17,13 to -14,24 ‰) but less linear, particularly for the crust (-16,41 ‰), that shows an increase of $\delta^{13}C$ value, if compared with the first layer (0-2 cm). One cannot, however, overlook the great σ intra-plot of NT data (particularly for the 2-4 cm layer): probably this could be due to the spatial difference between the 3 replicas (which have different

values within the same parcel) of the samples, or perhaps to a not perfect homogenization during the preparation phase (subsection 2.1.2).
T data are more homogeneous along the depth (with a mean value of -15,40 ‰), as might be expected after plowing.

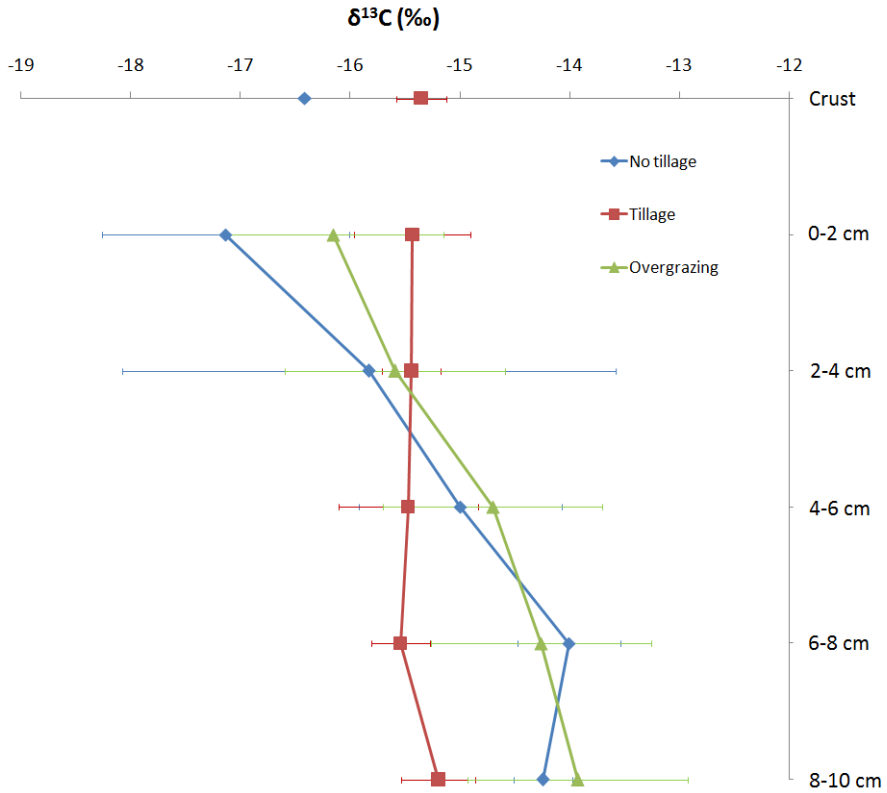


Figure 3.4: Comparison between NT, T and OV $\delta^{13}C$ values.

It is interesting that for this and previous analyzes it can be noticed differences between soil with and without plowing already from these small depth. However, it would be appropriate to investigate further the study considering greater depths.

3.2.4 Estimate of C and N stocks

Soil carbon stocks. Knowing the bulk density values (mass of soil/volume as a whole) of the different parcels, it is possible to assess the actual amount of C in the soil.
The bulk densities were measured on samples coming from the same parcels, but different from those on which EA-IRMS analysis was performed: 3 density measurement were made for NT and T parcels, 9 for OV parcel. The

mean values (ρ_b) are respectively 1,198, 1,140 and 1,216 g/cm^3. This values concern only the depth 0-5 cm, since it is possible to use only the C percentage data referred to the same depth.
Applying the formula $mg\,C/cm^3 = (\%C/100) \cdot 1000 \cdot \rho_b$ (where $\%C$ are the C percentages of the 3 NT and of the 3 T soil replicas for depth 0-5 cm) and averaging the values thus obtained, we achieve a rough estimate on the average carbon content in the different parcels.
The mg/cm^3 of C obtained from NT and T are respectively $29,95 \pm 7,77$ and $19,87 \pm 0,48$.
For OV soil we do not dispose of soil 5 cm depth, therefore the data were treated considering an average of the values first only of the layers 0-2 and 2-4 cm (0-4 cm), then of the layers 0-2, 2-4 and 4-6 cm (0-6 cm), in order to have a good approximation of 0-5 cm layer.
The calculation of mg/cm^3 of carbon was therefore carried out in the same way, obtaining a value of $34,04 \pm 3,75$ (layer 0-4 cm) and $29,34 \pm 3,21$ (layer 0-6 cm).
The results are shown in the graph 3.5.
Visually, the C content present in NT and OV soils (which give the same result) appears to be greater than that of T parcel, even taking into accout the large σ of NT mean value. This would be in line with the numerous studies carried out to determine the C stock in soils (e.g. Neto et al., 2010; Paustian et al., 1997; Sá et al., 2001), however it must be considered that these values are referred only to the 0-5 cm layer, therefore the result is not extensible to the lower layers, where instead seems to be an increase of C in T parcel (although slight). Moreover, as said, bulk density and C content were not measured on the same sample, thus these results should be interpreted with caution. For this reason, the calculation of σ between soil replicas was done choosing always the largest value.
In contrast also the crust was excluded from this analysis, where there is a large difference between C percentage values of NT and T: crust density is not available, but probably the amount of NT crust C significantly exceeds the amount of the other one.

Soil nitrogen stocks. N soil content was calculated in the same way as done for the estimate of C content (last paragraph of subsection 3.2.1), as shown in the graph 3.6.

The same considerations also apply to N, since the values reported in this graph are relatively similar to those of the graph 3.5 (with a different measurement scale), i.e. N amount for the depth 0-5 cm seems to be greater in NT and OV than T, however considering all the restrictions previously commented for the C amount.

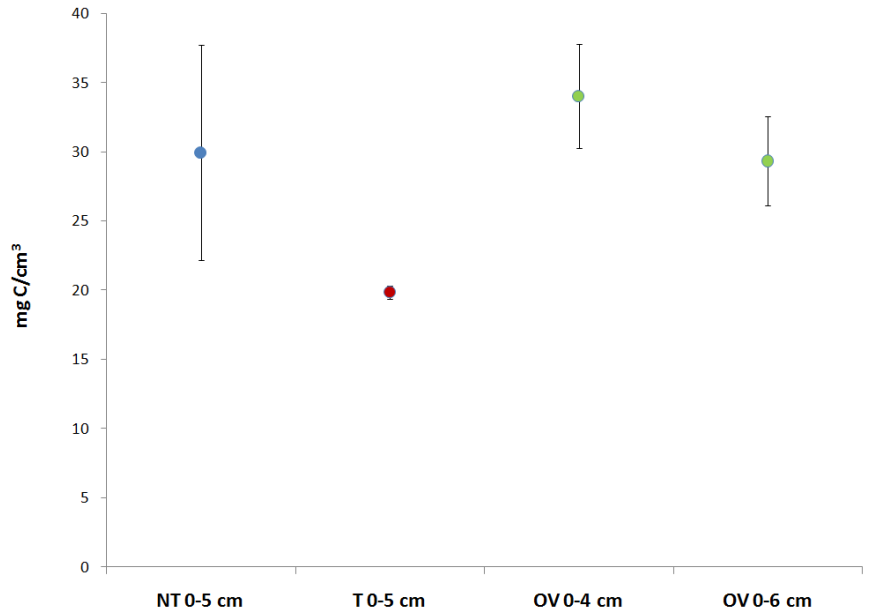


Figure 3.5: Comparison between NT, T and OV carbon content.

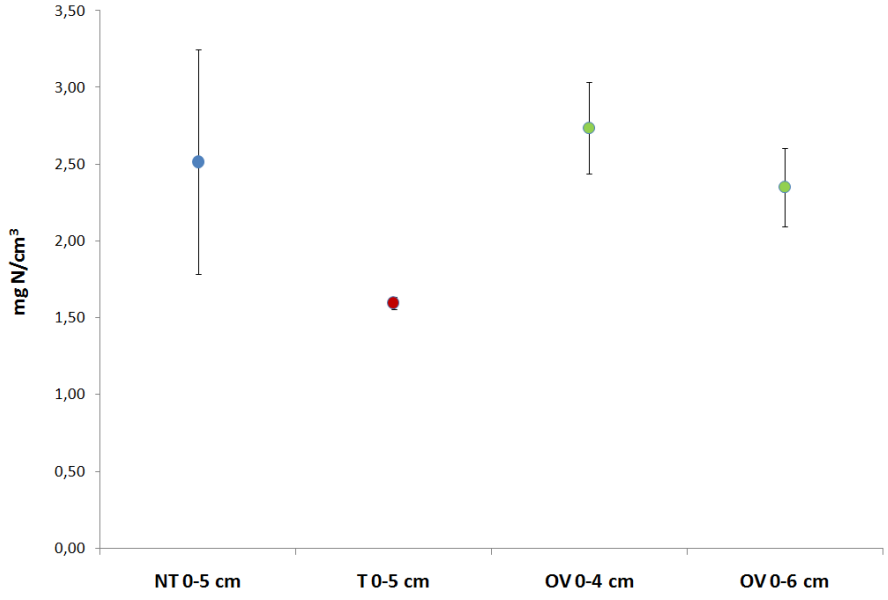


Figure 3.6: Comparison between NT, T and OV nitrogen content.

3.3 Py-GC-MS

The table 3.3 shows the principal molecules found in demineralized NT crust sample (figure 3.7), cracked with Curie point pyrolysis, separated by gas chromatograph and identified with mass spectrometry (quadrupole analyzer). The molecular family are marked with different symbols, explained in legend.
First 10 minutes of each analysis are not taken into consideration because these compounds are products resulting from the pyrolysis. The dominant peak (4) corresponds to 1,3,5-trimethylhexahydro-s-triazine, derived from TMAH (the height is not taken into account).
The mass chromatograms can be divided into two dominant regions. The first part (about 75 minutes) mainly comprises phenolic compounds probably derived from lignin and some of them may also derived from tannins (Galletti et al., 1995; Van der Heijden, 1994), such as methoxybenzene, 1-methoxy-4-methylbenzene, 1,2-dimethoxybenzene, 1-ethenyl-4-methoxybenzene, 1,2-dimethoxy-4-methylbenzene, 1,2,3-trimethoxybenzene, 4-ethenyl-1,2-dimethoxybenzene, 3,4-dimethoxybenzoic acid (methylester), 3-(4-methoxyphenyl)-2-propenoic acid (methylester), 3,4,5-trimethoxybenzoic acid (methylester), and 3-(3,4-dimethoxyphenyl)-2-propenoic acid (methylester). Going into more detail, for some compounds found in the sample we can also determine from which unit of lignin they derive (Chefetz et al., 2000): basic components of this polymer are p-hydroxyphenyl, guaiacyl, and syringyl. Methoxybenzene and 3-(4-methoxyphenyl)-2-propenoic acid derive from p-hydroxyphenyl, 3,4-dimethoxy-benzoic acid from guaiacyl, and 1,2-dimethoxybenzene and 1,2,3-trimethoxybenzene and 3,4,5-trimethoxybenzoic acid from syringyl. 1,2,4-thrimethoxybenzene is released not from lignin but from polysaccharides (Fabbri and Helleur, 1999).
In the first part we find also some compounds containing N: these molecules normally are natural plant components, but could come also from animal wastes, inputs of fresh soil organic matter (the parcels are subjected to free grazing, or overgrazing as regards OV plot).
The second part of chromatogram (after min. 75) contains several series of methylesters of alkenoic and alkanoic acid, mono- , di- and trihydroxyalkanoic acids and alkanedioic acids, which are well-known constituents released from cutin and suberin (de Leeuw et al., 1993; del Río and Hatcher, 1998; Nierop, 1998). More precisely, di- and trihydroxyalkanoic acids derived from cutin polymer: dihydroxyalkanoic acid are formed by microbial oxidation of fatty acids, with the intermediate formation of ω-hydroxyalkanoic acids (Hita et al., 1996). Suberin is composed of aliphatic and aromatic components and contains monomers with a higher chain length (C20-C30), such as fatty acids (alkanoic and alkenoic), ω-hydroxyalkanoic acids and alkanedioic acids (Kögel-Knabner, 2001).

The presence of long-chain fatty acids is due also to the microbial activity by-products (Chefetz et al., 2000). Alkanoic acids are a constant in all the mass chromatograms (mainly from chain C14 to chain C32); the dominant peak corresponds to hexadecanoic acid).
The relative abundance of the other acids is generally lower: chains of monohydroxyalkanoic acids are more visible from C16 to C28, particularly C23, C24 and C26; alkanedioic acids (peaks of lesser intensity) are detected mainly at the end of the mass chromatograms (C24, C26).
In the non-demineralized sample of NT Crust (figure 3.8) there are 3 principal sugar peaks, probably assigned to: 2,6-anhydro-1,3,4-tri-O-methyl-β-d-fructofuranose, methyl-d-galactopyranose and 2,5-anhydro-1,3,4,6-tetra-O-methyl-mannitol (as for all the other compounds containing carboxylic and phenolic hydroxyl groups, the methylation, if not already present, is caused by derivatization with TMAH). The other chromatograms of non-demineralized samples are not reported because there are no qualitative differences if compared to the corresponding chromatograms of the demineralized samples: the only differences are mainly quantitative.

Peak number	Identified compounds	Main mass fragments (m/z)
1	Ethylbenzene	91, 106
2	Styrene + Hexanamine	104, 59, 72
3	Methoxybenzene	108, 78, 65
4	1,3,5-Trimethylhexahydro-s-triazine	44, 86, 42, 128
5	1-Methoxy-4-methylbenzene	122, 121, 77
6	Butanedioic acid, dimethylester	84, 55, 115
7	1-Ethyl-4-methoxybenzene	121, 136
8	Octanoic acid, methylester	74, 87
9	1,2-Dimethoxybenzene +	138, 95, 123, 77
	1-Ethenyl-4-Methoxybenzene	134, 119, 91
10	1,4-Dimethoxybenzene	123, 138, 95
11	Dodecene	55, 56, 57, 69, 83
12	N-compound + Dimethyl-methoxyfuranone	142, 57, 57, 42
13	1,2-Dimethoxy-4-methylbenzene	152, 137, 109, 91
14	Tridecene	55, 56, 83
15	1,2,3-Trimethoxybenzene	168, 153, 110
16	4-Ethenyl-1,2-Dimethoxybenzene	164, 91, 149
17	1,2,4-Trimethoxybenzene	168, 153, 125
18	Tetradecene	55, 57, 83, 70
19	Pentadecene	55, 57, 83, 69
20	1,2-Dimethoxy-4-(1-propenyl)benzene	178, 107, 191
21	Dodecanoic acid, methylester	74, 87, 55
22	Nonanedioic acid, dimethylester	55, 83, 59, 74, 111
23	3,4-Dimethoxy-benzoic acid, methylester	165, 196
24, 25	Dimethoxy-(methoxyethenyl)benzene (isomers)	151, 194, 179
26	3-(4-Methoxyphenyl)-2-propenoic acid	161, 192, 133
27	Tetradecanoid acid, methylester +	74, 87, 143

Continued from previous page

Peak number	Identified compounds	Main mass fragments (m/z)
	3,4,5-Trimethoxybenzoic acid	226, 211, 155, 195
28	Nonadecene	56, 57, 69, 70
29, 30, 31	Pentadecanoic acid, methylester (isomers)	74, 87, 143
32	Trimethyl-pentadecanone	58, 43, 57, 55, 59
33, 36	Hexadecanoic acid, methylester (isomers)	74, 87, 143, 270
34	3-(3,4-Dimethoxyphenyl)-2-propenoic acid, methylester	222, 191
35	Hexadecenoic acid, methylester	55, 69, 74, 83
37, 38, 39, 40	Heptadecanoic acid, methylester (isomers)	74, 87, 143, 284
41, 42	Octadecenoic acid, methylester (isomers)	55, 83, 69, 264
43	Octadecanoic acid, methylester	74, 87, 255, 298
44	ω-Hydroxyhexadecanoic acid, dimethylester	55, 45, 74, 87, 69, 98
45	Nonadecanoic acid, methylester	87, 74, 143, 312
46	Hexadecanedioic acid, dimethylester	55, 98, 112, 241, 283
47	Dihydroxyhexadecanoic acid, trimethylester	71, 45, 95, 109, 55
48	Eicosanoic acid, methylester	74, 87, 143, 326
49	Dihydroxyoctadecanoic acid, trimethylester	55, 87, 95, 109, 155
50	ω-Hydroxynonadecenoic acid, methylester	55, 81, 67, 95, 121
51	Heneicosanoic acid, methylester	55, 57, 74, 87, 111
52	ω-Hydroxynonadecanoic acid, dimethylester	55, 57, 83, 87, 98, 141
53	Docosanoic acid, methylester	74, 87, 143, 354
54	ω-Hydroxyeicosanoic acid, dimethylester	55, 45, 74, 87, 83, 98
55	Trihydroxyoctadecanoic acid, tetramethylester	71, 81, 45, 201
56	Tricosanoic acid, methylester	74, 87, 143, 368
57	Trihydroxynonadecanoic acid, tetramethylester	71, 81, 45, 173, 201
58	ω-Hydroxyheneicosanoic acid, dimethylester	57, 55, 83, 97, 111
59	Tetracosanoic acid, methylester	74, 87, 143, 382
60	ω-Hydroxydocosanoic acid, dimethylester	55, 57, 83, 87, 97, 98
61	Pentacosanoic acid, methylester	74, 87, 57, 143, 396
62	Alkyl chain (unknown)	57, 83, 97, 111, 353
63	ω-Hydroxytricosanoic acid, dimethylester	55, 57, 83, 97, 111
64	Hexacosanoic acid, methylester	74, 87, 143, 410
65	ω-Hydroxytetracosanoic acid, dimethylester	55, 57, 83, 97, 98
66	Heptacosanoic acid, methylester +	57, 55, 74, 87, 97, 424
67	Tetracosanedioic acid, dimethylester	55, 98, 87, 74, 395
68	Hentriacontane (C31)	57, 71, 85, 99
69	Octacosanoic acid, methylester	87, 74, 57, 143, 438
70	ω-Hydroxyhexacosanoic acid, dimethylester	55, 83, 87, 98, 74, 111
71	Nonacosanoic acid, methylester +	57, 83, 97, 74, 452
	Alkyl chain (unknown)	55, 43, 81, 382, 396, 428
72	Hexacosanedioic acid, dimethylester	98, 55, 74, 87, 423
73	Tritriacontane (C33)	57, 71, 85, 99
74	Triacontanoic acid, methylester	87, 74, 57, 143, 466
75	ω-Hydroxyoctacosanoic acid, dimethylester	55, 57, 83, 87, 98
76	Alkyl chain (unknown)	57, 71, 85, 425, 440
77	Alkyl chain (unknown) +	57, 83, 82, 55, 97, 74, 87
	Hentriacontanoic acid, methylester	
78	Octacosanedioic acid, dimethylester +	98, 55, 74, 87, 372, 451
	ω-Hydroxynonacosanoic acid, dimethylester	
79	Alkyl chain (unknown)	57, 85, 71, 411, 426, 446
80	Dotriacontanoic acid, methylester	87, 57, 74, 143, 494
81	ω-Hydroxytriacontanoic acid, dimethylester	55, 57, 83, 87, 98

Continued from previous page

Peak number	Identified compounds	Main mass fragments (m/z)
82	Tritriacontanoic acid, methylester	57, 87, 74, 143, 508
83	Tetratriacontanoic acid, methylester	87, 57, 74, 143, 523
84	Hexatriacontanoic acid, methylester	87, 74, 57, 143, 551

Table 3.3: Products released from NT crust sample upon TMAH/Curie-point pyrolysis at 650 ℃.

Before comparing the chromatograms, it is necessary to highlight some critical issues: it is not possible to accurately quantify the compounds found in the samples, because the methylation with TMAH amplifies the signal of the molecules that contain functional groups such as hydroxyls (e.g. methylesters of ((poly)hydroxy)alkanoic acid), thereby increasing their proportion compared to the others (Nierop, 2001). Furthermore the demineralization process causes a loss (but only in terms of quantity) of the compounds present in the first part of the chromatogram (phenolic compounds, with lower molecular weight), therefore underestimated.
However it is possible to do a relative comparison of the main families of compounds by using a reference: hexadecanoic acid (C16, peak 36 in figure 3.7), ubiquitous in soils, is the highest peak in all the chromatograms, so it can be use as a relative reference to estimate the abundance of the other compounds in the sample (Py-GC-MS is a semi-quantitative technique).
Commenting on the figures 3.9 and 3.10, in T crust acid signals are stronger than those of phenolic compounds, whereas in NT crust we can detect greater peaks of phenolic groups (always comparing the peak heights with that of the hexadecanoic acid), with a particular contribution of 3-(4-methoxyphenyl)-2-propenoic acid, probably coming from lignin. NT crust seems to have a greater molecular diversity than T crust, with a greater lignin/lignocellulosic (polysaccharides) contribution not found in any other chromatogram.
In 0-5 cm layers (figures 3.11 and 3.12), on the contrary of crust layers, for NT sample fatty acids and ω-hydroxyalkanoic acid signals (mainly suberin contribution) are higher if compared to those of phenolic compounds; however in T sample the proportions of the various compounds are smaller in relation to hexadecanoic acid, much more than for the NT one (phenols barely detectable).
OV 0-2 cm sample (figure 3.13) shows a profile similar to NT crust, although the signal intensity is lower (due to analysis conditions), with a good contribution of phenolic compounds found in the first part of the chromatogram. We can say the same for OV 2-4 cm sample (3.14), but with a slight increase of acid components compared to phenolic compounds.

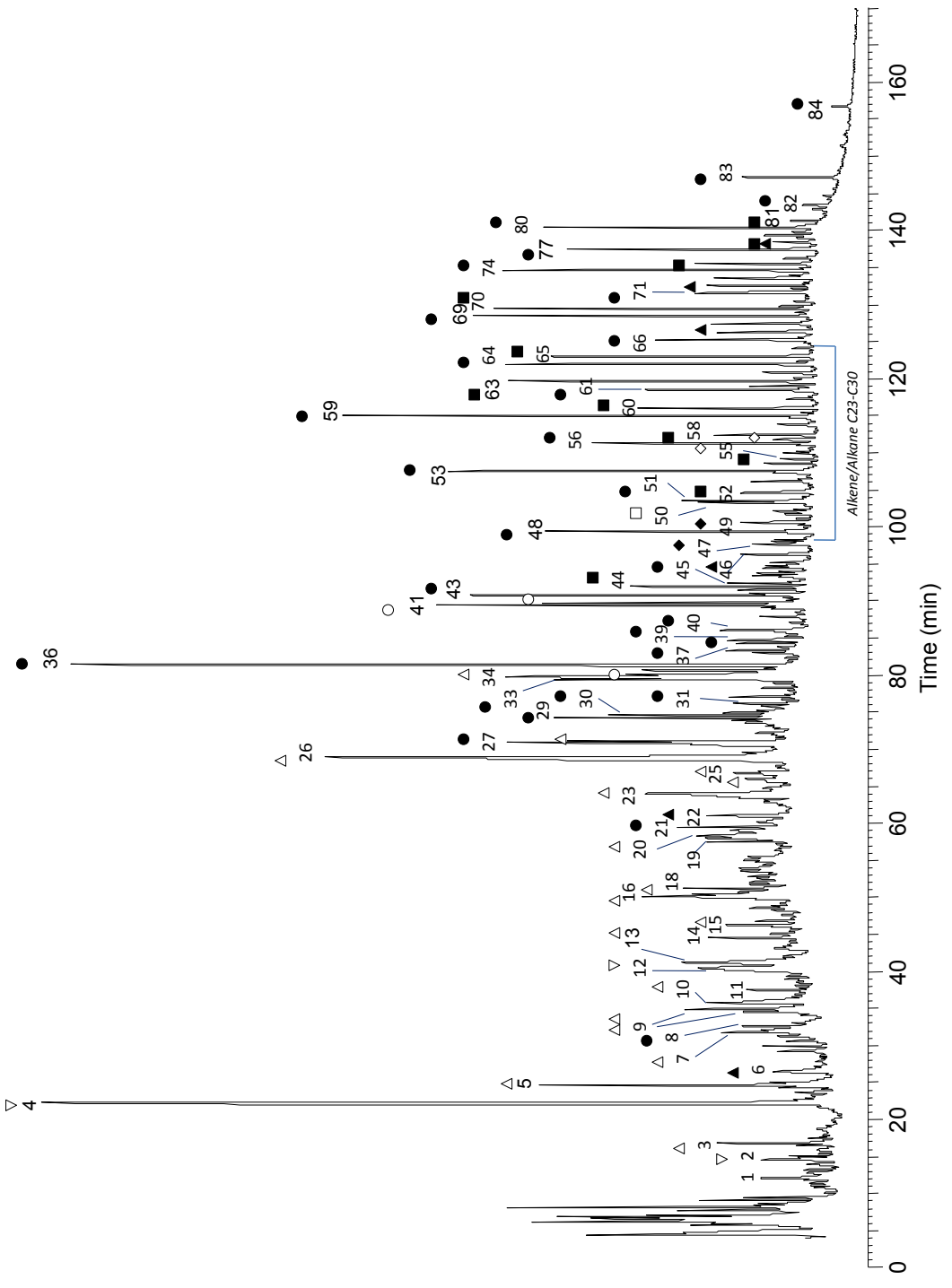


Figure 3.7: NT crust chromatogram. Peak numbers are reported in table 3.3. Main families are phenolic compounds (△), N compounds (▽), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (◆) and tri- (◇) hydroxyalkanoic acids, and alkanedioic acids (▲).

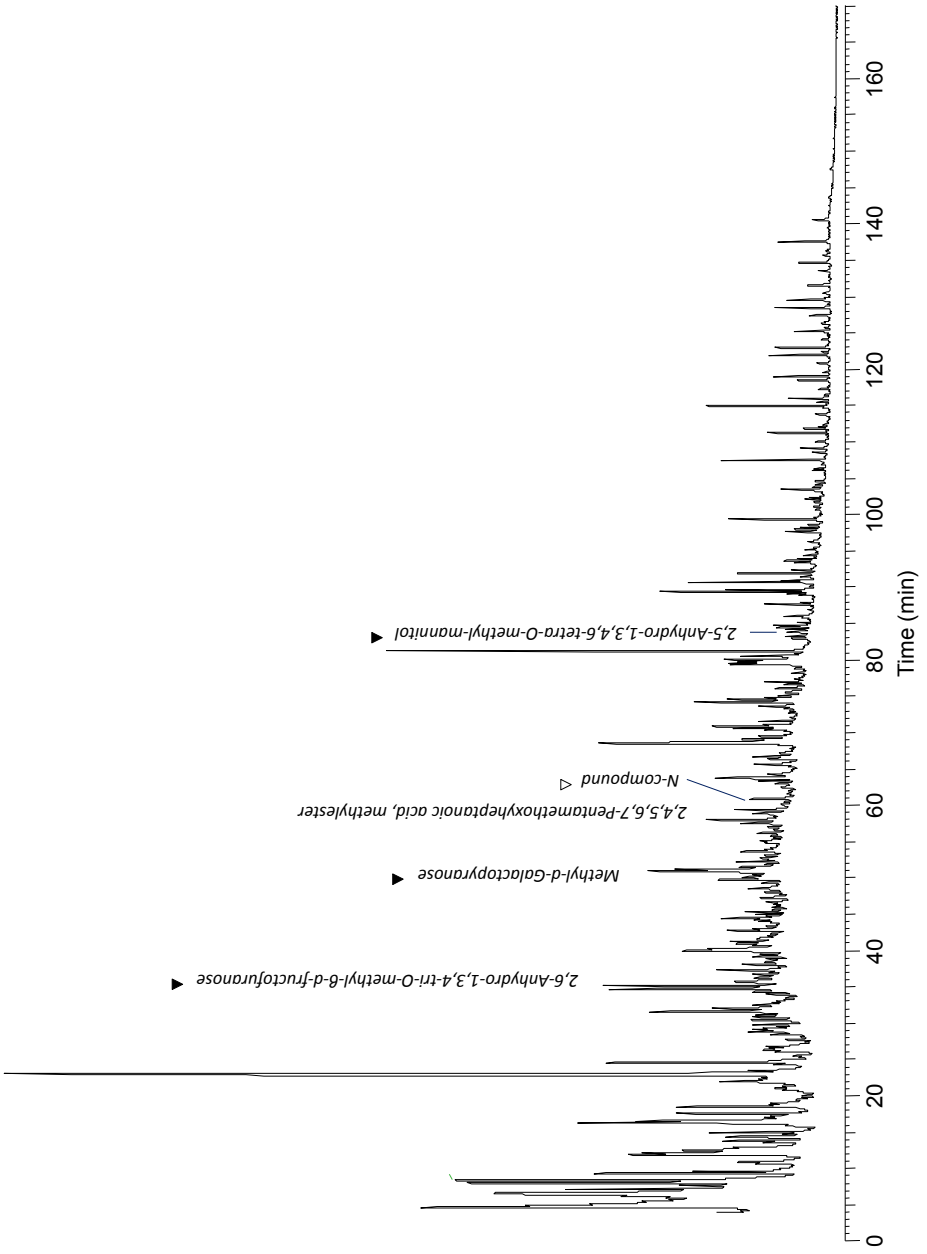


Figure 3.8: Non-demineralized NT crust chromatogram. It can be found compounds not well detectable in the corresponding demineralized sample, such as sugars (▼) and N compounds (▽).

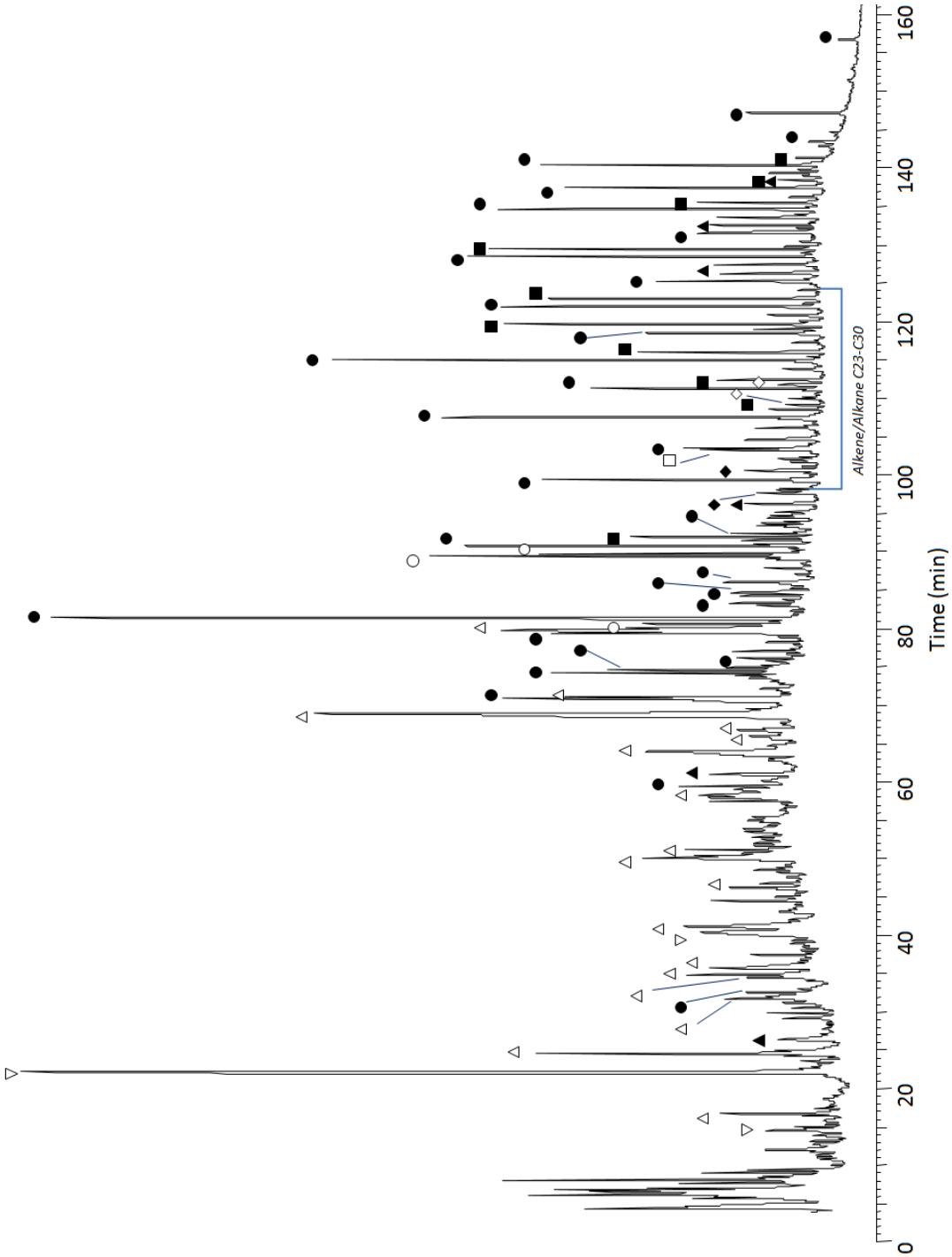


Figure 3.9: NT crust chromatogram (without peak numbers). Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (♦) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲).

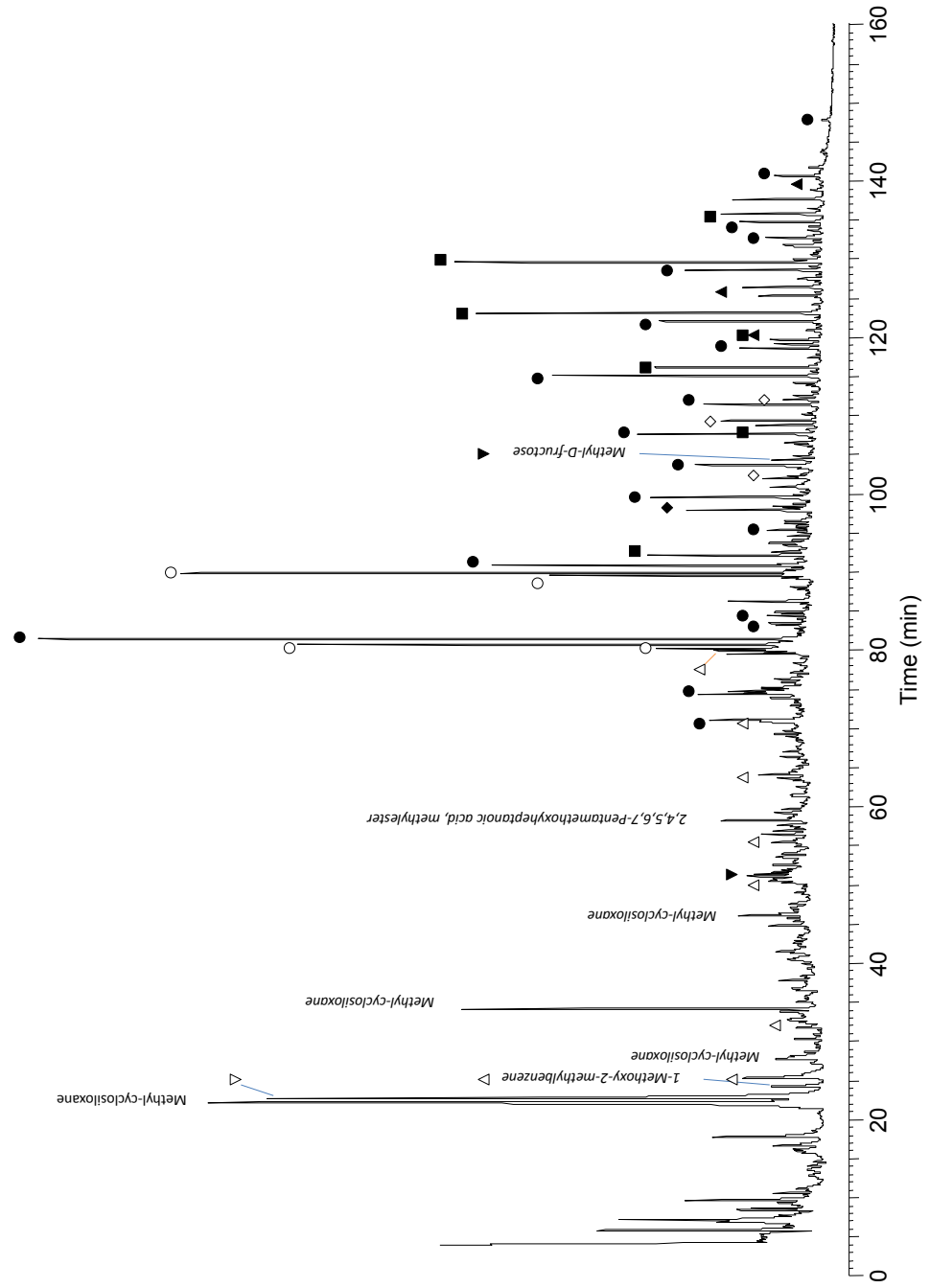


Figure 3.10: T crust chromatogram. Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (⧫) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲). The detection of cyclo-siloxanes is due to contaminations caused by chromatographic column constituents.

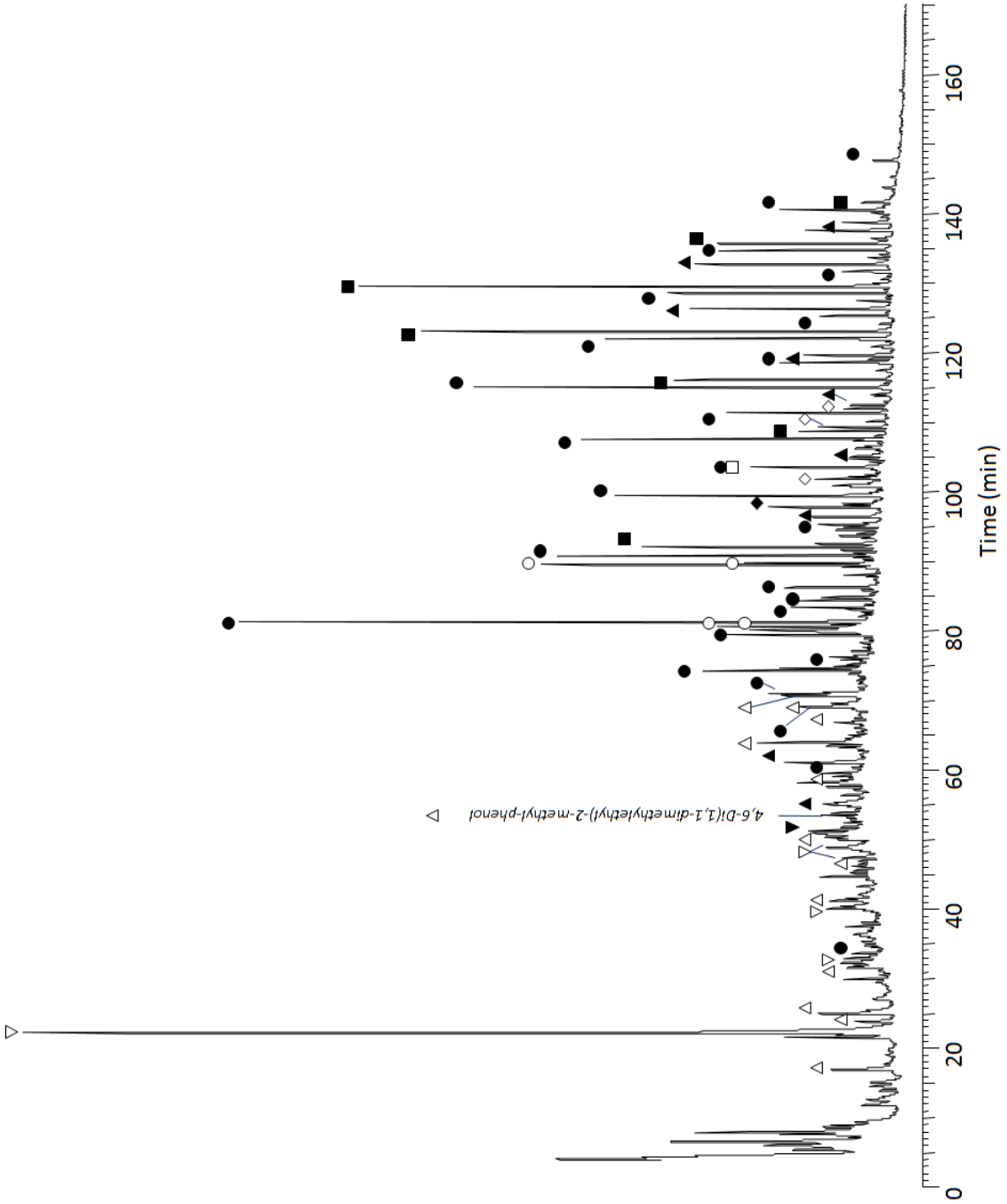


Figure 3.11: NT 0-5 cm chromatogram. Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (⧫) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲).

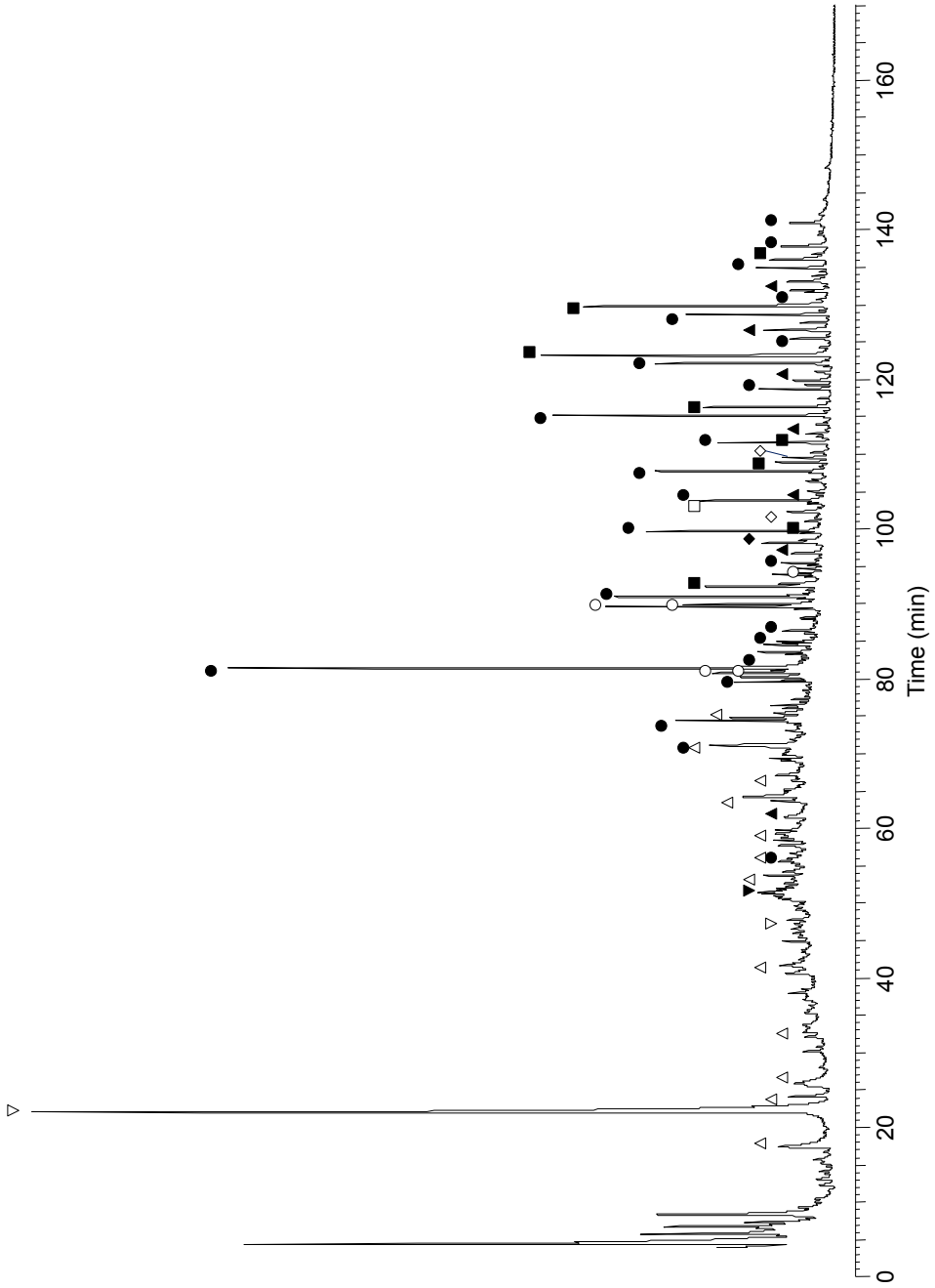


Figure 3.12: T 0-5 cm chromatogram. Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (⧫) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲).

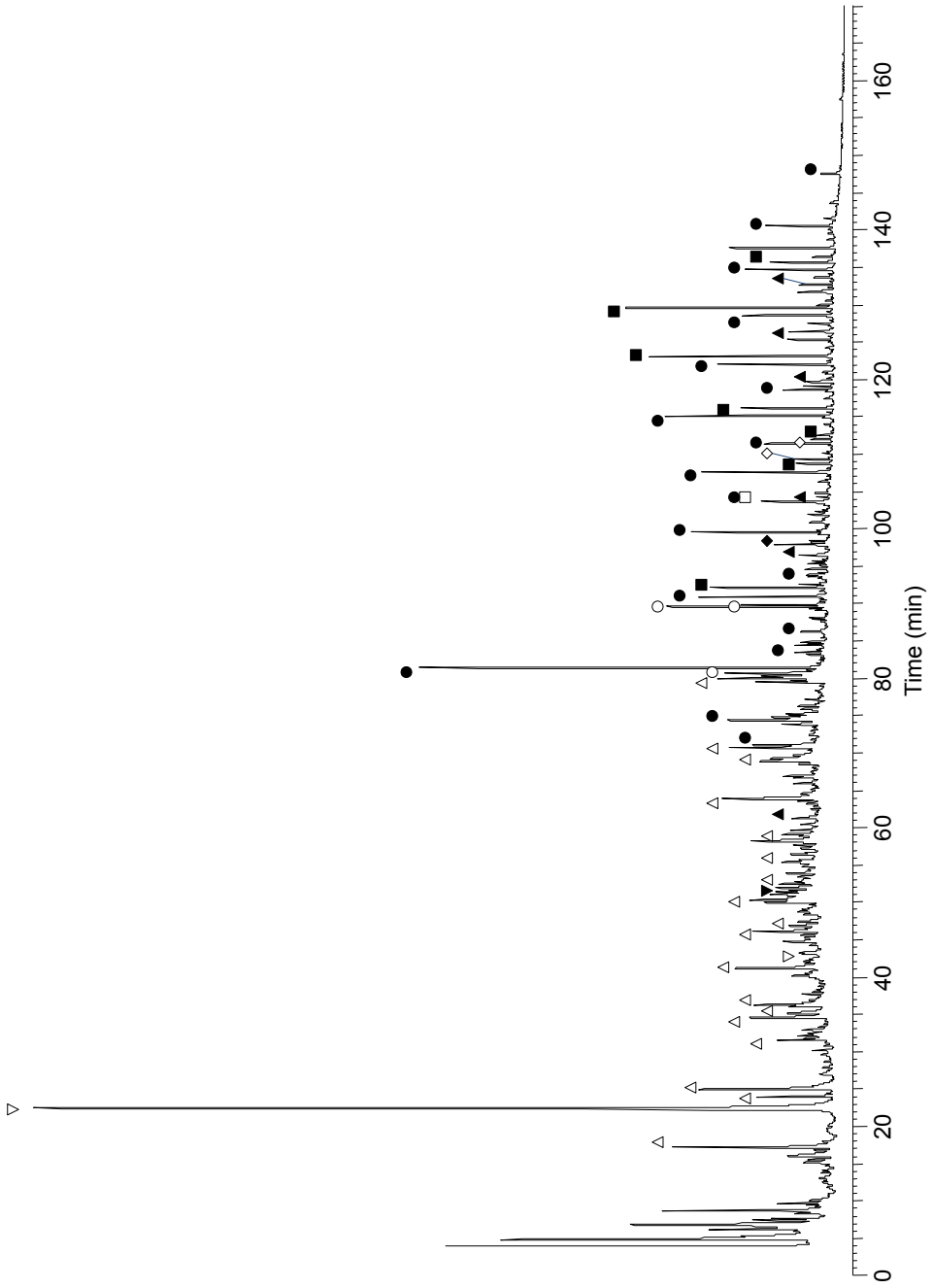


Figure 3.13: OV 0-2 cm chromatogram. Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (⧫) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲).

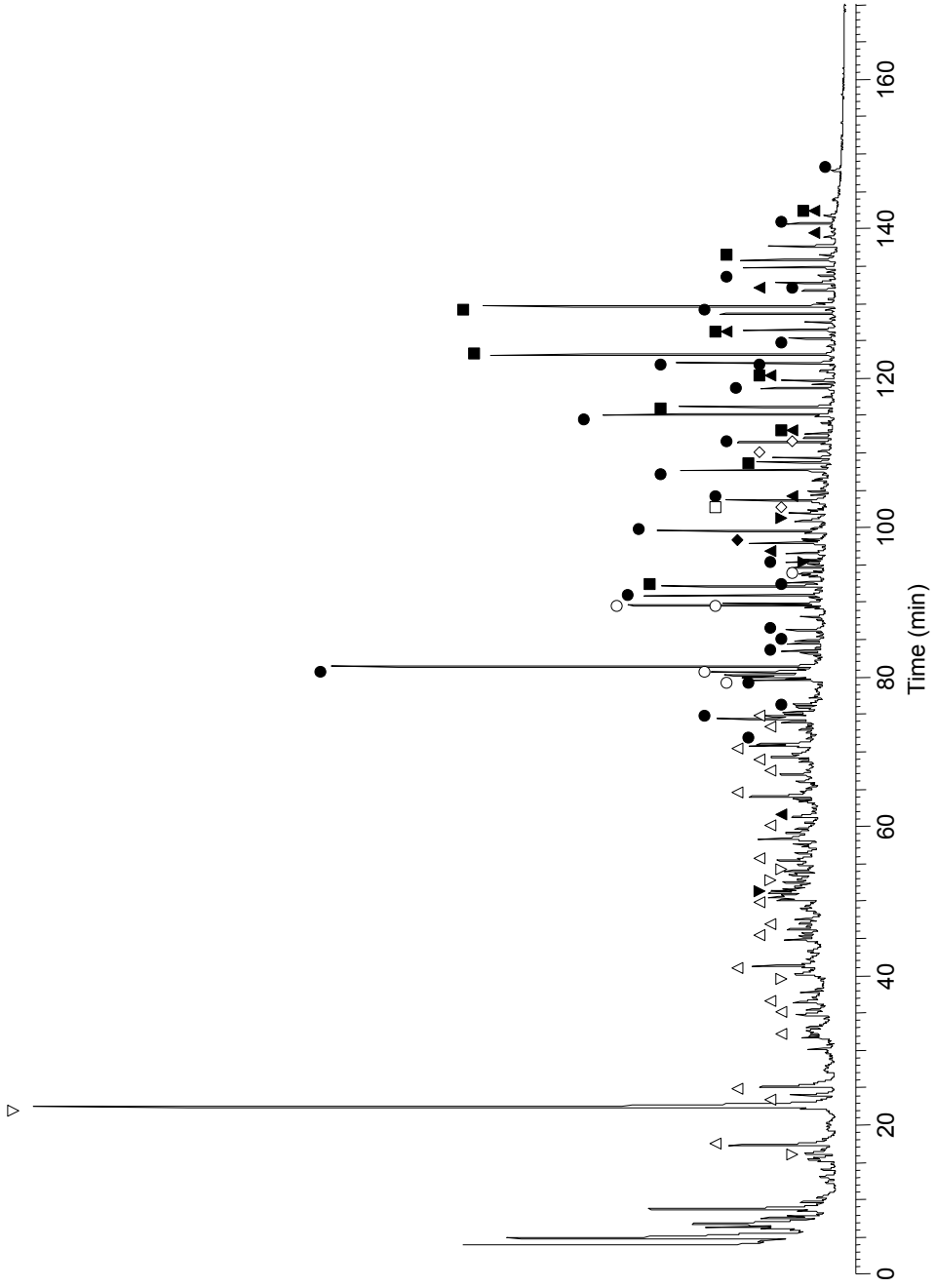


Figure 3.14: OV 2-4 cm chromatogram. Main families: phenolic compounds (△), N compounds (▽), sugars (▼), alkanoic (●) and alkenoic (○) acids, ω-hydroxyalkanoic (■) and alkenoic (□) acids, di- (⧫) and tri- (◊) hydroxyalkanoic acids, alkanedioic acids (▲).

3.4 NMR

Figure 3.15 shows a ^{13}C NMR spectrum, divided in 4 chemical shift regions, with an assignement of the principal resonance lines to functional groups. This spectrum was obtained from the analysis of T crust.
A/O-A ratios of demineralized samples are presented in graph 3.16, obtained as explained in section 2.4.
All the spectra (figure 3.17) have the same resonance peaks, but with different proportions between chemical shift regions, highlighed later in graphs shown in figure 3.18.

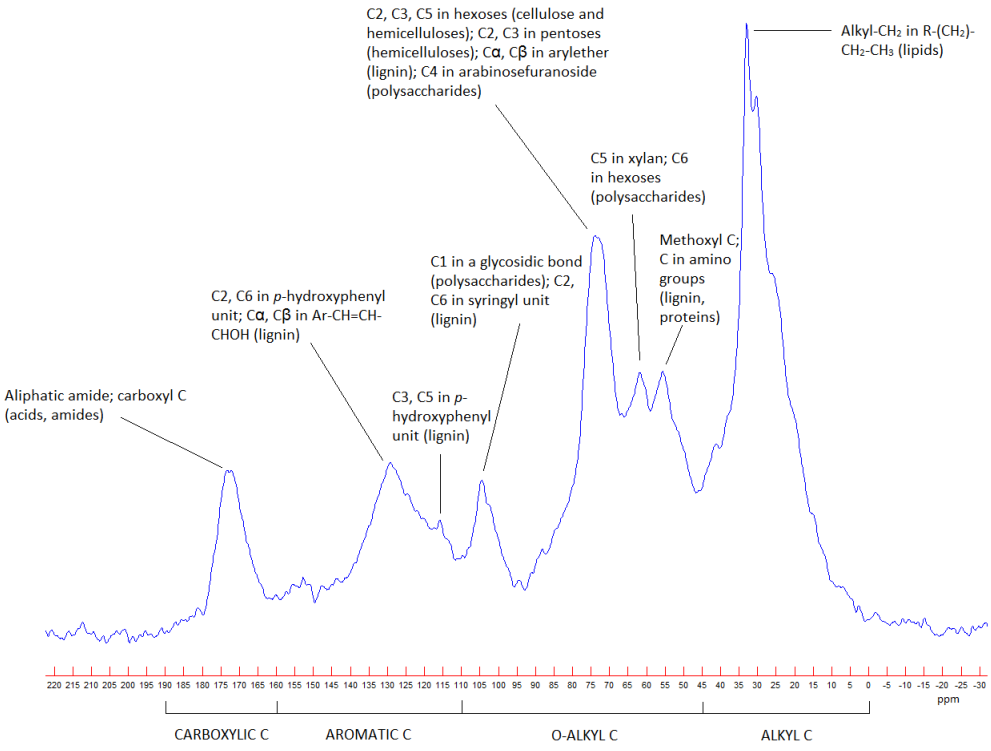


Figure 3.15: Assignment of the resonance lines to functional groups of the ^{13}C NMR spectrum.

The chemical shift region 0-45 ppm (alkyl C) covers unsubstituted C in paraffinic structures. Signal at 30 ppm show polymethylene C in long chain aliphatic structures (e.g. lipids, cutin), whereas branched structures produce signals around 20-30 ppm. Amino acid C also contributes to signal intensity between 17 and 50 ppm.
Aliphatic alcohol and ether structures (O-alkyl C), such as those found in

carbohydrates, have resonance between 45 and 110 ppm. The C2, C3, C5 in hexoses (celluloses, hemicelluloses) and C2 and C3 in pentoses (in hemicelluloses) contribute to the dominating signal at about 72 ppm. The shoulders at approximately 56 and 63 ppm are caused respectively by methoxyl C, C in amino groups (in lignin and proteins) and by C6 in hexoses or C5 in pentoses (e.g. xylan). The signal at 105 ppm is characteristic for the anomeric C1 of polysaccharides in a glycosidic bond (Wilson et al., 1983), with the contribution of C2, C6 in syringyl unit, from lignin (Wilson, 1987; Hatcher, 1987). The signals of aromatic carbon (between 110 and 160 ppm) with a chemical shift especially at 130 ppm are predominantly classified as C atoms of lignins (e.g. C2, C6 in p-hydroxyphenyl unit). Signal at 172 ppm refers to aliphatic amide and carboxyl C contribution (acid, amides, from 160 to 220 ppm).

Analyzing figure 3.16, NT crust shows an A/O-A ratio (0,66) decidedly smaller than T crust (0,89): this suggests that effectively the SOM of the first has a lesser extent of decomposition, as explained in the work of Panettieri et al. (2013). This may be due also to the greater input of fresh organic matter in NT soil.
The A/O-A ratio of the underlying layers is quite different from the crust, especially for the 0-5 cm layer: the ratios are reversed with a value for T sample (0,81) lower than that of NT one (0,97): visibly NT soil presents the most obvious change, with a net increase in the values. At 5-10 and 10-15 cm depths the ratios are slightly lower for T, but hardly these differences can be judged as significant.
This may be in line with the results obtained from EA-IRMS, that show a homogeneous profile for T soil: probably the O-alkyl components found in greater quantities in NT crust but lower in underlying layers, in T soil are better redistributed along the profile. Another hypothesis could be related to the fact that during the demineralization process were lost a larger amount of O-alkyl compounds in NT 0-5 cm sample than the others: however regarding NT pyrolysis mass chromatogram we do not noticed big differences in qualitative terms before and after the demineralization (there are about the same chemical compounds) and it is impossible to compare quantitatively these analyzes, because demineralized samples provide a stronger signal than the untreated ones.
It should however take into account, for all the demineralized samples, that during the acid treatment probably the fulvic acids (humic acids of lower molecular weight and soluble at wide pH range) were lost through the elimination of supernatant, since soluble at wide pH range. Fulvic acids, analyzed by Watanabe et al. (1989) with ^{13}C NMR, consist mainly of O-alkyl, aromatic and carboxylic compounds, therefore the A/O-A ratio should be further reduced.
In any case it is better to treat the results obtained from the crusts sep-

arately from the others, because they have the most significant difference between A/O-A ratios. OV samples, although we have only the 0-2 and 2-4 cm depths, show a trend more similar to what would be expected from a soil without plowing, i.e. with a relatively low A/O-A ratio if compared with a soil under T; for 0-2 cm depth the ratio (0,64) is even lower than NT crust value.

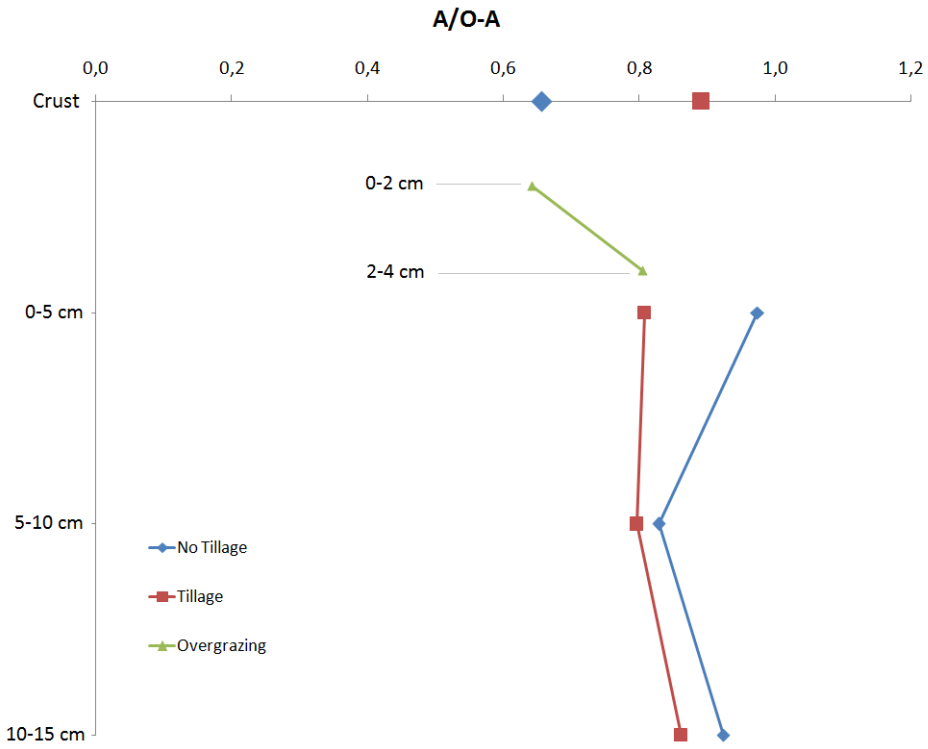


Figure 3.16: Alkyl C/O-Alkyl C ratios for NT, T and OV soil samples.

Figure 3.17 compares all the NMR spectra: the two main peaks are around 30 and 72 ppm.
Regarding the first layers, we note that O-alkyl region of NT crust and OV 0-2 cm predominates over alkyl region, mostly due to the peak at 72 ppm (arylether of lignin, arabinosefuranoside of polysaccharides and pentoses and hexoses of cellulose and hemicelluloses). In NT crust it can be noticed also that the peak at 56 ppm is slightly greater than the 63 ppm one, probably due to the greater amount of lignin compared to polysaccharides.
In all the spectra is always present the large peak of alkyl region around 30 ppm, rather predominant for T crust, NT 0-5 cm, T 0-5 cm and for all the deeper layers. In T 0-5 cm the contribution of polysaccharides, 63 ppm, seems to be higher than the contribution of lignin, 56 ppm.

Other signals, less relevant, common in all the spectra and present with approximately the same proportions are: 105 ppm peak, caused by syringyl units from lignin and polysaccharides; 128 ppm peak, caused by p-hydroxyphenyl units from lignin; 172 ppm peak, caused by carboxyl C and, secondly (given the results of Py-GC-MS), aliphatic amide.

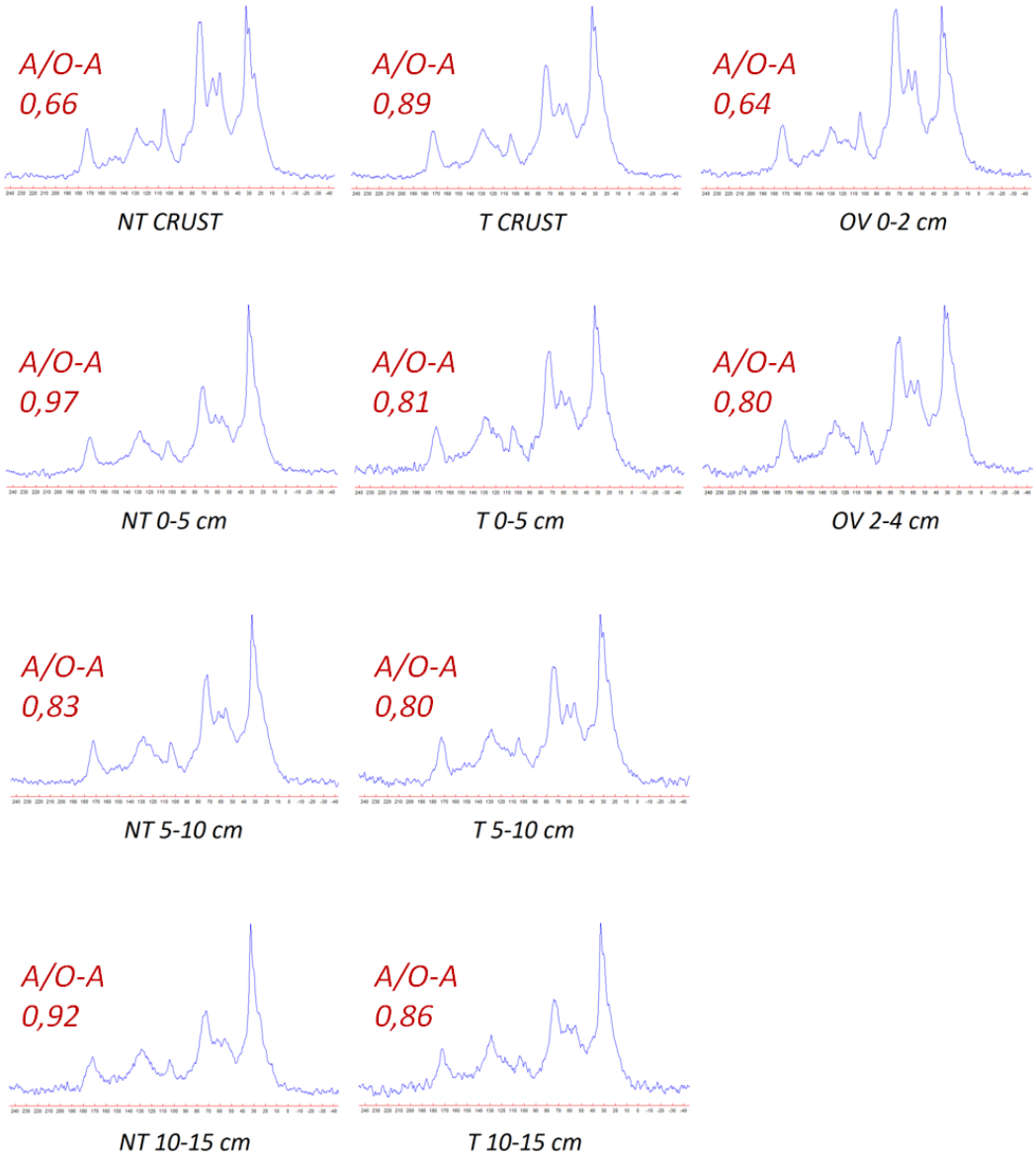
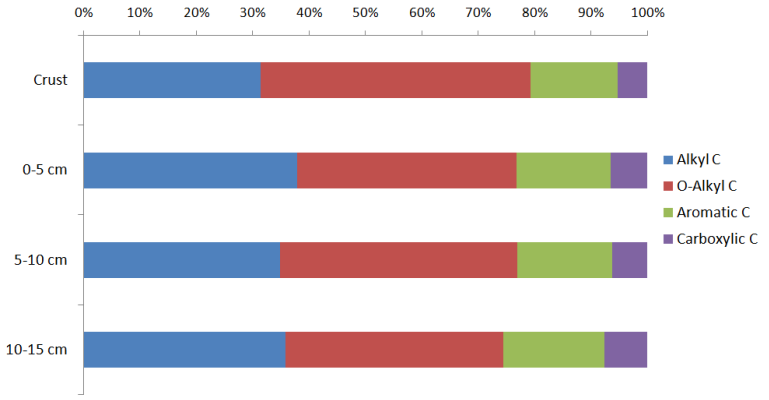
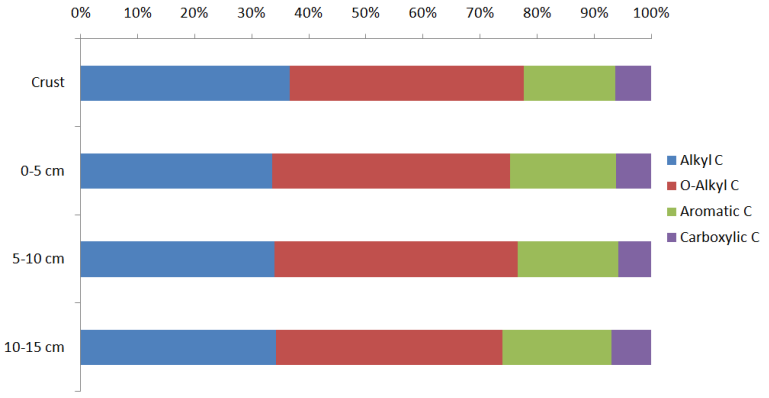


Figure 3.17: ^{13}C NMR spectra of soil samples. Chemical shift in ppm.

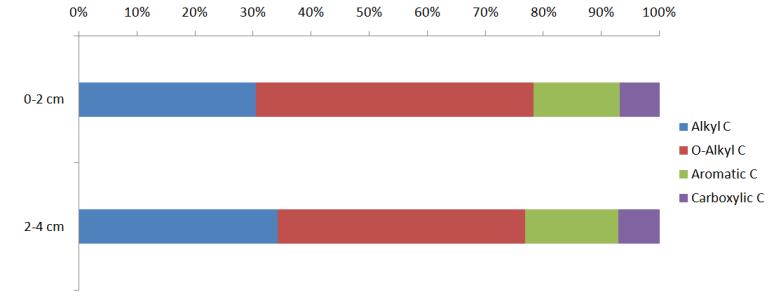
Graphs in figure 3.18 synthesize all the spectra, assigning the percentages of the total abundance for each chemical shift region. As explained above, O-alkyl C region is predominant in NT crust and OV 0-2 cm.
Regarding the smaller regions, aromatic C percentages for NT and OV have a slight increase with depth, especially for NT (from 5,3 to 7,6 %). Agreeing with Baldock et al. (1997), contrary to the traditional view that humic substances with an aromatic core accumulate as decomposition proceeds, changes in the aromatic region were variable: aromatic C, presumably derived from lignin structures, accumulates as the carbohydrates cellulose and hemicellulose are utilised, but then disappears with further decomposition to leave a residue with high contents of alkyl C (Bracewell and Robertson, 1987; Hempfling et al., 1987; Baldock et al., 1992; Zech et al., 1992).
Carboxyl C region (the less abundant) shows about the same trend of aromatic C percentages (not significant difference).



(a) *NT*.



(b) *T*.



(c) *OV*.

Figure 3.18: C distribution in the different chemical shift regions.

3.5 Data summary

3.5.1 EA-IRMS, Py-GC-MS and NMR discussion

Summarizing the results obtained from the analyzes, the biggest differences emerge regarding the crust layers: NT crust shows a greater abundance O-alkyl (lower A/O-A ratio, so a reduced degradation of SOM and/or a greater input of fresh organic matter) than T crust; Py-GC-MS analysis highlights the presence of numerous phenolic compounds derived from lignin. In addition NT crust has a higher C content (5,34 versus 1,76 %).
NT 0-5 cm has instead a higher A/O-A ratio than T 0-5 cm, but the Py-GC-MS data explain that the former has a greater molecular diversity and consists mainly of acids but also phenolic compounds (higher contribution of suberin, cutin and lignin). Besides EA-IRMS analysis and the calculations done with the bulk density measurements confirm that in NT sample a higher amount of C was found, estimated at around 30 mg C/cm^3 (versus 20 mg C/cm^3 of T sample), however taking into account all the precautionary considerations done in subsection 3.2.1.
OV samples, as NT crust sample, have smaller A/O-A ratios and a higher C content than T layers, with a greater contribution of lignin compounds (mainly phenolic groups) in 0-2 cm layer.
Recapping the data, the first layers of no-plowed parcels (NT and OV) have a greater C amount, a lower A/O-A ratio (except NT 0-5 cm), a greater abundance of phenolic compounds (using hexadecanoic acid as reference) and a greater molecular diversity.

3.5.2 Link with CO_2 emissions

By evaluating C release in atmosphere, I compared CO_2 emissions from T and NT soils, with a Paired t-test (one-tailed, NT-T<0), resulted from a meta-analysis of 39 studies that reported the emission values of the 2 agricultural practices (Chaplot V., personal communication). The CO_2 emissions of T soils are significantly lower than those of NT soils ($p = 0,00055$).
These studies and results of 2013 campaign prove that soil parcels under NT release less CO_2 than those under T (figure 1.1). Parallel to this, as it has been said repeatedly, we notice a greater C content in NT (and OV) soil than in T soil, particularly evident in the crust and in the upper layers.
The fact that the contribution of the acids in relation to the phenolic groups is greater in T samples, can be correlated with a higher microbial activity in the parcel. In fact microorganisms utilize both phenolic and acid compounds as C source, but they are not able to re-synthesize the first (whose signals are very weak in T samples), while alkanoic and alkenoic acids may also be by-products of their activity, as mentioned above (section 3.3). The

hypothetical more intense microbial activity in T soils may cause a reduction of SOC stock, due to a higher respiration (higher CO_2 release).
Another factor that may influence the CO_2 emissions is the higher amount of lignin compounds in NT parcel, due both to greater contribution of plant residues (a priori, there are no differences in biomass between the parcels) and lower microbial activity. Lignin in fact plays a significant role in the C cycle, sequestering atmospheric C into the living tissues of woody perennial vegetation (Rahman et al., 2013), and it is one of the most slowly decomposing components of dead vegetation (except in the presence of specific wood-degrading fungi).
The use of T cultural practice can therefore lead, in time, to a diminution of SOC stock, related to the intense activity of aerobic microorganisms that occurs after plowing, which makes oxygen more available to them.
The presence of the crust layers causes an increase in CO_2 emissions for both parcels (1.2), which suggests a higher microbial activity of both layers. But we must take into account that in NT crust we found a C content much greater: considering that the emissions are about the same, this means that NT crust structure allows to store more SOC.

Chapter 4

Conclusions

The results obtained from this work highlight that NT soil has a greater C and N stocks, taking into account the first layers, and a higher molecular abundance and diversity than T soil.
OV parcel does not show particular differences compared to the NT one. It is not possible therefore to associate with this practice a particular benefit to the soil, as hypothesized in subsection 2.1.1.
The higher contribution of phenolic group in NT parcel along with the lower CO_2 emissions allow to make general assumptions on the C cycles that occur in soil profiles. The figure 4.1 summarizes the different results obtained during this study and presents a model consistent with the proposed hypotheses on soil functioning.
Assuming that NT and T parcels produce and provide to the subsoil the same biomass amount, in NT plot it can be hypothesized a greater total SOC amount due to the higher intake of fresh plant OC. In fact lignin components (phenolic groups found with Py-GC-MS) have a more important contribution in no-plowed parcel. This is linked to the probable lower microbial activity (confirming the analysis of 2013): microorganisms, as esplained in section 3.3, utilize both phenolic and acid compounds as C source, re-synthesizing only acid products, that contribute only in part to reform their biomass.
In T plot the acid contribution is higher in relation to that of phenol compounds, and this is probably due to the higher microbial activity that use principally the re-synthesized acids to increase the microbial biomass. As a result, the intense activity causes an increase in CO_2 emissions, so a lower total SOC stock.
It will be important to deepen the analyzes on NT crust layer: its mass chromatogram shows a molecule distribution quite different from the others, as well as the highest C content found in the samples. This does not exclude the possibility that it has a biological origin.

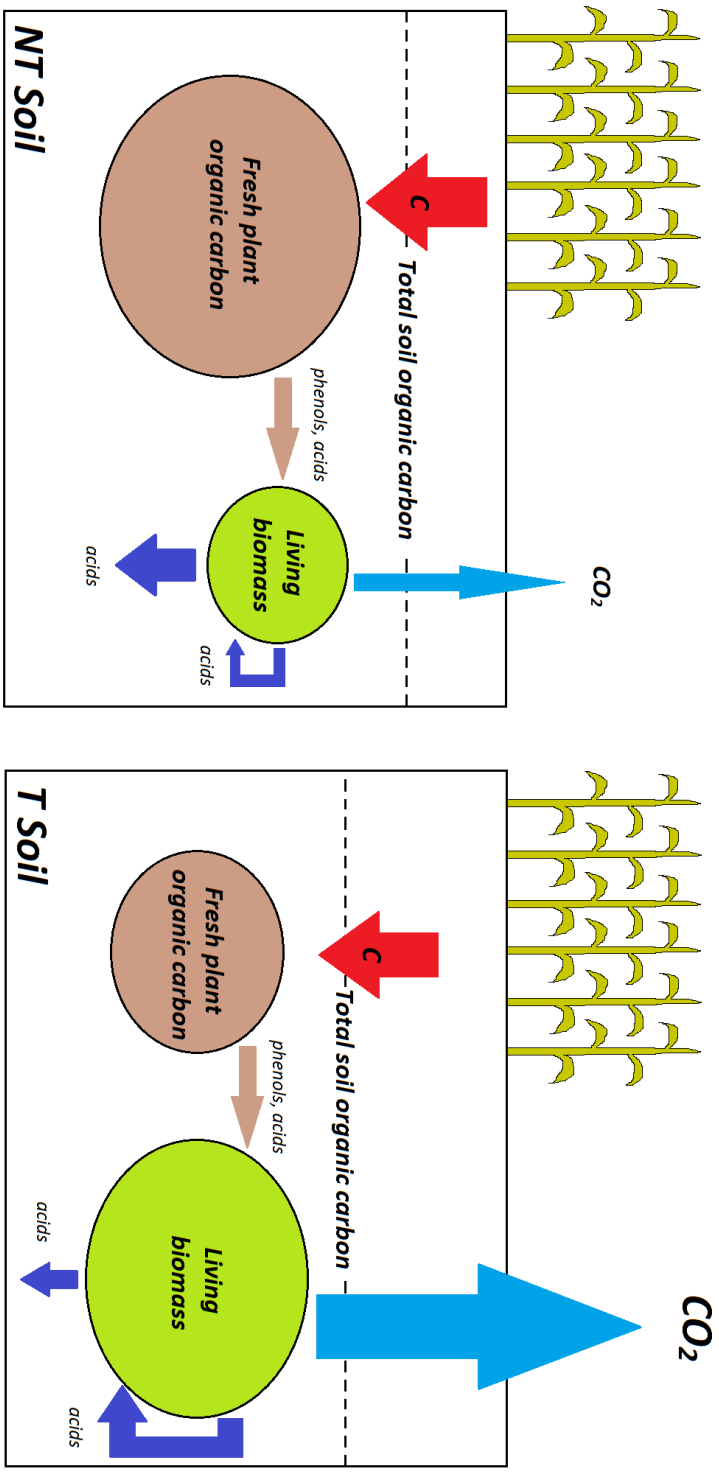


Figure 4.1: Scheme of hypothetical carbon cycles in NT and T soils.

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