Improving our Understanding of Carbon Cycling in Agroecosystems by Studying $\delta^{13}C$ Signatures in Soil Carbon Stocks and Fluxes

Results from a Swedish Long-Term Field Experiment

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2014

Acta Universitatis agriculturae Sueciae
2014:5

Cover: *Lives of Grass*, artwork by Mathilde Roussel-Giraudy (reprinted with permission)

ISSN 1652-6880
ISBN (print version) 978-91-576-7958-1
ISBN (electronic version) 978-91-576-7959-8

Print: SLU Service/Repro, Uppsala 2014

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Abstract

Soil carbon (C) storage has gained much attention in the past decade due to its potentially huge impact on climate change mechanisms. Particular focus has been paid to possible feedback mechanisms, whether soil can be considered a sink or source of C and how soils can be managed in order to mitigate climate change. However, there is still a lack of knowledge about the contribution of different C sources to soil organic carbon (SOC) formation, particularly in the subsoil. As the subsoil can store approximately as much C as the topsoil, such information is crucial. In a series of studies described in this thesis, data from the Ultuna long-term field experiment established in 1956 were used to study the effects of different organic amendments and mineral fertilizers, which were found to have influenced topsoil and subsoil in several ways. Cultivation of maize, a C_4 crop, began in the experiment in 2000, but prior to that only C_3 crops were grown. By exploiting the natural difference in C isotopic signature between the metabolisms in these two plant types, the role of these two sources in soil organic matter turnover could be quantified. The isotopic composition of C fluxes and stocks in the Ultuna experiment revealed the contribution of different sources to different C pools. Old (>10 years) SOC resulted contributing as much as young (<10 years) SOC to soil respiration. A set of soil respiration measurements with a cavity-ring-down spectrometer directly in the field at different times over the growing season allowed the relative contributions of autotrophic and heterotrophic respiration to be determined. Another set of measurements on soil samples from 0 to 50 cm depth was used to estimate the contribution of different SOC sources within the profile, finding a high contribution of root-derived C on total SOC in the topsoil and upper subsoil. By utilizing the topsoil C isotope and SOC data the root humification coefficient with two different methods could be calculated, suggesting that contribution of root-derived C on total SOC is often underestimated. The measured changes in the δ^{13}SOC signature over the years in European bare fallow plots receiving virtually no C input during several decades allowed the natural δ^{13}C enrichment occurring in SOC to be measured and modelled. The results validated those of the natural abundance-based techniques over the mid- to short-term. The natural δ^{13}SOC enrichment was modelled by calibrating a Rayleigh distillation function. The enrichment kinetic resulted similar in all the sites, and the general calibration could be extended to other studies.

This thesis studied the C cycle in a managed long-term field experiment from several perspectives and across different scales, so providing valuable information of potentially high importance for understanding the general dynamics of C in soil. During the 53 years of the Ultuna long-term field experiment, significant differences between the treatments have developed, with clear C accumulation or depletion in soil to 35 cm depth. Thus, SOC changes below ploughing depth should be considered in SOC balance studies and as a viable C sequestration strategy.

Keywords: SOC, subsoil, $\delta^{13}C$, stable C isotope, C sequestration, humification, $\delta^{13}C$ enrichment

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Dedication

To Professor Giovanni Ferrari, for having shown a bored teenager the vibrant beauty of the red shades of Oxisols.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Menichetti, L., Ekblad, A. & Kätterer, T. (2013). Organic amendments affect $\delta^{13}C$ signature of soil respiration and soil organic C accumulation in a long-term field experiment in Sweden. *European Journal of Soil Science* 64, 621-628

II Menichetti, L., Ekblad, A. & Kätterer, T. Soil carbon accumulation along the soil profile in a long-term field experiment in Sweden. (Submitted to *Agriculture, Ecosystems and Environment*).

III Menichetti, L., Kätterer, T., Christensen, B.T., Chenu, C., Barré, P., Vasilyeva, N.A. & Ekblad, A. Shifts in $\delta^{13}C$ with loss of soil organic carbon in long-term bare fallow experiments. (Submitted to *Oecologia*).

IV Kätterer, T., Bolinder, M., Andrén, O., Kirchmann, H. & Menichetti, L. (2011). Roots contribute more to refractory soil organic matter than aboveground crop residues, as revealed by a long-term field experiment. *Agriculture Ecosystems and Environment* 141, 184-192.

Papers I and IV are reproduced with the kind permission of the publishers.

The contribution of Lorenzo Menichetti to the papers included in this thesis was as follows:

I Main author. Planned the analysis together with the co-authors. Performed the soil sampling and the gas flux analyses. Performed data analysis. Performed writing with the assistance of the co-authors.

II Main author. Planned the analysis together with the co-authors. Performed the soil sampling. Performed data analysis. Performed writing with the assistance of the co-authors.

III Main author. Consolidated the dataset. Performed data analysis. Performed writing with the assistance of the co-authors.

IV Performed soil sampling. Participated in the writing together with the co-authors.

Abbreviations and definitions

C_4	Plant with an intermediate PEP carboxylase step in photosynthesis, meant to localise C fixation far from high oxygen concentrations and to reduce photorespiration. Adaptation typical for lower latitude plants.
C_3	Plant with direct photosynthesis.
$\delta^{13}C$	Ratio of ^{13}C to ^{12}C relative to the international standard of Vienna Pee Dee Belemnite according to the equation: $\delta^{13}C = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right)$ where $R = \frac{^{13}C}{^{12}C}$
$\delta^{15}N$	Ratio of ^{15}N to ^{14}N relative to the international standard on atmospheric nitrogen according to the equation: $\delta^{15}N = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right)$ Where $R = \frac{^{15}N}{^{14}N}$
HSD	Honestly Significant Difference
LSD	Least Significant Difference
LTBF	Long-Term Bare Fallow
LTE	Long-Term Experiment
MCMC	Monte Carlo Markov Chain
PLFA	Phospholipid-derived fatty acids

RMSE	Root Mean Squared Error
SOC	Soil Organic Carbon

1 Introduction

Carbon (C) is by far the most abundant element, excluding water, in life forms as we know them. It is therefore an element connected with anything that is biologically related.

Soil is a particular ecosystem, and some authors view it as a living entity (Phillips, 2009). It is therefore not surprising that soil C, besides representing the largest organic element cycle in soil by mass, is the element most tightly connected with the whole biosphere. Soil organic C (SOC) influences soil structure, erodibility and cation exchange capacity and provides energy to microorganisms, controlling the life of every organism in soil. The C ultimately provides both fuel and engine building blocks that keep the soil alive, and it is therefore critical for maintaining soil functions. Soil functions in turn have a direct or indirect positive impact on human life, and can be defined as ecosystem services. In a global perspective, soil is the major reservoir of organic C (Stockmann *et al*., 2013). In the perspective of climate change and the need to reduce the amount of greenhouse gases (GHG) in the atmosphere, SOC sequestration is one ecosystem service that has been studied over recent decades (Lal, 2011). SOC sequestration capacity is the capacity of the soil to act as a sink, as a consequence of the balance between C influxes (mainly in the form of organic matter) and effluxes (mainly in the form of CO_2). The possibility to maintain or increase soil C stocks depends on the capability to increase the influxes or reduce the effluxes. Estimating or measuring changes in C stocks are therefore relevant for policy decisions and might have a strong impact on human societies.

The amount of C stored in the subsoil is a crucial aspect to be considered. Estimates of global SOC stocks vary between approximately 1500 Pg to 1 m depth and 2300 Pg to 3 m depth (Jobbagy & Jackson, 2000), but are difficult to quantify with high precision (Lorenz & Lal, 2005). Uncertainty is also high regarding the relative contribution of different sources to the more stabilised SOC, as a process-based understanding of SOC stabilisation is still under development (Kleber *et al.*, 2011). A precise estimate of how much C from several C sources enters the SOC pool is a crucial parameter for predicting the outcome of different management regimes.

Existing knowledge has been summarised into mathematical models that can be conceptualised in several possible ways. Although SOC is a continuum (Bosatta & Ågren, 1999), it is often conceptualised for practical purposes as the sum of several discrete pools (*e.g.* Andrén & Kätterer, 1997, Coleman et al, 1997). Each pool is defined by a certain decay constant and the rate at which it receives inputs. Although this is only a semi-empirical representation of the SOC balance, it is a useful way of summarising knowledge in a practical form.

In agricultural systems, both sides of the SOC balance can potentially be managed. Management option that increase net primary production will generally lead to increased SOC stocks (Kätterer et al., 2012). However, the quantification of the proportion of any C input that will remain in soil or a soil C pool after a certain time (i.e., the humification coefficient) is still a major challenge. Most difficulties arise because of the continuous nature of SOC, but also because of the complexity of the system and difficulties in measuring single processes often interacting in a non-linear way. Using C isotopes is popular in soil science because one can mark a specific substance without major interference with the system and follow the fate of the substrate directly in the field. Soil C stable isotopes, ^{12}C and ^{13}C, which are present in different ratios in nature, are suitable to monitor the fate of several organic substrates in soil.

In this thesis, fluxes of maize-derived C were studied by utilising the natural isotopic abundance generated by the two major photosynthetic systems present in plants, C_3 and C_4 (Balesdent & Mariotti, 1996). The C inputs from maize cultivation, mainly in the form of root exudates and dead roots, enter the SOC pools, leaving a detectable $\delta^{13}C$ signature at different soil depths.

Through a mass balance calculation it was possible to estimate to what extent maize rhizodeposition had influenced the formation of SOC in the soil profile. The same type of balance calculation was applied to soil respiration data in order to estimate the contribution of CO_2 mineralised from maize material and the amount of residual maize C in soil.

The C amount derived from maize could then be related to the amount of SOC formed since the beginning of the Ultuna long-term experiment. As the amount of C input through addition of amendments and crop yield was known, it was possible to derive the root C inputs and calculate the contribution of root C to SOC since the start of the experiment in 1956. The isotopic mass balance and the total C mass balance were compared and integrated, providing information on C kinetics over time scales from decades to weeks.

The limits and opportunities of this technique were tested using the changes in the isotopic signature of SOC in a bare fallow treatment over the duration of the long-term experiment (LTE). Other long-term bare fallow treatments in Europe were also used in the evaluation. The results can significantly improve knowledge of ^{13}C kinetics in soil.

Overall, this thesis describes the C cycle from several perspectives, considering the long-term kinetics, the origins of SOC and short-term kinetics leading to soil respiration, in an agricultural long-term experiment. The results can broaden our perspective on the C cycle in soil.

2 Aims

This thesis investigated stocks and fluxes of various SOC pools (Figure 1), with the aim of answering the following questions:

- How have approximately 50 years of different treatments affected topsoil and subsoil C in a Swedish long-term field experiment?
- Is the root C input relevant for soil C accumulation in the whole soil profile? And if so, how much does it amount to? To what extent does root C contribute to soil C effluxes?
- Is ^{13}C:^{12}C ratio a reliable method for estimating the kinetics of different SOC pools over the long-term? How can estimates be improved?
- How can we influence SOC balances through agricultural management?

These questions were investigated in Papers I-IV, specific objectives of which were to:

- Determine the contribution of different C sources to soil respiration
- Determine the contribution of different C inputs to SOC stocks over the soil profile
- Model the natural $\delta^{13}C$ change occurring in SOC in several long-term bare fallow experiments
- Determine the contribution of different C inputs to SOC

Most of the research questions were formulated as mathematical hypotheses by combining functions describing SOC turnover. The difference in the natural abundance of ^{12}C and ^{13}C between C_3 and C_4 plants allowed two different SOC pools, defined by age since maize has been cultivated in the experiment since the year 2000, to be distinguished.

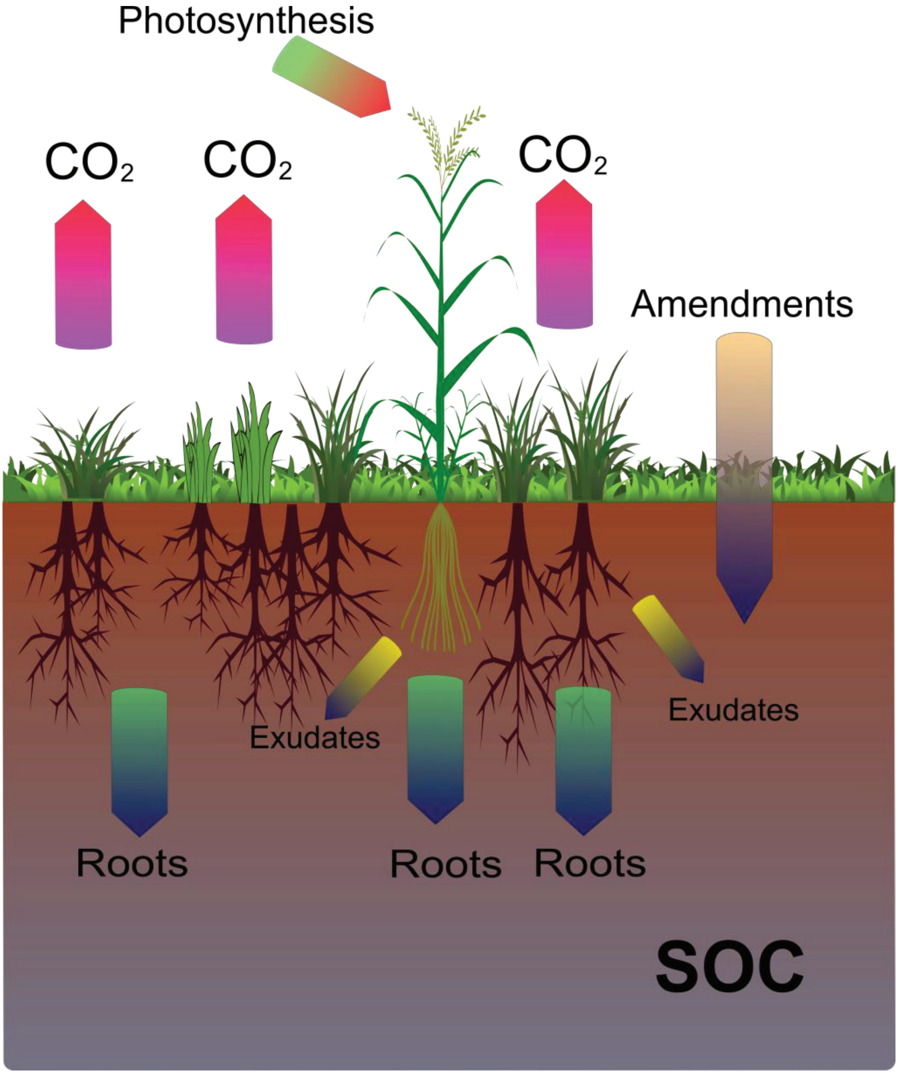


Figure 1. Schematic representation of the C pools considered in this thesis. SOC represents the whole soil organic carbon content.

3 Methods

3.1 The Ultuna Long-Term Experiment

The core of this thesis work was the Ultuna long term field experiment (LTE) (Figure 2), which has been managed by the Swedish University of Agricultural Sciences (SLU) since 1956. The site had been in agricultural use for at least 300 years before the beginning of the experiment. Since then, plots have been cultivated manually. The experimental site is located in Ultuna, close to Uppsala (59.82°N, 17.65°E). According to the Köppen classification (Peel, 2007) the climate is Dfb (warm summer hemiboreal). The site has mean annual precipitation of 570 mm and mean annual air temperature of +5.4°C. The topsoil (0-20 cm) is a clay loam with 36.5% clay, 41% silt (0.002-0.06 mm) and 22.5% sand (0.06-2 mm) and has been classified as a Eutric Cambisol (IUSS Working Group, 2007). The parent material consists of post-glacial sediments. In 1956 the soil had an organic C content of 1.5%, an N content of 0.17% and a pH(H_2O) of 6.6.

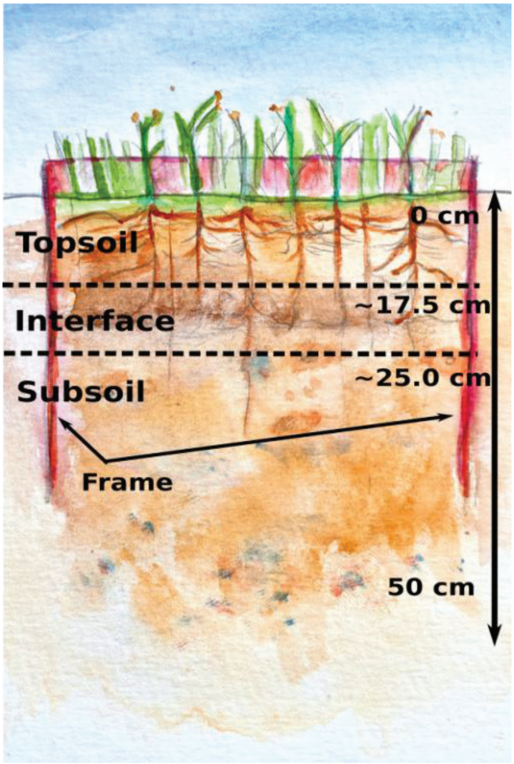


Figure 2. A schematic illustration of the Ultuna soil profile.

From 1956 to 1999, C_3 annual crops such as oats, spring barley, sugar beet, oilseed rape, turnip rape and white mustard were cultivated and had an average $\delta^{13}C$ signature of -28.0 ±0.1‰. In 2000 these crops were replaced with forage maize, a plant with a C_4 photosynthetic cycle and an average $\delta^{13}C$ signature of -12.3±0.1‰.

The experimental design consists of 15 treatments in four replicate plots in a randomised block design (Table 1). Each plot is 2 m × 2 m, separated by 40 cm high steel frames inserted to a depth of 30 cm (Figure 2). Approximately the same amount of C (~4 Mg ha^{-1}) is added in 10 of the treatments in autumn every second year as different organic amendments (Table 1). Inorganic N fertiliser is added annually during spring at a rate of 80 kg N ha^{-1} yr^{-1} in the fertilised treatments. The experiment also contains a control treatment (neither N fertiliser nor organic amendments) and a bare fallow treatment (kept free from vegetation). All plots are fertilised annually with 22 kg P and 35-38 kg K ha^{-1}. Above-ground crop residues are harvested by cutting the crop close to the soil surface and all above-ground biomass is removed. A sample archive stores samples from topsoil, plant materials and amendments taken every second year since 1983 and intermittently since 1956.

Table 1. *The 15 treatments of the Ultuna LTE.*

Code	Treatment applied
A	Without organic matter, without N, bare soil
B	Without organic matter, without N cultivated
C	Without organic matter, and 80 kg N ha^{-1} as calcium nitrate
D	Without organic matter, and 80 kg N ha^{-1} as ammonium sulphate
E	Without organic matter, and 80 kg N ha^{-1} as calcium cyanamide
F	4 t ha^{-1} C in straw, every two years, without N
G	4 t ha^{-1} straw C, every two years, and 80 kg N ha^{-1} as calcium nitrate
H	4 t ha^{-1} C as green manure every two years, without N
I	4 tons ha^{-1} peat every two years, without N
J	4 t ha^{-1} C as farmyard manure every two years, without N
K	4 t ha^{-1} C as farmyard manure + P every two years, without N
L	4 t ha^{-1} C as sawdust every two years, without N
M	4 t ha^{-1} C as peat every two years, and 80 kg N ha^{-1} as calcium nitrate
N	4 t ha^{-1} C as sawdust every two years, and 80 kg N ha^{-1} as calcium nitrate
O	4 t ha^{-1} C as sewage sludge every two years, without N

3.2 The Long-Term Bare Fallow network

In order to model natural $\delta^{13}C$ enrichment and examine how it can be influenced by climate and edaphic conditions, five sites within the European Long-Term Bare Fallow (LTBF) network (Barré *et al.*, 2010) were used (Figure 3). Each site in the network fulfils the following criteria: 1) The experimental plots have been kept bare (vegetation-free) and without organic amendments for more than 25 years; 2) there have been regular measurements of SOC in the plough layer; and 3) there is a soil archive with soil samples taken at intervals since the establishment of the experiment, allowing for analyses of the $\delta^{13}C$ signature of SOC remaining after different periods of time. The five sites in the study are located at Ultuna (Sweden), Askov (Denmark), Kursk (Russia) and Versailles and Grignon (France). The Ultuna site has been

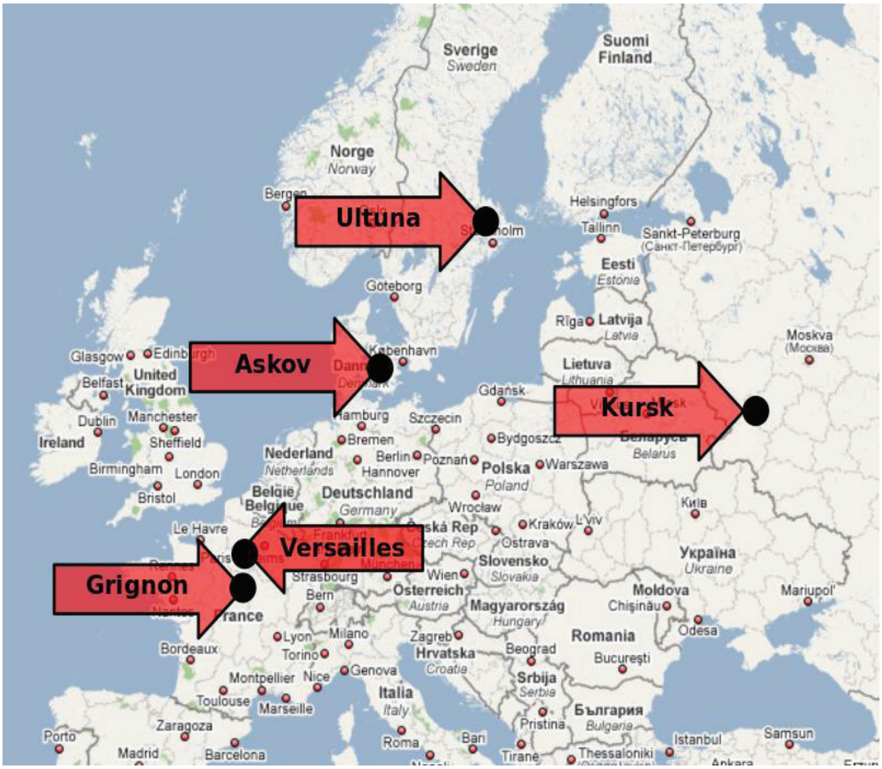


Figure 3. Location of the sites of the LTBF network.

described above and the other four sites are briefly described below. The analytical methods are described in detail in Paper IV.

3.2.1 Askov

The experiment was situated next to the B4-field of the Askov Long-Term Field Experiments on Animal Manure and Mineral Fertilizers (Denmark), at 55° N. The climate is classified as Cfb according to the Köppen classification. Subsamples were retrieved from the soil archive of a field experiment with bare fallow initiated in 1956 and terminated in 1985. The soil is classified as a Haplic Luvisol (IUSS Working Group, 2007), with 77% sand, 13% silt and 10% clay. The dominant minerals of the clay fraction are illite and smectite. The site was brought under arable use around 1800. The experiment included four replicate plots with bare fallow. Further details can be found in Christensen *et al*. (2011).

3.2.2 Grignon

The Grignon site is a fertilisation experiment initiated in 1956 in the gardens of the Chateau de Grignon, France, at 48° N. Before 1875, land use was unmanaged grassland. The climate is classified as Cfb according to the Köppen classification. The soil is a silty loam classified as a Haplic Luvisol (Colbach et al., 2000; IUSS Working Group, 2007), developed within a colluvial carbonate deposit. Before the start of the experiment, the site had been under grassland since before 1875 (Barré et al., 2010). All plots are dug by hand twice a year to a depth of 25 cm, and kept free from vegetation. The site has six different treatments, including bare fallow, with six replicates of each. Soil organic C concentrations have been measured by dry combustion. Further details can be found in Morel et al. (1984).

3.2.3 Versailles

The Versailles fertilisation experiment was initiated in 1928 in the garden of Chateau de Versailles, France, at 48° N. Previous land use was unmanaged grassland and forest before the 17th century. Climate according to the Köppen classification is the same as in Grignon. The soil is classified as a Eutric Cambisol (Guenet *et al*., 2011; IUSS Working Group, 2007), developed within the aeolian loess covers characterising the Paris Basin (Barré *et al*., 2010). All plots are dug by hand twice a year to a depth of 25 cm, and kept free from vegetation. The site has 42 plots, of which 10 are kept as bare fallow. Of these

10 plots, six have continuous measurements and were included in this study. Soil organic C concentrations have been measured by dry combustion. Vasilyeva *et al*. (submitted) found an increase in charcoal content during World War II. The charcoal in Versailles, closer than Grignon to the urban area of Paris, might be the effect of bombing. Thus, for soil from the Versailles experiment, $\delta^{13}C$ was determined for bulk soil and also for the clay- and silt-sized fractions. These two particle size fractions were observed to contain insignificant amounts of charcoal. Data on the soil size fractions are from Vasilyeva *et al*. (submitted).

3.2.4 Kursk

The Kursk site is on a silty loam at 51° N classified as Haplic Luvisol (IUSS Working Group, 2007) which had developed under grassland. Previous land use at the site was grassland with hay harvest and pasture for the last four centuries. The climate is classified as Dfc according to the Köppen classification. A experiment was started in 1947 on a 60 m x 100 m plot. The bare fallow plot was tilled annually to a depth of 17-18 cm by horse-drawn implements until the mid-1970s and by tractor-drawn implements to a depth of 22-24 cm since then. Weeds are eliminated upon emergence by harrowing. Over the years, the SOC content in bulk soil has been determined by dry combustion using different equipment.

3.3 Analytical methods, sampling and collaboration studies

The methods applied allowed changes in different SOC pools to be tracked from multiple scales and perspectives. The main parameters studied were:

- SOC content and main soil parameters in different years, based on direct sampling and on archived soil samples. Soil respiration measurements during the growing season.
- ^{12}C:^{13}C ratio (expressed as $\delta^{13}C$ signatures) of SOC in different years and at different soil depths, of organic inputs to the soil and of soil respiration.

To characterise the soil profiles in the different treatments, soil sampling with an auger was carried out after harvest in September 2009. Samples were taken at increasing depth intervals of: 0-15, 15-17.5, 17.5-20, 20-22.5, 22.5-25, 25-27.5, 27.5-30, 30-35, 35-40 and 40-50 cm and stored at 5 °C until further treatment. From each depth interval, a sample of 10-15 g was taken by aggregating five subsamples (~2-3 g each) taken at approximately 1 cm intervals. The bulk sample was then dried at 105 °C for 12 hours, homogenised and milled with an agate mortar and pestle, and subsequently analysed.

To determine physical conditions in the soil profile, penetration resistance to about 45 cm depth was measured in September 2009 with an Eijkelkamp penetrologger (model P1.52, Eijkelkamp, Arnhem, Netherlands).

The SOC and N content in topsoil was analysed with an elemental analyser (Leco CN-2000, St. Joseph, Michigan).

All the $\delta^{13}C$ values were expressed in parts per thousand (‰) relative to the international standard of Vienna Pee Dee Belemnite (V-PDB), where $\delta^{13}C = (R_{sample} - R_{standard})/R_{standard}$ and R is the ratio of ^{13}C to ^{12}C. The $\delta^{13}C$ signatures of solid samples were measured with an elemental analyser (model EuroEA3024; Eurovector, Milan, Italy) coupled online to a continuous flow Isoprime isotope-ratio mass spectrometer (GV Instruments; Manchester, UK). Details of sampling and the calculations are reported in Papers I and II.

Soil samples from 1956 and 1999, plant materials (leaves and stems) of the crops grown from 1975 to 2009 and samples of the amendments from the same period were taken from the historical archive and also analysed for total C and $\delta^{13}C$. Six data points (1979, 1987, 1989, 1991, 1993 and 1995) were used for C_3 plants and two points (2005 and 2009) for C_4 plants. The $\delta^{13}C$ values for the amendments were measured by averaging direct measurements of samples

Figure 4. Field measurement of CO_2 with the Picarro cavity-ringdown spectrometer. (photo: L. Menichetti)

from 1975, 1979, 1989, 1993, 1995 and 2005. The resulting δ^{13}C values for farmyard manure, green manure and sewage sludge were -27.9±1.2, -27.4±0.9. and-25.7±0.1, respectively. The δ13C value for peat was taken from Gerzabek et al. (1997), who measured it on material from the Ultuna field site.

The δ^{13}C signatures of gas samples were measured directly in the field in May, August, September and October 2011 with a Picarro cavity-ringdown G1101-i laser absorption spectrometer (Picarro, Inc, Santa Clara, U.S.A.). The instrument is relatively lightweight and robust, and was utilised in a field setup (Figure 4). The Picarro was powered with alternating current from a petrol generator. Given the relatively compact dimensions of the instrument and its simple configuration, it could be operational in the field in less than one hour. A preliminary field test was performed in summer 2009 to confirm the suitability of the method. The respiration chamber was an opaque plastic cylinder with a detachable lid sealed with an O-ring, (13.5 cm tall, 24 cm diameter). The lid was removed during insertion of the chamber to minimise disturbances due to pressure changes and a plastic wind shelter was used to minimise disturbances from wind-induced pressure changes in the chamber during measurements. Details on the sampling loop and the calculations are reported in Paper I.

The effect of root contribution on topsoil was studied with a mass balance approach (Paper IV). The SOC contents measured in the LTE from 1956 to 2009 were utilised to determine SOC fluxes. Data have been obtained with several techniques over the years, and the consistency of the dataset was tested by comparing the measurements with those presented by Gerzabek *et al.* (1997), who re-analysed archived soil samples from five treatments for the period 1956-1993 using an element analyser (Carlo Erba 1500). The correlation between the two datasets (expressed as R^2) was found to be >0.9 with a slope very close to unity, confirming the suitability of the archived SOC data.

In order to correct for the change in depth of the cultivated part of the soil profile due to the change in bulk density which had occurred in the different treatments since the start of the experiment, the calculation considered all parts of the mass balance in each plot. The mass of amendments added during the experiment and the soil exports due to sampling were recorded in the experiment archive. The silicon export due to crop harvest was estimated based on the recorded amounts of plant material and grain harvested and was assumed to be 10 mg g^{-1} dry matter for grain, 67 mg g^{-1} for straw and 25 mg g^{-1} for silage maize. An allometric function proposed by Bolinder *et al.* (2007) was used to estimate the C input to the soil produced by roots from the yield data recorded in the historical dataset. The calculation was performed considering the equivalent soil depth. All the C inputs in the experiment could therefore be estimated.

Based on the C changes over time, a single-pool model (described by first-order kinetics) was fitted to these data according to the equation:

$$SOC_t = \frac{\sum_{j=1}^{j=7} H_j I_j}{k} + \left(SOC_0 - \frac{\sum_{j=1}^{j=7} H_j I_j}{k} \right) e^{-kt} \qquad (1)$$

where SOC_t and SOC_0 are SOC at time t and time 0, respectively, H_j represents the humification coefficient of the j^{th} input, I_j represents the amount (Mg ha^{-1}) of the j^{th} input and k represents the decomposition rate constant of SOC. The different inputs have been grouped in 7 classes (j) and the resulting equation has been solved simultaneously for the 15 treatments considered.

Equation 1 therefore describes the decay of the inputs that enter the SOC, which are defined by their humification coefficient, and the decay of the remaining SOC. By calibrating this function, a specific humification coefficient for each C form added to the soil could then be calculated. The humification factors were calculated by RMSE minimisation. More details on the methods used can be found in Paper IV.

Soil respiration during the 2012 sampling campaign was measured with a field infrared gas analyser (IRGA), model EGM-4 (PP-Systems, Boston, US).

3.4 Data analysis

Numerical analyses were conducted with the latest version of the R statistical software (R Development Core Team, 2012). Data treatments included analysis of variance (ANOVA) followed by a post hoc test (Fisher's least significant difference (LSD) test or Tukey's honestly significant difference (HSD) test). A preliminary exploration of possible correlations in the datasets was frequently performed by principal component analysis (PCA), although the results were not reported.

More specific data analysis techniques were utilised as specified in papers I-IV. The main calculations in papers I-III were performed inside a Bayesian statistical framework in order to ensure as precise an estimation of the errors as possible. The Bayesian framework was implemented utilising a Monte-Carlo Markov chain (MCMC) approach based on the Metropolis-Hastings search

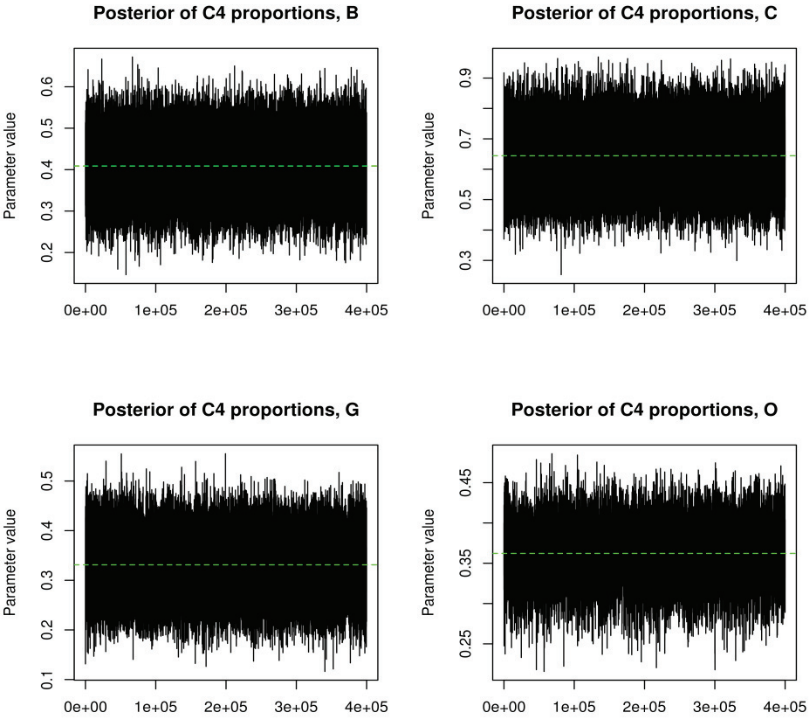


Figure 5. Example of a random walk performed by a Metropolis-Hastings search algorithm (the parameter value in this case is referred to the C4-derived proportion in SOC, deriving from a mass-balance equation). The dashed line represents the average value of the Monte-Carlo Markov chain.months.

algorithm (Figure 5). The application of Bayesian statistics through stochastic methods allows more precise error propagation than deterministic methods (Kruschke, 2013), since it can consider the error in the measurements involved in the calculations in a much more detailed way than can be achieved by deterministic techniques. This is particularly useful when working with techniques based on ^{13}C natural abundance, as these techniques are often reliant on very narrow isotopic differences and proper quantification of noise significantly improves the resolution power of the analyses (Parnell *et al.*, 2010).

Several functions were used in this project to describe the processes under study:

- The mixing of sources with different isotopic signatures was described with mass balance equations (see Papers I, II and IV for additional details)
- The effect of temperature on soil respiration was described with an Arrhenius equation modified according to Lloyd & Taylor (1994)
- The effect of moisture on soil respiration was described with the function developed by Moyano *et al*. (2011) in Paper I and according to the moisture module of the PATCIS model (Bauer, 2008; Moncrieff & Fang, 1999) for the normalisation of soil respiration measured during the 2012 sampling campaign.
- The natural isotopic enrichment process was described with a Rayleigh distillation equation (Rayleigh, 1896) (see Paper III for additional details).

The functions were combined to describe the hypotheses and subsequently to test them against the measured data. The calibration procedures used included optimum climbing algorithms such as the Hooke-Jeeves optimisation and MCMC-based algorithms such as the Metropolis-Hastings search algorithm described above.

The retention coefficients as the fraction of added organic C left in the soil were defined for each specific material. Such values were not considered here as a function of time, and were therefore specific to the time frame considered in each paper. These values express the humification potential of a certain material. The retention coefficients express the same concept of the humification factors calculated in Paper IV.

The specific respiration rate was defined as the amount of C respired per unit of soil C, and expressed the potential of a certain material (or C pool) to be respired as CO_2 and released into the atmosphere.

4 Highlights of the results

4.1 Soil CO_2 fluxes

The analysis of soil respiration rates in the different treatments revealed specific trends over time. Rates were significantly different between treatments

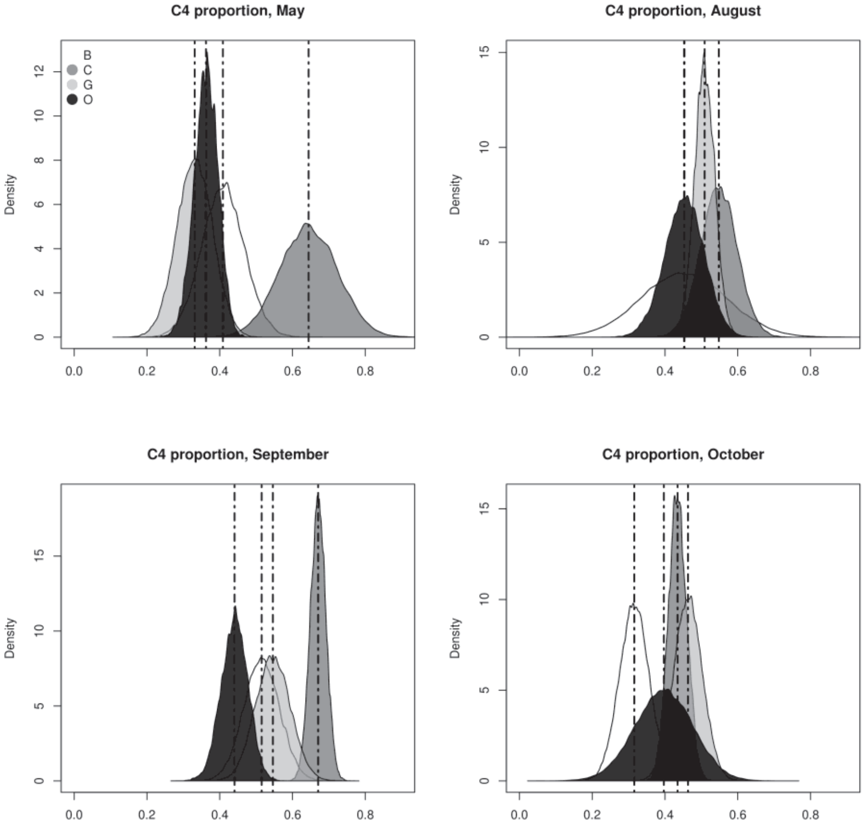


Figure 6. Probability distribution of the proportion of soil respiration from C_4 material in the different sampling months.

in May, at the beginning of the growing season. Soil C fluxes were ranked according to the SOC distribution in the treatments (which also reflected the quality of SOC, with richer treatments being characterised by less recalcitrant and younger C), but the differences between the treatments progressively decreased over time.

The proportion of C_4-originated release was calculated within a Bayesian statistical framework, assessing the probability of each value across a posterior distribution (Figure 6). The partitioning of C fluxes in soil respiration revealed that the respiration rates of young plant materials were 10-20 times greater than those of older material, although soil respiration derived from of older (>10 years) organic matter contributed significantly to total soil respiration, resulting in 32-56% of total soil respiration. The respiration rates of C_4 and C_3 plant materials were influenced by experimental treatments and in particular by organic amendments. Respiration rates were higher from amended treatments, suggesting that amendments may trigger an increase in decomposition by influencing the decomposability of plant residues or by affecting the microbial environment. More details about the results can be found in Paper I.

4.2 Seasonality of soil CO_2 fluxes

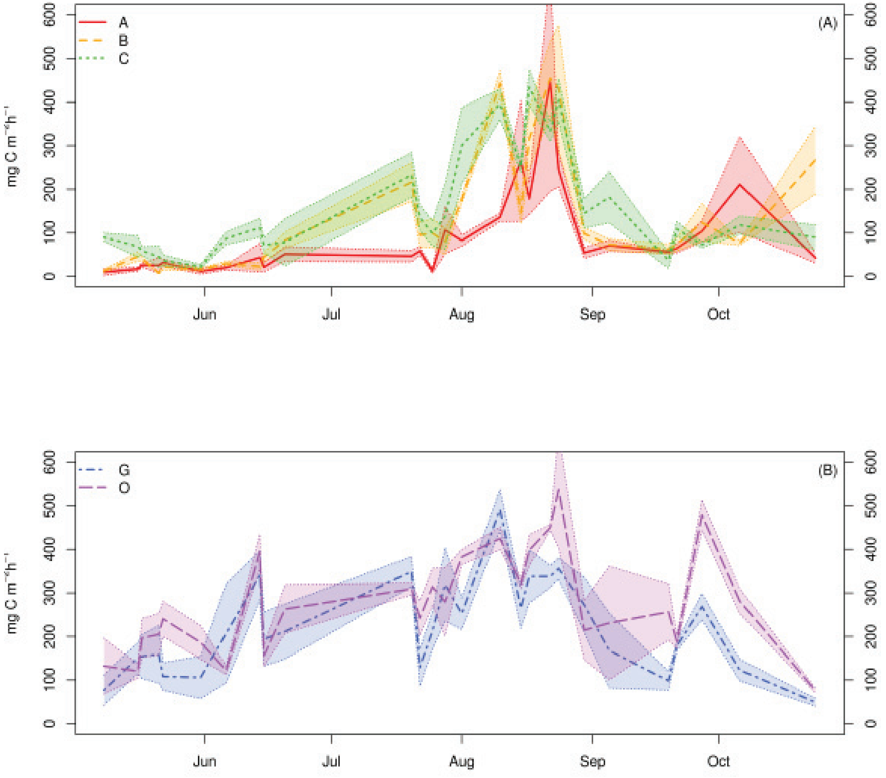


Figure 7. Soil respiration measured during 2012 in the non amended (a) and amended (b) treatments considered.

Intensive sampling was carried out from May to October 2012 to measure seasonal variations in soil respiration in five treatments (A, B, C, G and O; see Table 1 and Figure 7), selected to cover the whole range of SOC in the experiment). In general, soil respiration rates followed the amount of SOC present, with poorer treatments like the A, B and C respiring less than amended and richer treatments like the G and O (Figure 8).

In order to normalize the results for possible differences in temperature and moisture content in soil caused by the different treatments, a model combining the Arrhenius-type function for temperature (Lloyd & Taylor, 1994) and the moisture module of the PATCIS model for moisture (Bauer *et al.*, 2008; Moncrieff & Fang, 1999) was used, calibrated with a Bayesian approach as described above. The standardised respiration at 10°C was then normalised by the amount of SOC to give a specific respiration rate for each treatment over time. Each respiration rate was described by a probability function resulting from the Bayesian calibration, and the data compared with a Kolmogorov-Smirnov test (Figure 13) in order to assess the differences between the probability distributions. During the first months after spring thaw (May, June

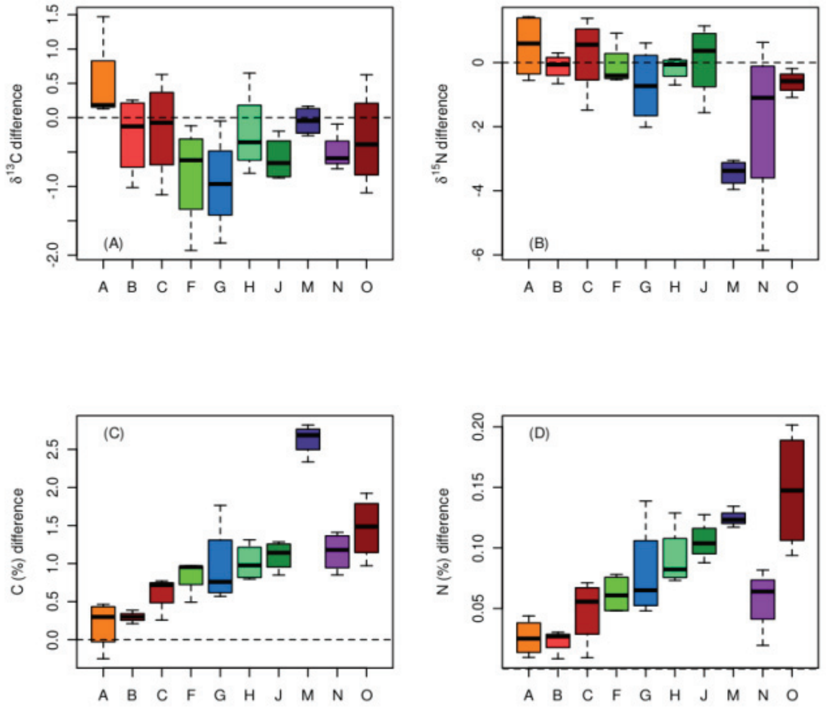


Figure 8. Differences in main variables measured between topsoil and subsoil for $\delta^{13}C$, $\delta^{15}N$, C content and N content. Treatments(reported on the x-axis) refer to Table 1

and July), soil respiration rates were still distributed according to SOC content, while in September and partially in August, the poorer treatments (A and B) had rates comparable to those with higher SOC contents (C, G and O).

4.2 Soil C stocks within the soil profile

The effect of the different treatments on the accumulation of SOC stocks due to root deposition within the soil profile was measured using the natural difference in ^{13}C content between C_3 and C_4 plants. The influence of 10 years of maize cultivation was measured by considering the change induced in the $\delta^{13}C$ signature over depth. The ^{15}N content was also measured, but it did not provide new information as treatments were better characterised by their $\delta^{13}C$ signature (Figure 9). The effect of maize cultivation was significant down to 35 cm depth and maize roots significantly contributed to SOC in the topsoil (Figure 9, Paper II).

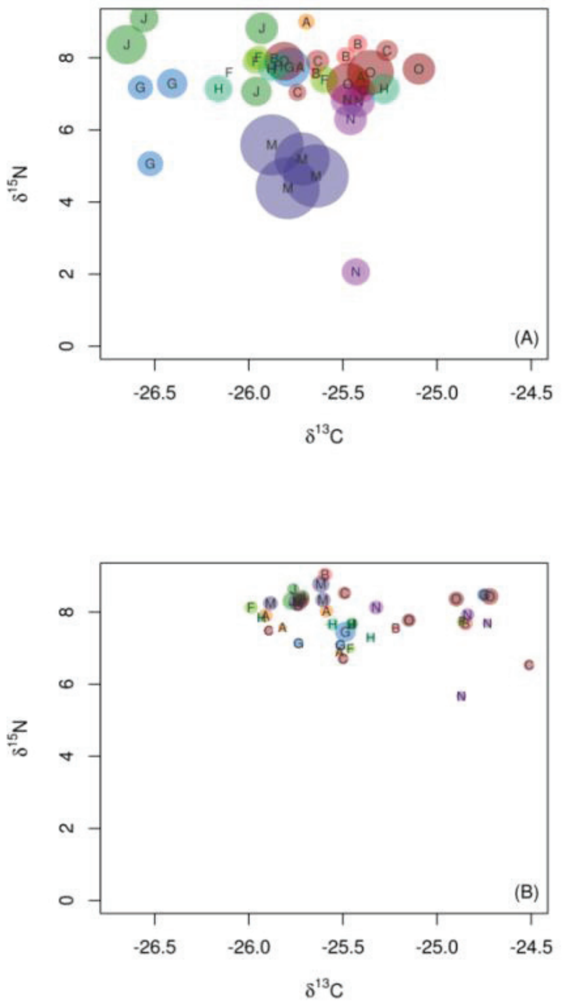


Figure 9. $\delta^{13}C$ and $\delta^{15}N$ profiles of topsoil (A) and subsoil (B).

The treatments caused differences in SOC content and quality. The fertilisation treatments, particularly those with a higher effect on yields, generated a positive C balance, resulting in higher C stocks (Papers II and IV).

The differences in topsoil C concentration after about 50 years of treatments were highly significant. In the two extremes, topsoil C concentration had decreased by more than one-third in the bare fallow treatment, while it had more than doubled in the peat+N treatment.

The changes in the chemical composition and amount of C stored also produced related changes in the bulk density of the treatments and this was reflected in the equivalent soil depth, which varied from 19.5 to 27 cm. Considering the changes in bulk density through the equivalent soil depth, total C stocks in the topsoil varied from 27 Mg ha^{-1} in the bare fallow treatment to 97 Mg ha^{-1} in the peat+N treatment.

The humification or retention coefficient of the material derived from roots in the last 10 years, identified by the signature of the maize, resulted in a value of 0.30±0.09 when estimated through analysis of δ^{13}C. The humification coefficient of root-derived material estimated through C mass balance resulted in a value of 0.27±0.09. The latter estimate was based on a longer period, but the two estimates were surprisingly close. More details about these results can be found in Papers II and IV.

4.3 Natural δ^{13}C enrichment

The study of the δ^{13}C signature in five different bare fallow treatments from the LTBF network scattered across Europe, on an area that covered 11° latitude and 34° longitude and a period of 84 years, allowed the natural change in δ^{13}C that occurs naturally in soil to be quantified. Although isotopic methods based on natural abundance ^{13}C levels are a well-established tool for studying SOC, changes in the ^{13}C levels are often ignored in calculations, although this can add a significant amount of error in the calculations (Clay *et al.*, 2007).

All bare fallow soils showed substantial losses of SOC (of 33-65%) and a related δ^{13}C increase (of 0.01-0.02 ‰ per year) (Figure 10 and 11). Similarities among the enrichment curves in the different sites were assessed preliminarily through linear generalised additive models (GAM), a nonparametric regression technique that can be considered neutral toward the shape of the relationship. All sites showed a similar trend, as shown in Figure 10.

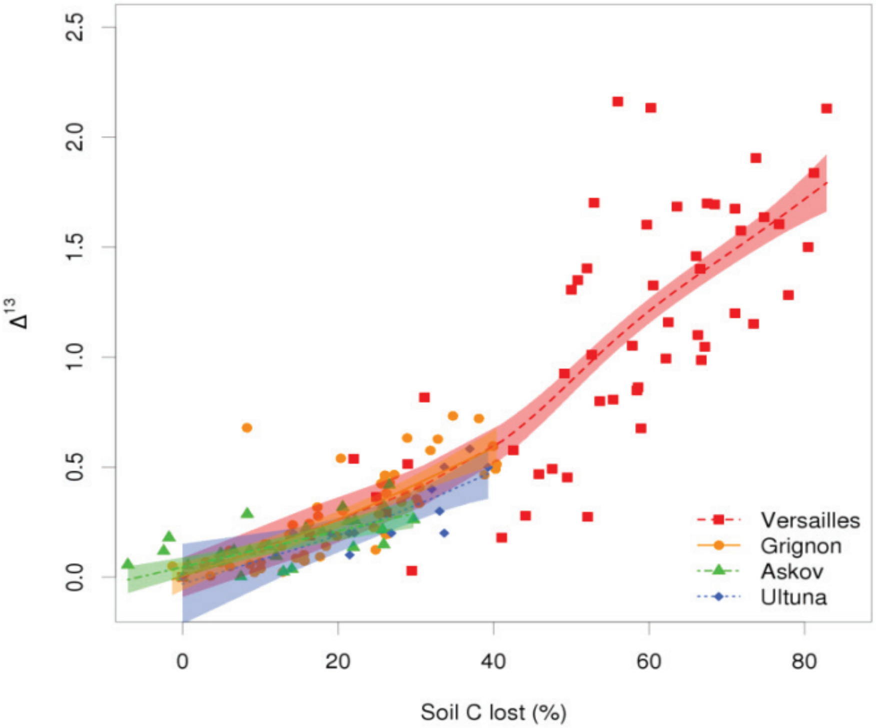


Figure 10. A nonparametric (Generalized Additive Model) regression of the observed δ^{13}C trends over C losses. Shaded areas show 95% confidence interval.

A Rayleigh distillation function (Rayleigh, 1896) was then used to describe the change. The estimated mean fractionation coefficient (ε) was found to be -1.16±0.27‰ across all sites. Differences in climate and land use history seemed to have a small influence on the fractionation, except for the Versailles site, where the presence of black carbon strongly influenced the kinetics of δ^{13}C.

A mechanistic model could not be used to explain the observed enrichments, as several possible processes lead in the same direction and interact. Several of these processes are discussed and additional details on the results, the discussion and the calibrated function are provided in Paper III.

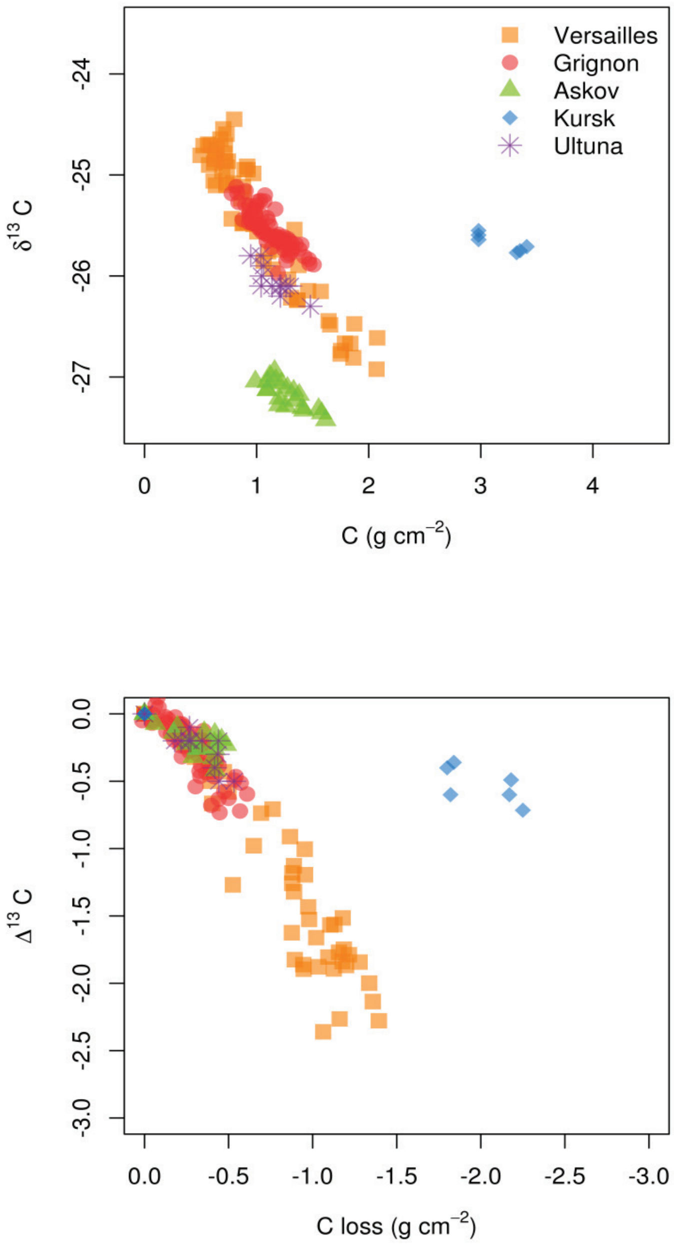


Figure 11. The $\delta^{13}C$ of SOC in the five bare fallow experiments plotted against the C content (above) and changes in $\delta^{13}C$ (Δ) plotted against the changes in C content (below).

5 Discussion

5.1 Soil C stocks along the soil profile

The $\delta^{13}C$ signature change due to maize was found to be significant to a depth of 22.5 cm, showing that rhizodeposition from maize had a significant impact on SOC already after 10 years.

The residues, mainly roots, coming from maize cultivation contributed significantly to the SOC in the topsoil and differences between the treatments were significant in most of the treatments studied. The effect on subsoil was much less evident and was detectable only in the C-enriched treatments M and O, which also gave higher yields and therefore higher rhizodeposition. This suggests that the amount of C introduced by rhizodeposition into the subsoil of agricultural ecosystems is much lower than that in the topsoil. Nevertheless, data from penetrometer measurements showed significant differences between treatments in the subsoil below 41 cm depth, suggesting that some structural modification due to SOC translocation had also taken place in the deeper layers of the soil (as the texture is the same in all the treatments and we can exclude a difference in clay accumulation).

The humification (or retention) coefficients for maize material over all treatments calculated in Paper II were compatible with those calculated in Paper IV regarding root contribution since the start of the experiment, although the first calculation was based on 10 years and could therefore be expected to be slightly different.

The root humification coefficient was higher than values generally reported in the literature (see for example Plénet et al., 1993 and Bolinder et al., 1999), suggesting some kind of protection mechanism acting on root-derived C in soil other than the recalcitrance of the material itself. These results are in line with the hypotheses proposed by Rasse et al. (2005), who suggested that root-derived C is protected by physico-chemical and physical interactions. In particular, the C in fine roots is considered to enter the soil directly at the scale of physically protected C, inside aggregates or fine pores (Rasse et al., 2005;

Mendez-Millian et al., 2010). The data in this thesis suggest that the contribution by roots to SOC is often underestimated.

5.2 Soil C stocks and soil respiration

The proportion of C4-derived C in total SOC ranged from 4.9% to 8.1% after 10 years. Similar proportions were calculated, based on isotopic signatures, by Pausch & Kuzyakov (2012), who reported that 5% of SOC was C4-derived after two years of maize cultivation. The origin of soil respiration followed a more complex pattern.

The large C_4 proportion recorded in the +N treatment in May could have been related to a flush of more easily mineralised root-derived C caused by the thawing of frozen soil. The same flush could occur in the two amended

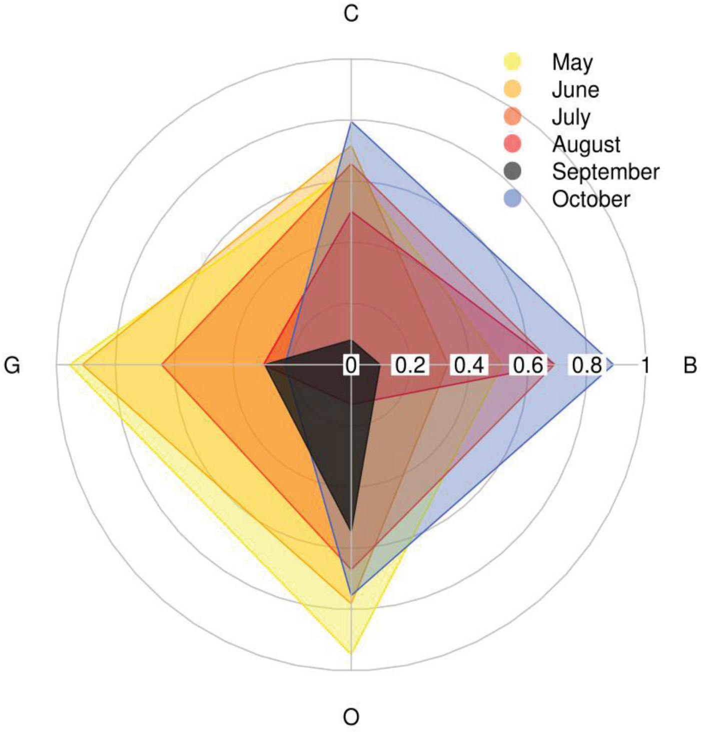


Figure 13. Results of a Kolmogorov-Smirnov test expressing the distance of the probability distribution of specific C respiration in the different treatments (B,C,G and O) from the A treatment during different months. Letters represent the treatments as described in Table 1. A value of 1 indicates two completely different distributions, a value of 0 two identical distributions.

treatments, but in those cases it would be masked by the signature of the amendment-derived material. Laboratory experiments on soils from the Ultuna experiment revealed a C flush following freeze-thawing cycles, showing that microbial C contributed 65% of the CO_2 flush that occurred upon thawing (Hermann & Witter, 2002).

It was possible to calculate the proportion of C_4 autotrophic and heterotrophic respiration considering the difference between September, when the plots were vegetated, and October, after harvest. The autotrophic contribution to soil respiration in the non-amended treatments was in general comparable to values measured using root exclusion methods in agricultural systems (Suleau *et al.*, 2011; Lohila *et al.*, 2003), but was not proportional to the yields in the treatments and was found to be higher in the non-fertilised treatments. The proportion of respiration from heterotrophic degradation of C_4 material instead roughly followed the trend in yield. It was smaller in the Control treatment, as expected, because of the much smaller root inputs. In the +N treatment, which is closer to a typical agricultural field, heterotrophic C_4-C respiration was greater and comparable with heterotrophic respiration coming from older SOC.

Specific respiration coming from young plant materials was one order of magnitude greater than that coming from older material, showing a huge influence of maize roots on soil respiration in the topsoil and pointing out the high activity of this younger pool compared with the more stabilised material older than 10 years. Nevertheless, soil respiration deriving from older (>10 years) C_3 organic matter contributed significantly to total soil respiration, in proportions that were roughly comparable with those from younger C_4 material.

The C-specific respiration rates showed an interesting variation over the seasons. At the supposed peak, in September, the C-specific respiration from the bare fallow treatment (A) roughly matched that from the other treatments (Figure 13). This might be a consequence of the higher temperature sensitivity that is often reported for older SOC (*e.g.* Biasi *et al.*, 2005; Vanhala *et al.*, 2007; Hartley & Ineson, 2008; Lefèvre et al., 2013). A possible explanation for the observed results is nonlinear interactions of temperature and moisture conditions, which vary in the different plots mainly due to crop coverage, with the soil microbial population. Soil enzyme activity can be an important rate-limiting step in soil organic matter decomposition (Sinsabaugh, 1994) and variations might affect C flows. It has been observed that the amount of more stabilised C utilised by soil microorganisms increases with elevated temperature (Dalias *et al.*, 2001; Waldrop & Firestone, 2004). This might be related to the higher temperature sensitivity of enzymes involved in the decomposition of substrates with high molecular weights (Trasar-Cepeda *et al.*, 2007). Waldrop & Firestone (2004) also found a relationship between temperature increase and ratio between oxidative and hydrolytic enzyme activity, which might explain the higher degradation of more stabilised SOC at higher temperature.

A possible source of error that should be considered is diffusion of respired CO_2 below the frames separating the plots. Gas diffusion is much faster in air than in soil according to Fick's law (Webb & Pruess, 2003), so the CO_2 gradient formed should be from the subsoil to the atmosphere (Davidson *et al.*, 2006). Nevertheless, some local gradients allowing diffusion of CO_2 from one plot to another might be present. Part of the increased respiration measured in the bare fallow plots might have been due to this interference, which is most likely quite small.

5.3 The effects of the treatments after more than 50 years

More than 50 years of fertilisation and addition of amendments caused differences in the soil in several respects. The accumulation of SOC and the changes in bulk density produced a visible difference in the height of the plots (Paper IV). The microbial communities in the different treatments have become quite specific in the topsoil (Börjesson *et al.*, 2011). This is particularly true for some extreme treatments, such as the sewage sludge treatment, which is characterised by the presence of heavy metals and low pH (Witter, 1996).

The SOC also differed in quality and amount. The amendmended treatments, although receiving the same amount of C, produced different accumulation of SOC stocks and material already partially humified. For example, peat addition lead to higher SOC stocks as a result of the higher humification coefficient. The contribution of roots also resulted in quite important differences. According to the analysis of C stable isotopes (Paper II), in 10 years maize had contributed approximately 3-10% to total SOC. Comparable results were obtained through a mass balance approach for the root contribution over the whole duration of the experiment (Paper IV).

The effect of the treatments on subsoil was much less evident, however, both in terms of microbial ecology (Börjesson *et al.*, 2011) and in terms of SOC (Paper II). No significant differences were found in the composition of the microbial community below 27 cm. There was significant treatment effect on SOC down to 35 cm, but this was significant only between the richest, peat and the sewage sludge treatments, and poorest (bare fallow, control) treatments. This suggests that the accumulation of SOC in the subsoil, as well as the decrease that could be expected in poor treatments like the bare fallow, is relatively slow and on a time scale of decades.

5.4 Natural $\delta^{13}C$ enrichment: Problem to be solved or opportunity to be taken?

The ^{13}C enrichment of SOM over time has been known for a long time (Balesdent & Mariotti, 1996), but has often been considered negligible in partitioning studies. This can be true on really short time scales, but as soon as the time scale embraces more than one decade, this effect becomes relevant and can lead to a relatively big error in calculations based on stable C isotope mass balance (Clay *et al.*, 2006). Consideration of natural ^{13}C enrichment is therefore important in order to improve the precision of calculations.

Moreover, understanding the mechanisms behind such enrichment, which are debated in the literature, could lead to an improvement in the use of stable isotopes in soil science research. The opportunity offered by long-term bare fallow is therefore precious for studying this phenomenon in the field and over a relatively long time scale.

By fitting the Rayleigh function to a dataset spanning over 11° of latitude (Paper III), we updated the calculation suggested by Balesdent & Mariotti (1996). The Rayleigh relationship accurately described the observed trends. However, although a simple Rayleigh function can already be used for correcting the calculations from other sites, the function cannot discriminate between the two processes and understanding of the mechanisms behind the natural ^{13}C enrichment of SOM is still incomplete. There are several mechanisms that could explain the observed enrichment. One set relates to the true (kinetic)

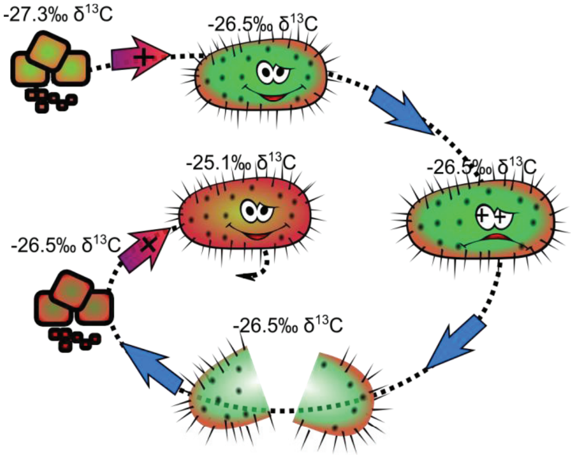


Figure 14. A possible enrichment mechanism: preferential substrate preservation (here pictured as microbially mediated).

isotopic fractionation (Figure 14) that occurs in cells during metabolic processes (*e.g*. Blair *et al*., 1985). Another set of mechanisms (Figure 15) is related to the preference of decomposer organisms for certain substrates (*e.g*. Werth & Kuzyakov, 2010). The anaplerotic re-fixation of CO_2 by carboxylation reactions is another process that could explain the enrichment of microbial material, as eventual C assimilated from the air rather than from the SOC would come from a much enriched substrate. The enrichment could also be explained by a simple SOC decay model which considered C pools with different isotopic signatures decaying at different rates (Paper III, Figure 15). Such a model is a good example of a possible mechanism behind the enrichment, but the multi-pool enrichment model is based on an initial difference in the $\delta^{13}C$ signature of different SOC pools, which still needs to be explained. There are several possible reasons in the history of a specific site that could cause such differences, for example a particular geological history, but it is difficult to formulate a general, site-independent rule. Moreover, the similarities between the enrichment kinetics we found remain unexplained in this case. A relevant possible cause for a $\delta^{13}C$ spectrum in SOC is the Suess effect, related to the gradual ^{13}C depletion that has been observed in atmospheric CO_2 due to emissions from fossil fuels (which have on average a signature much more depleted than the atmosphere, being on average around -28 ‰). This effect is likely to have some influence in the $\delta^{13}C$ stratification found sometimes in forest soils (Boström *et al*., 2007), but in well-mixed soil layers such as the plough layer of an agricultural system this effect is probably smaller. Some of the bare fallow experiments analysed in this thesis are also quite old, from 1929, and were therefore initiated when the effect of the industrial emissions in the global atmosphere were not yet as dramatic as in the recent decades. A specific analysis of plant residues from the Rothamsted archive by Zhao *et al*. (2001) revealed a pronounced effect of the

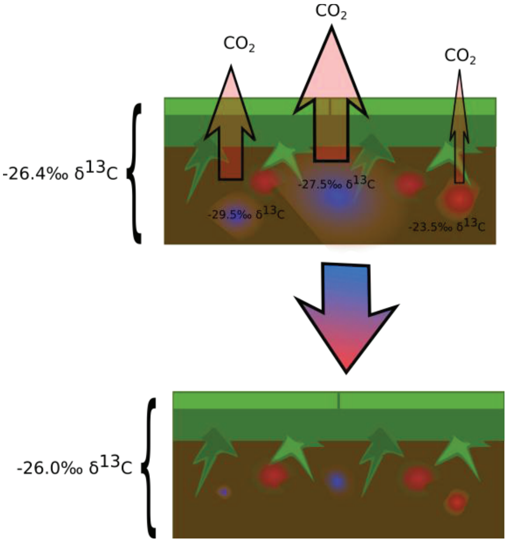


Figure 15. A possible enrichment mechanism: preferential substrate utilisation.

atmospheric enrichment on plants only after 1960, and therefore the Suess effect on the experiments we considered is likely to be negligible.

The Rayleigh function has already been used in modelling the natural ^{13}C enrichment of SOM observed with depth (Wynn *et al.*, 2005), but in that case too, the function represented an aggregate effect. Although for applied purposes the Rayleigh function works well, a more mechanistic understanding of such enrichment is important for inclusion of the enrichment process in isotopic C models. This enrichment is likely to be in part or completely a consequence of biological activity (Yang et al., 2014), in particular selective preservation of dead microbial cells (Dijkstra *et al.*, 2006), and a detailed understanding of this process could be a link between biological activity, SOC stabilisation and SOC decay. Exact determination of ^{13}C kinetics in soil could also allow historical information about SOC decay to be obtained from actual soil profiles, where an increase in ^{13}C content with depth is usually observed (Wynn *et al.*, 2005, 2006). The attempts in this thesis to build a generic model for ^{13}C enrichment should be considered a first step, but also the start of a promising direction for soil science research and, in particular, for SOC modelling research.

6 Conclusions

In the years that have elapsed since the start of the Ultuna long-term field experiment, significant differences between the treatments have developed, with some treatments showing clear C accumulation or depletion in the topsoil. Agricultural management thus had a direct influence on SOC stocks.

The SOC in the subsoil was significantly affected by the treatments down to 35 cm. The contribution of maize-derived material was found to be significant down to 22.5 cm depth, resulting therefore in SOC derived from the last 10 years of maize cultivation being concentrated mainly in the topsoil. Increasing the SOC stocks in the subsoil seems therefore to be a viable option for soil C sequestration strategies, particularly if planned on an adequate time scale.

The accumulation of SOC in the topsoil proved to be very sensitive to the treatments. Treatments that included organic amendments, preferentially already partially humified or digested, but also N fertilization caused accumulation of SOC. Agricultural management appears therefore to be highly important for soils being a sink or source of atmospheric C.

This thesis shows the contribution of roots to SOC formation, and suggests that root inputs are one of the main variables determining both SOC accumulation and soil respiration. Root-derived SOC seems to be more protected and the origin of more SOC than is generally reported, which indicates that the contribution of roots is often underestimated in SOC balances.

The quantitative measurements of SOC accumulation (or, in some cases, losses) described in this thesis can improve actual SOC decay models, providing accurate data and concepts.

The bare fallow treatment was of particular importance for determining ^{13}C kinetics. The bare fallow measurements, together with other bare fallow experiments across Europe in the LTBF network, allowed the natural ^{13}C enrichment that occurs in the soil over long time scales to be quantified and modelled. This model can be used directly in natural abundance ^{13}C studies on other agricultural sites to improve the precision of the calculation, but also represents a potential first step toward a more mechanistic understanding of ^{13}C

kinetics in soil and poses interesting and challenging questions about the underlying causes of the observed/modelled kinetics.

The seasonal decomposition pattern of SOC posed questions which remain to be answered. Specific respiration during autumn was similar in bare fallow and richer treatments despite lower SOC quality in the bare fallow. This suggests nonlinearity in the relationship between SOC age and its decomposability and could possibly be caused by higher temperature sensitivity of older SOC. However, the particular experimental design of the Ultuna LTE imposed some limitations that made difficult to obtain clear results, and more investigation on this topic is needed.

7 Potential for more studies

The main focus in this thesis was on understanding the changes in SOC stocks after several years or decades of different agricultural management. One of the core techniques used was the natural difference in ^{13}C content between C_3 and C_4 plants. This technique proved quite effective in following the root influence on SOC stocks over the profile, producing promising results. Such effects can be expected to operate on multi-decadal time scales, but we were able to detect it already after 10 years after the shift to maize. A continuation of the experiment and subsequent sampling to depth after some decades from now to obtain values to compare with our results will offer a unique perspective on the long-term root influence on SOC along the soil profile. Considering the scientific value of the Ultuna Long-Term Experiment and the probable duration of the project in which this thesis work was performed, which will extend for many years from now, we suggest the introduction of profile sampling every 10 years. A time series of isotopic measurements over the soil profile would constitute an invaluable tool for assessing root effects on deeper horizons.

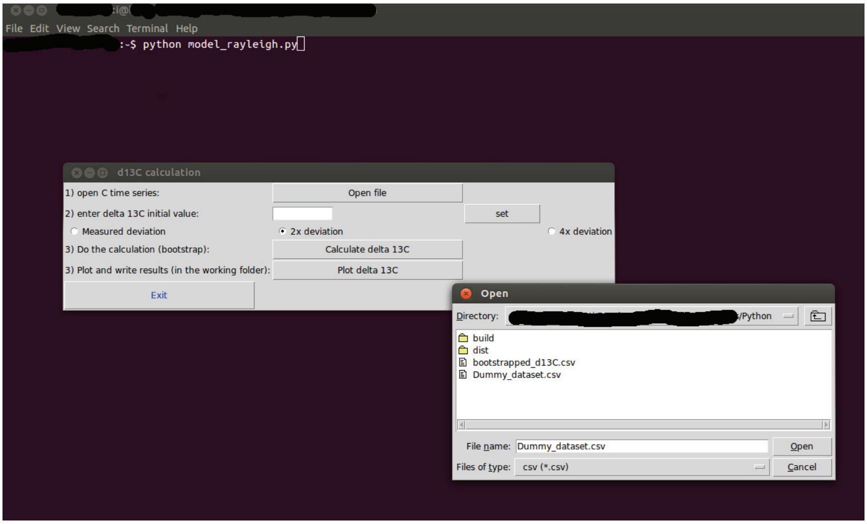
The bare fallow treatment probably produced the most interesting results in the whole experiment, since it contains SOC that is more than 50 years old and has always been kept in field conditions. Together with the other bare fallow treatments in the LTBF network, these particular plots might therefore contain a crucial key for understanding the mechanisms of SOC recalcitrance and for answering the fundamental questions recently posed by the scientific community (*e.g.* Kleber *et al.*, 2011). An important direction for future research is to investigate all the possible mechanisms that are acting on that older SOC, first testing the assumption of SOC recalcitrance and then searching for correlations and causal connections between the decomposition kinetics of older SOC and other factors. Since SOC decomposition is the result of biological processes in interaction with soil particles, priority should be given to the relationships between soil metabolic indicators, decomposition and soil physical protection. A really promising direction in this perspective is the detailed study of soil specific activity, which could be indicated for example by its exoenzyme pools, in relation to older SOC decomposition.

As shown it was possible to study natural ^{13}C enrichment thanks to the particular conditions of the experiments in the LTBF network, and the detailed investigation of such processes might also contain some important keys for understanding SOC kinetics. Since the natural ^{13}C enrichment seems to be a process driven by SOC decomposition and could be relatively independent of edaphic and climatic variables, a consistent part of such a process may be a constant presence in soils that links together SOC decomposition and time and such linkage has global validity. A more detailed understanding, and a subsequent mechanistic model, of the mechanisms leading to the natural ^{13}C enrichment that occurs in soil might therefore connect these mechanisms with a variable that is nowadays easy to measure and readily available even with really small amounts of sample. Such efforts should be directed toward discriminating how much the possible causes for the observed enrichment, namely microbially-mediated enrichment and historically caused initial inhomogeneity in the isotopic composition of different C pools, affect the observed kinetics.

8 Appendices

A version of the Rayleigh function containing our calibration has been coded as a Python script, in order to offer a graphical user interface (Figure 16) for rapidly assessing the suitability of the method for predicting $\delta^{13}C$ signature from recorded SOC decay. The file can be found here: http://www.slu.se/Documents/externwebben/nl-fak/mark-och-miljo/VnMb/d13C_enrichment_rayleigh_model.zip.

A Python environment needs to be installed on your machine in order to be able to run the program. For any problem or broken link, please contact Lorenzo Menichetti at ilmenichetti@gmail.com.



```
[redacted]:~$ python model_rayleigh.py
```

1) open C time series:
2) enter delta 13C initial value:
○ Measured deviation ● 2x deviation ○ 4x deviation
3) Do the calculation (bootstrap):
3) Plot and write results (in the working folder):

Directory: [redacted]/Python

build
dist
bootstrapped_d13C.csv
Dummy_dataset.csv

File name: Dummy_dataset.csv
Files of type: csv (*.csv)

Figure 16. Screenshot of the program for predicting $\delta^{13}C$ shifts from SOC changes.

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Acknowledgements

I am grateful to my main supervisor, T. Kätterer, for having given me the opportunity of learning all that I have learned during my PhD studies, and in particular for the freedom he gave me to acquire all the tools I needed to enjoy doing science. And…well, for his constant but never oppressive presence, for his imaginative thinking, for all the discussions we had, and particularly for having suggested to someone that did not even know what could be called "research" to carry on and keep going with my studies when, in the beginning, I was thinking of giving up.

I want to thank all my supervisors, particularly A. Ekblad, who fought hard to counterbalance my tendency to speculation and kept me tightly locked on the solid ground of stable C isotope, and O. Andrén, one of the best persons I have met during the last four years and that I still regret I did not work closely enough with. I want to thank all my co-supervisors, H. Kirchmann who first incited me to work hard to publish, and G. Renella, who keeps following me since before my graduation. And all the people with whom I have discussed and who have contributed to this work, and they are many, from the close colleagues of my group (thanks M. Parvage, V. Kainiemi, J. Rasmussen, G. Börjesson, M. Bolinder and K. Hamnér) to the people in the department (thanks M. Rappe George and K. Steffens for all the discussions I obliged you to have…and basically anybody working here).

I want also to thank the charisma of a few scientists I have met or listened to, among them I want to list J. Schimel and K. Beven, who without knowing it motivated me with their example.

And I want to thank the system administrators of our department, without whom nothing would work here and that I bothered constantly with some weird request or the other; thanks to R. Persson and H. Karlsson, probably the most important people in our department.

...and thanks to A. Cannizzaro, who fed me on a daily basis and tolerated my presence in the same 35 m^2, particularly in the last months.