

Masterthesis

# Change of carbon and nitrogen contents and their respective isotopes $\delta^{13}\text{C}$ , $\Delta^{14}\text{C}$ and $\delta^{15}\text{N}$ in soils of the Hainich Nationalpark

Zur Erlangung des Grades Master of Science  
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## Selbstständigkeitserklärung / Statement of Authorship

Ich erkläre hiermit, dass die vorliegende Arbeit selbstständig und nur unter Verwendung der aufgeführten Literatur angefertigt wurde.

I declare that I completed this work on my own and that information which has been directly or indirectly taken from other sources has been noted as such.

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Ort, Datum

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Manuel Rost

## Danksagung

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## Abstract

Forests are seen as important sinks for the greenhouse gas CO<sub>2</sub>, whereat fertilization through N deposition and increasing atmospheric CO<sub>2</sub> concentrations play an important role. If this leads to a long-term change in carbon storage remains unknown and will depend on how additional carbon is conserved after entering soil via biomass. At present, sorption of OC to mineral surfaces is regarded as the most important mechanism of OC stabilization in soils. However, this mechanism also provides partially fast turnover times. To find out how C and N sequestration in soils of widely pristine forests will change, we sampled 10 soil cores of 0-50 cm depth in the Hainich national park in 2016. After analysis of OC, TN and the isotopes <sup>13</sup>C, <sup>14</sup>C and <sup>15</sup>N, we compared the results to earlier studies of 2004 and 2009. Additionally, soil samples of 0-5 cm and 10-20 cm were separated into free light (FLF), occluded light (OLF) and heavy fraction (HF) via density fractionation and were analysed for OC, <sup>13</sup>C and <sup>14</sup>C.

Our results show a decline in  $\Delta^{14}\text{C}$  in bulk soil in 0-5 cm from 2004 with  $86 \pm 12 \text{ ‰}$  to  $24 \pm 7 \text{ ‰}$  in 2016. The average annual decline of  $\sim 5.2 \text{ ‰}$  resembles thus atmospheric CO<sub>2</sub> levels. In deeper soil from 20-50 cm, we observed a steeper decline in  $\Delta^{14}\text{C}$  which indicates shortened turnover times of OC in bulk soil and hints to processes which we cannot explain yet. Density fractionation has shown a decline of  $\Delta^{14}\text{C}$  and OC stocks from 2009 to 2016 in FLF in both 0-5 cm and 10-20 cm as well as an increase of OC stocks in the OLF from 2009 to 2016, indicating that the light fractions may be dynamically active.

## List of Abbreviations

LD-PE	low density polyethylene
C	Carbon
OC	organic Carbon
IC	inorganic Carbon
TC	total Carbon
N	Nitrogen
TN	total Nitrogen
FLF	free light fraction
OLF	occluded light fraction
HF	heavy fraction
SPT	sodium poly tungstate
PP	poly propylene
MPI BGC	Max Planck Institute for Biogeochemistry
SOM	soil organic matter
SOC	soil organic carbon
NPP	net primary production
ECM	ectomycorrhiza
AM	arbuscular mycorrhiza
RuBisCo	Ribulose-1,5-bisphosphat-carboxylase/-oxygenase
SE	standard error
StD	standard deviation



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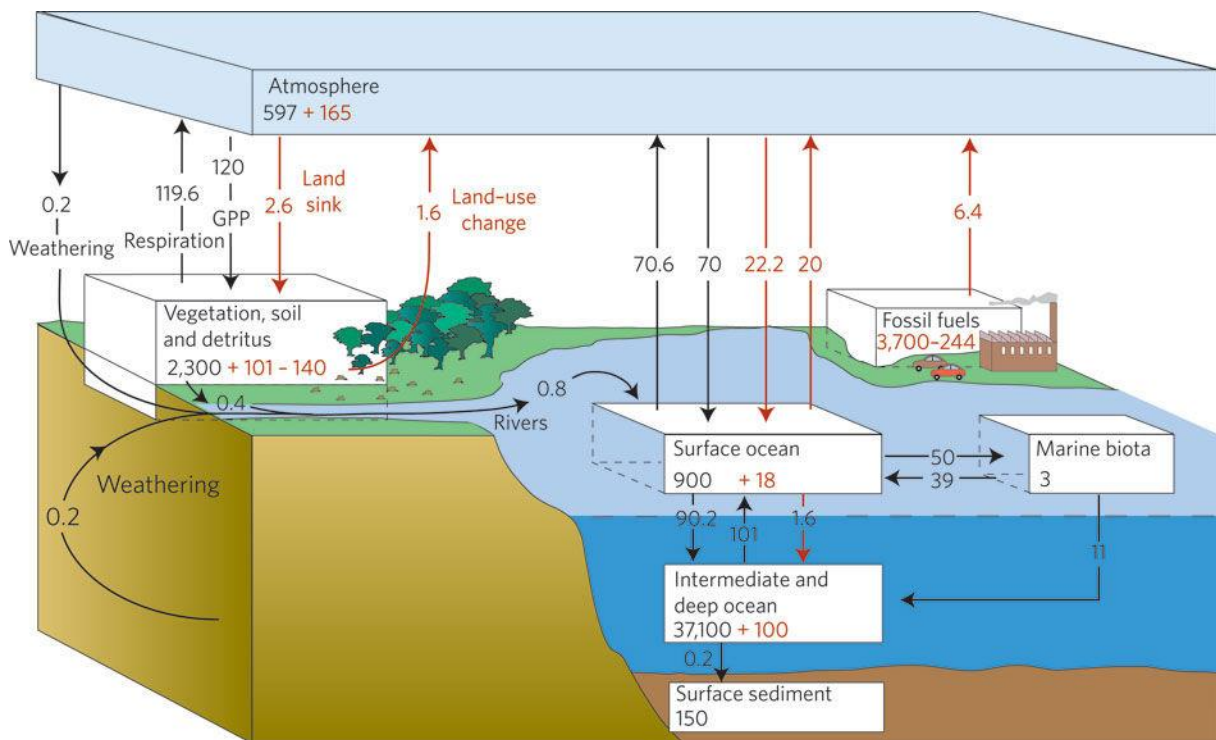
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# 1 Introduction

## 1.1 The role of soils in the global carbon cycle

Soils are the conversion product of their respective genesis factors: parent rock material, climate, organisms and relief over time. Through complex interactions of these factors, pedogenic processes of transformation and translocation occur and form the respective soil marks. Soil takes on many important roles in the ecosystem of our planet. It provides environment for organisms, hosts regulation processes such as filtering and functions as a storage and is in this way source as well as sink for many organic and inorganic substances (Blume et al, 2010).



**Figure 1: Global carbon cycle for the 1990s showing the main fluxes [Gt y<sup>-1</sup>] reservoirs of carbon [Gt], (Gent, 2012), (source of image: [http://www.nature.com/nclimate/journal/v2/n1/fig\\_tab/nclimate1299\\_F1.html?foxtrotcallback=true](http://www.nature.com/nclimate/journal/v2/n1/fig_tab/nclimate1299_F1.html?foxtrotcallback=true))**

As figure 1 shows, the main participant of the global carbon cycle is the ocean, where carbon storage is driven by the partial pressure of atmospheric CO<sub>2</sub> and a chemical equilibrium between water and atmosphere. The carbon stored in the ocean exists as inorganic carbon, consisting of dissolved CO<sub>2</sub> (less than 1 %) and bicarbonate or carbonate anions (about 99 %) (Houghton, 2007). While marine biota also plays a relevant role in ocean carbon storage, the main sequestration to the intermediate and deep ocean is chemically driven (Houghton, 2007). Next to the ocean, the terrestrial systems with soil, vegetation and detritus adhere the second largest pool of carbon, whereat the carbon reposition here is driven by

photosynthesis and respiration. As shown in Figure 1 by Gent (2012) and also mentioned by Killham (1994), the terrestrial systems store carbon up to four to five times the pool of the atmosphere, whereas it is also the biggest producer of atmospheric CO<sub>2</sub> through respiration.

About 80 % of terrestrial organic carbon (OC) which actively takes part in the carbon cycle, is bound in soils, while only 20 % is bound by vegetation (Blume et al, 2010). According to Killham (1994), the soils of temperate forests hold a soil organic carbon pool of up to 110 t ha<sup>-1</sup> compared 80 t ha<sup>-1</sup> of tropical rainforests, showing that soils (of temperate forests in particular) act as a carbon sink (Killham, 1994).

While photoautotrophic organisms as plants, algae and some microorganisms fix atmospheric carbon (CO<sub>2</sub>) via photosynthesis, heterotrophic organisms as animals and microbes consume, alter and respire photosynthesis products (see also Figure 2). As carbon and energy source, soil organic carbon contributes to a high diversity for soil fauna and flora, respective to its abundance (Blume et al, 2010). The non-respired and unmineralized part of carbon is fixed in soil organic substances (SOC) or soil organic matter (SOM). Through different stabilization processes respective to the organic base material, SOM is more or less protected from decay, consumption and respiration. This results in different amounts and turnover rates of SOM (Heimann, 1993). According to Killham (1994) half of the organic carbon (50 %) in soils is present in aromatic forms or compounds, with a further 20 % associated with nitrogen, 15 % in form of carbohydrates and another 15 % as fatty acids and alkane carbon. SOM can be divided into three pools, insoluble, soluble and biomass whereas the insoluble pool constitutes the biggest pool with about 90 % total soil organic carbon (Killham, 1994). When looking into turnover times, it is also important to differentiate between the SOM species in the soil and the way in which they are stored. According to Schmidt et al (2011), the molecular structure of SOM only has a secondary role in determining residence times and that C stability mainly depends on its biotic and abiotic environment. Thus, the vulnerability of SOM to degradation will depend on the nature of disturbance as well as the (de/) stabilization mechanisms present in the ecosystem (Schmidt et al, 2011).

Although seemingly well researched, at least with many studies and different approaches, regarding turnover times, it remains largely unknown why some SOM persists for millennia whereas other SOM readily decomposes within decades (Schmidt et al, 2011).

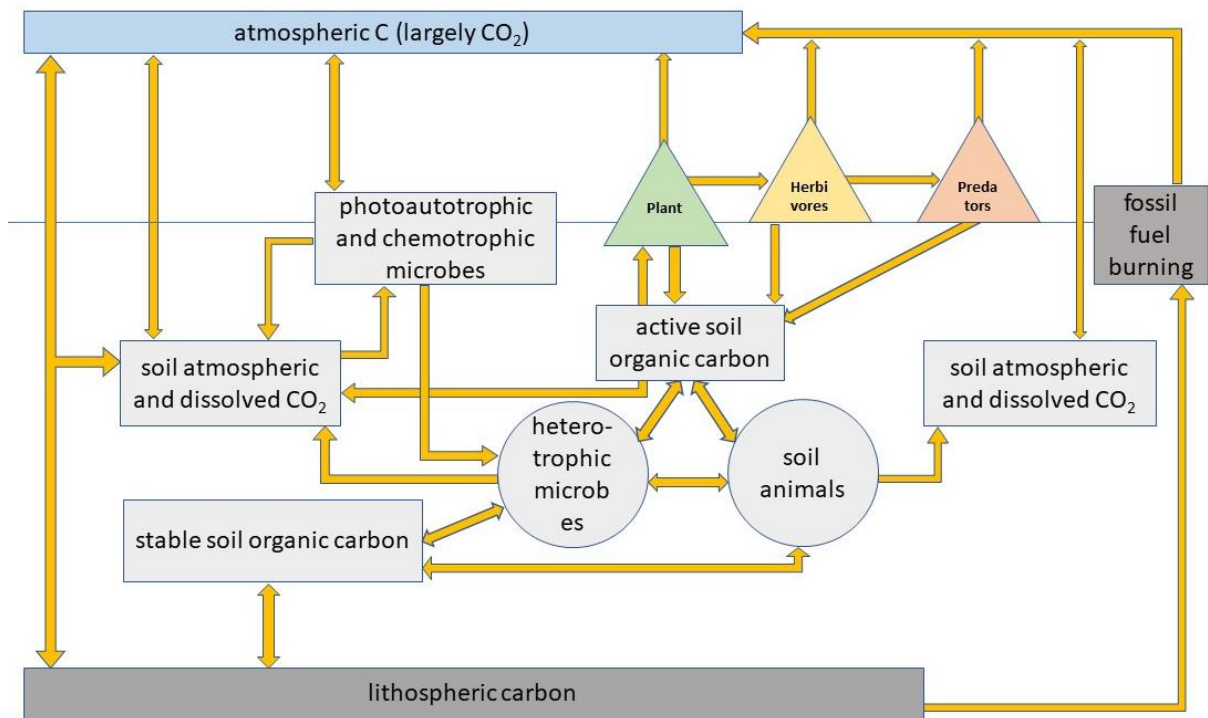


Figure 2: Schema of the soil carbon cycle (altered, after Killham, 1994)

## 1.2 Links between carbon and nitrogen cycle

As the cornerstone of nucleic acids and amino acids, nitrogen is essential for all life. As a macronutrient, it is used to compose any kind of cell (mostly in combination with carbon, hydrogen, oxygen and other elements) (Killham, 1994). The most common occurrence of nitrogen is its most inert form  $N_2$  - of which 78 % of our atmosphere is composed. In its most reduced form, as ammonium ( $NH_4^+$ ), as well as in its most oxidized form as nitrate ( $NO_3^-$ ), nitrogen is directly available for plants, chemo- and photoautotrophic organisms whereas nitrate has by far a greater mobility than ammonium. The latter tends to bind to electronegatively charged soil particles and is thus retarded (Blume et al, 2010). A negative side effect of the mobility of nitrate is the process of leaching nitrate to groundwater or aquatic systems, resulting in a loss of nitrogen (Vitousek & Howarth, 1991). The processes in which gaseous nitrogen is introduced into the respective ecosystems are either ammonification or nitrification. Both occur natural and are microbiotic driven, often in plant symbiosis with either fungi, archaea or bacteria. Also known as microbiotic driven, is the processes of denitrification which withdraws fixed nitrogen from soils and releases it to the atmosphere.

While nitrogen also occurs in mineral form (sodium nitrate or Chile saltpeter, not common in Europe) and can also be fixated through lightning (very small ecosystem contribution), the

biggest alteration to the nitrogen cycle was manmade. Through combustion of fossil fuels and the emission of nitrogen oxides but more importantly through the production of artificial nitrogen fertilizers via the Haber-Bosch process, the transfer of nitrogen from the unreactive atmospheric pool to the biologically available pool doubled (prior to human alteration) and will increase even further with ongoing population growth (Vitousek et al, 1997; Galloway, 1998)

In soils of natural, undisturbed systems such as deciduous temperate forests, plant production and biomass accumulation are limited by the supply of bioavailable nitrogen (Vitousek & Howarth, 1991; Vitousek et al, 1997). Allowedly increasing CO<sub>2</sub> has a fertilizing effect on plants but cannot be exploited by terrestrial ecosystems with a nitrogen shortage.

### 1.3 Objectives

Forests are viewed as carbon sinks and the sorption of OC to mineral surfaces is regarded as the most important mechanism to stabilize OC in soils. Recent studies have reported respiration of relatively old SOM due to changes in environment, indicating that SOM presumably is not as inert or inactive as assumed (Schmidt et al, 2011). Thus, it is questionable how C and N sequestration in soils respond to rising CO<sub>2</sub> concentrations and climate changes over time. The aim of this study was to gather supplementary data based on the previous forest soil inventory from 2004 and 2009 (Schrumpf et al, 2014) and to contribute to a monitoring of soil organic carbon.

By sampling, analysis and comparison of ten soil cores from a deciduous old growth forest with former measurements, this study shall provide insight into changes of C and N storage/input in deciduous forest soil over time and into the distribution and stability of C and N in soil and its fractions

With the analysis of the isotopes of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$ , as an indicator for C age, we furthermore want to gain an understanding of the stability and availability of the SOC pools and possible involved processes as well as insight of C and N input.

## 2. Materials

### 2.1 Study site

The sampling campaign for this master thesis was conducted in the UNESCO designated Hainich National Park in west Thuringia. The Hainich is located south of the city Mühlhausen. In the north. The National Park is bordered by the small towns Mülverstedt, Weberstedt and Flarchheim and in the west by the municipality Bischofroda. South of the National Park area runs the Motorway A4 by Hörselberg.

The Hainich itself is a 25 km long and 4 km wide, mostly of limestone consisting mountain range. Patzelt (1998) described the Hainich as flat bulge with a steep south-western flank and a flat east to south-east slope with an average height of 400 m above sea level.

The several sampling sites were located in the core zone of the National Park near the Craulaer Kreuz.

## 2.2 Geology and hydrogeology

The Hainich belongs to the Muschelkalk landscapes of the inner Thuringian basin. Its bedrock mostly consists of limestone which is overlaying a deposition of "Röt" (upper Buntsandstein) whereat the latter can be only seen in the deeper cut valleys of Heyerode and Hallungen outside of the National Park area (Patzelt, 1998). The limestone is divided into the lower Muschelkalk, also referred to as Wellenkalk with about 100 m thickness, then following the middle Muschelkalk with anhydrite and dolomite, topped by the upper Muschelkalk with about 60 m thickness. The upper Muschelkalk is defined by thick banks of so called Trochitenkalk followed by ceratite layers, consisting of alternated beddings of limestone and clay marl layers. Eponymus is here the ceratite, a fossilized ammonite (Patzelt, 1998). Located above the upper Muschelkalk, are the layers of Keuper, consisting of sand-, silt- and clay stones with intercalations of limestone and dolomite. Formerly widespread, these layers were worn out in the area of the Hainich over time and are to be found in flat slopes, the transitions of the Hainich into the Mühlhäuser basin and in sinks in the west side of the Hainich near "Berka vor dem Hainich" and near Mihla (Patzelt, 1998). On the surface of the Keuper, as well as on the Muschelkalk, Loess was partially distributed and deposited during the last glacial period (Patzelt, 1998).

Due to its steep south-west and shallower north-east slope, its elevated height and as a geological bulge, the Hainich mountain range functions as a watershed and separates the water bodies of the Werra and the Unstrut (Patzelt, 1998). The Werra contributes to the river system of the Weser while the Unstrut flows to the Elbe. According to Seidel (2003) each geological layer contributes differently to the hydrologic conditions. The depositions of the Muschelkalk, in contact to sufficient amounts of water, tend to develop Karst systems and anhydrite, transformed to gypsum when exposed to water, tends to be washed out, resulting in possible sinkholes. On the other hand, ceratite layers pose as aquifuges. While the Muschelkalk formation, especially the limestone and dolomite of the upper Muschelkalk, contributes major to thuringian aquifers, the Hainich itself is rather a water shortage region in its plateaus (Seidel, 2003).

## 2.3 Climate

Despite the little height difference of 250-300 m, relative to the height level of the nearby rivers Werra in the west and Unstrut in the northeast, the Hainich shows a different climate than its surrounding environment. Yearly as well as monthly temperature means of the Hainich altitudes are about 1.5-2 °C below the average temperature of Mühlhausen (Patzelt, 1998). Regarding precipitation, the Hainich also behaves different than the neighbouring basin of Mühlhausen with 600 mm and the inner Thuringian basin with < 500 mm. The annual mean precipitation of the Hainich amounts to about 700 mm due to the steep western exposure which causes a windward-leeward effect (Patzelt, 1998; Koch 1953). Fischer et al (2010), mentions an annual mean temperature of 6.5 – 8 °C and an annual mean precipitation of 500 – 800 mm for the Hainich-Dün landscape while Knohl et al (2003) reports 750-800 mm and 7.5-8 °C at 483 m NN, near the Craulaer Kreuz.

## 2.4 Soil type and properties

Since soil appearance depends on the existing host rock, in the Hainich Loess and limestone lead to the development of mainly Eutric Cambisols, Luvisols and also Stagnosols. Commonly, the soil in the Hainich has a loamy and/or clayey texture (Fischer et al, 2010).

## 2.5 History

Before establishment as a National Park, the Hainich has been used as a military training area from 1935 to 1993 which prohibited a forestry use. Prior to usage as a military base, the Hainich was used as a coppice with standard-systems and has therefore not been exposed to clear cut, resulting in a wide range of tree age classes up to 250 years (Knohl et al, 2003).

# 3. Methods

## 3.1 Soil sampling

The sampling of the ten soil cores was done in two days with no changes in weather conditions on the 25<sup>th</sup> and 26<sup>th</sup> of May 2016. All samples were taken with a motor-driven drill (Cobra Combi, Atlas Copco AB, Nacka, Sweden) using 8.5 cm steel pipes (Eijkelkamp) and were stored in LD-PE bags. Before sampling, the selected drill area was prepared with a 25 cm times 25 cm metal frame to collect the organic layer covering the mineral soil. The litter covering the mineral soil was carefully collected and stored in LD-PE bags. After drilling, cores were opened and segments of 10 cm and 5 cm for the upper soil were cut and stored in LD-PE bags. The soil depths sampled were 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm. Samples of 50-60 cm and 60-70 cm were also taken but not analysed.



### 3.2 Sample preparation

Prior to processing, samples were stored at 4 °C. Roots > 1 mm in diameter and stones of a diameter > 4 mm were sorted from the undried, soil-moist samples which were then dried at 40° C and sieved to 2 mm. The samples of 0-5 cm and 10-20 cm depth were subsampled prior to drying and sieving for density fractionation. For all samples, the dry weight was determined by drying processed subsamples for 48 h at 105 °C. For further analysis, all samples were ground using a ball mill.

### 3.3 Density fractionation

Of every sample of 0-5 cm and 10-20 cm duplicates were weighed in 25 g portions and filled in 500 ml PP centrifuge cups. The cups were then carefully filled with 125 ml SPT solution of 1.6 g cm<sup>-3</sup> density and after one hour resting time centrifuged at 4500 rpm for 15 min. After centrifuging, the lighter and floating FLF was collected using a pipette. Centrifuging was repeated until all floating FLF particles were collected. The FLF was then separated from the SPT solution by filtering with rinsed 0.45 µm PALL Life Sciences Supor® 450 polyethylene sulfon membrane filters. SPT solution was reused after filtering and refilled into the respective sample. The collected FLF was then washed with deionized water until showing a conductivity < 50 µS cm<sup>-1</sup>, filled into wide-necked flasks and dried at 40 °C.

To separate the OLF from the sample an ultrasonic sonotrode was calibrated and used to emit an energy of 900 J ml<sup>-1</sup>. For this, the mean value of the sonotrode energy was determined measuring the heat generation by ultrasound treatment of deionized water, resulting in an average output of 104.9 J s<sup>-1</sup> using the following formula.

$$P = \frac{\Delta Q}{\Delta t} = \frac{m_{water} \times c_{water} \times \Delta T}{\Delta t}$$

With P = Power [J s<sup>-1</sup>], Q = thermal energy [J], t = time [s], m = mass [g], c = heat capacity of water = 4.18 J g<sup>-1</sup>K<sup>-1</sup> and T = temperature [K].

To calculate the time necessary to convey the needed energy, the following formula was used.

$$\frac{900 \frac{J}{ml} \times 125 ml}{104.9 \frac{J}{s}} = 1072.44 s = 17 m 52s$$

During ultrasonic treatment, the samples were cooled in ice and water to prevent overheating of the samples in order to prevent combustion or disintegration of OC. After sonification for a

total of 17 minutes and 52 seconds, the samples were centrifuged and the floating OLF was collected using pipettes. Due to clay particles clogging the filters, magnesium chloride was added to the sonicated samples to coagulate the clay particles. The filtered OLF was then washed with deionized water until showing a conductivity  $< 50 \mu\text{S cm}^{-1}$ , filled into wide-necked flasks and dried at  $40 \text{ }^\circ\text{C}$ .

To yield the HF, the remaining samples were centrifuged and the remaining SPT solution was carefully decanted. After this, the samples were washed and centrifuged repeatedly until showing a conductivity  $< 50 \mu\text{S cm}^{-1}$ , filled into wide-necked flasks and freeze dried.

While the HF samples were ground using a ball mill, the lighter fractions FLF and OLF were ground by hand with an agate mortar

### 3.4 Sample analysis

#### 3.4.1 C/N analysis

Carbon and Nitrogen analysis was done by the ROMA Lab (Routine Measurements & Analysis of Environmental Samples) using a "Vario MAX" elemental analyser for C and N (Elementar Analysensysteme GmbH, Hanau). For total carbon (TC) and total Nitrogen (TN) measurements, 250 mg of ground soil samples were filled in small ceramic cups, overlaid with tungsten trioxide ( $\text{WO}_3$ ) and analysed. To determine the inorganic carbon (IC), another batch of the respective sample was weighed in cups and combusted at  $500 \text{ }^\circ\text{C}$  for 8h in a muffled oven, eliminating all OC. After cooling down, these samples were also overlaid with tungsten trioxide. With this the organic carbon (OC) could later be determined by subtracting the IC from the TC result of the analysis.

For the actual analysis, the sample (held in an automatic sample feeder) is transferred into the combustion tube of the vario MAX with helium as carrier gas and heated to  $1100 \text{ }^\circ\text{C}$ . Helium as inert carrier gas is necessary to flush out air carbon dioxide and nitrogen. For the combustion the carrier gas helium, is temporarily mixed with pure oxygen to induce oxidation. After complete combustion, the gas mixture flows into a tungsten filled and to  $830 \text{ }^\circ\text{C}$  heated reduction tube, packed with silver wool, to eliminate excess oxygen and to reduce nitrogen oxides to nitrogen. Possible halogens in the gas mixture are eliminated by the silver wool. The gases of the mixture are separated by a purge and trap system, similar to gas chromatography, which retards the carbon dioxide. After nitrogen was measured by the thermal conductivity detector, the carbon dioxide is released and measured the same way. The respective measured signals are integrated and, via calibration of the integral to the respective element, calculated to an absolute concentration of the element. With ongoing

measurements, a correction for trends was done. (Handbook vario MAX CN) (ROMA Lab, MPI BGC) (Hilke et al 2012)

### 3.4.2 <sup>13</sup>C and <sup>15</sup>N and analysis

Analysis of the isotopes <sup>13</sup>C and <sup>15</sup>N was done by the Isolab of the MPI BGC with an isotope ratio mass spectrometer (Delta C or Delta+XL, Finnigan MAT, Bremen, Germany) coupled to an elemental analyser (NA 1100, CE Instruments, Milan, Italy). For the respective isotopic analysis, the samples were weighed in tin capsules. <sup>13</sup>C samples were acidified with 120 µl sulphurous acid to disassociate possible carbonate.

In irm-EAMS, the sample material is combusted to CO<sub>2</sub> and N<sub>2</sub>, ionized, accelerated to a given energy and collimated into a focussed beam. In the mass analyser, the ion beams are then separated from each other by a magnetic field on the basis of their mass whereat higher or lower energetic ions can also be removed from the beam with proper placement of slit apertures if necessary. The detection of the isotopes in the beams then occurs in Faraday cups, where the current intensity of the respective ion beam is measured (Sharp, 2017).

The δ<sup>15</sup>N notation in ‰, shown below, is commonly used as well as the δ<sup>13</sup>C notation in ‰.

$$\delta^{15}_N = \left[ \frac{(15_N/14_N)_{Sample}}{(15_N/14_N)_{Standard}} - 1 \right] 1000$$

$$\delta^{13}_C = \left[ \frac{(13_C/12_C)_{Sample}}{(13_C/12_C)_{Standard}} - 1 \right] 1000$$

### 3.4.3 <sup>14</sup>C analysis

Analysis for the <sup>14</sup>C isotopes was done by the <sup>14</sup>C Analysis Facility of the MPI BGC with a 3 MV Tandatron 4130 AMS <sup>14</sup>C system (HVEE, Netherlands). The necessary removal of inorganic carbon was also done by the <sup>14</sup>C Facility.

With accelerator mass spectrometry (AMS), isotopes as <sup>14</sup>C can be detected and measured directly by separation and acceleration of charged ions. In a specialized chemical preparation of the sample, the carbon is extracted and graphitized. The processed sample is ionized, negatively charged in a sputter-ion source and accelerated by high voltage application into an electromagnetic field where isotopes as <sup>12</sup>C, <sup>13</sup>C, and <sup>14</sup>C are separated by their mass and

electric charge by a 90°- analysing magnet. Injected into a tandem accelerator, the sample material is stripped in a vacuum chamber by changing the charge from negative to positive. With this, distracting molecules (as  $^{13}\text{CH}$  and  $^{14}\text{N}$  e.g.) are broken up and removed. The carbon ions are then further accelerated, subsequently arranged by their charges in an electromagnetic field and separated by their masses (12, 13, 14) with a 55° magnet. The  $^{14}\text{C}$  isotope is then detected using a gas ionisation detector which measures the kinetic energy emitted by the ions in the carrier gas while the stable isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$  can be analysed by measuring the current intensity of the ion beam in faraday cups (Steinhof et al, 2004; AMS C14-Labor, Erlangen).

The nomenclature  $\Delta^{14}\text{C}$  used in this study (see formula below), reports the  $^{14}\text{C}/^{12}\text{C}$  ratio of the sample measured in year  $y$  to an absolute standard (oxalic acid) that does not change with time and is corrected for decay between 1950 and the year  $y$ . It is also corrected for mass-dependant isotope fractionation to a common  $\delta^{13}\text{C}$  value of -25 ‰ (Trumbore, 2009).

$$\Delta^{14}\text{C} = \left[ \frac{\left[ \frac{^{14}\text{C}}{^{12}\text{C}} \right]_{\text{sample}, -25}}{\left[ 0.95 \frac{^{14}\text{C}}{^{12}\text{C}} \right]_{\text{OXI}, -19} e^{\left( \frac{y-1950}{8267} \right)}} - 1 \right] 1000$$

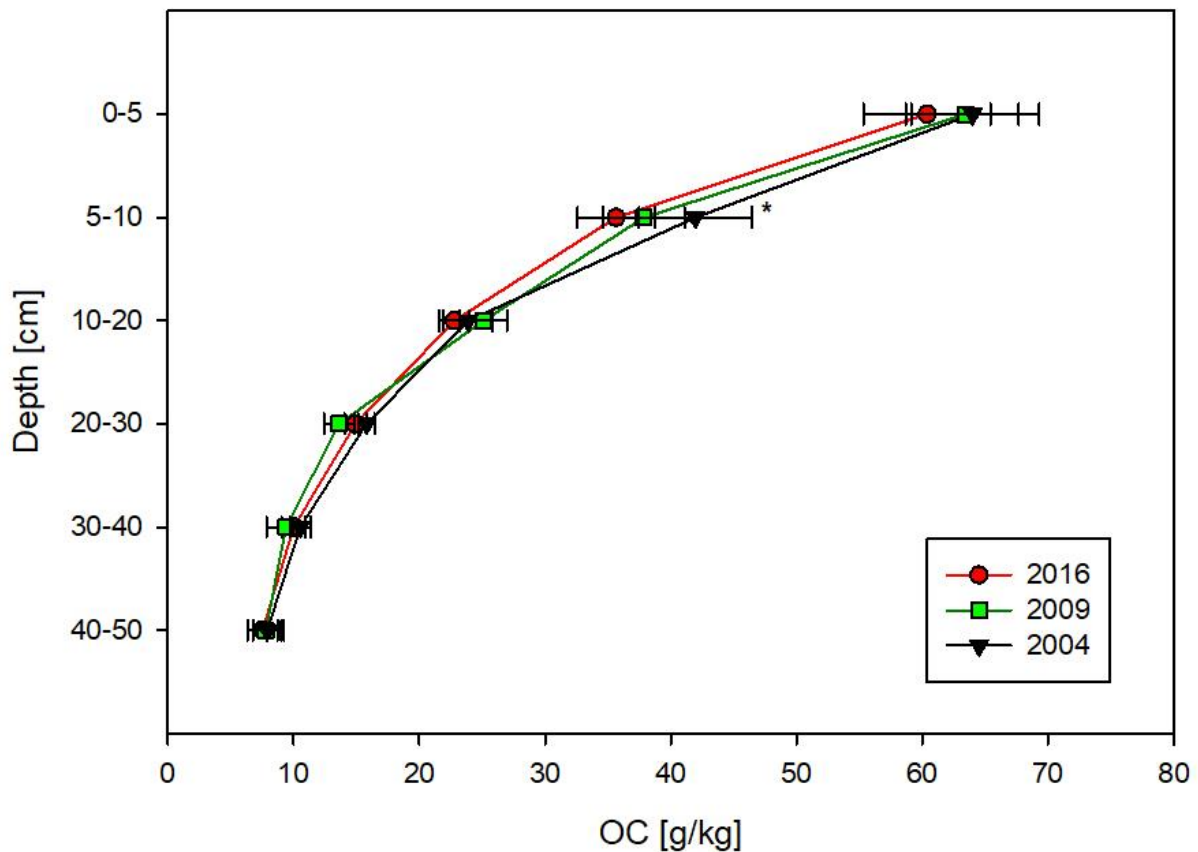
#### 3.4.4 Statistics

For every Parameter and between the sampled years 2016 and 2009 as well as 2016 and 2004, paired t-tests including tests on normal distribution after Kolmogorov-Smirnov were done. When normal distribution was not given, a Wilcoxon signed rank test was done instead. Since a paired t-test demanded equally sized and paired sample groups between controlled years, for all exceptions a normal t-test was done. All tests were done with a confidence interval of 95 % in Sigmaplot 12.5. For one extraordinary result of  $\Delta^{13}\text{C}$  in sample P92 5-10 cm in 2004, an outlier test was done after Grubbs, which defined said result with a probability of 95 % as an outlier. The tested result was then removed from evaluation.

## 4 Results

All shown results are the calculated means of the repetitions of the sampled cores and their respective standard error unless mentioned otherwise.

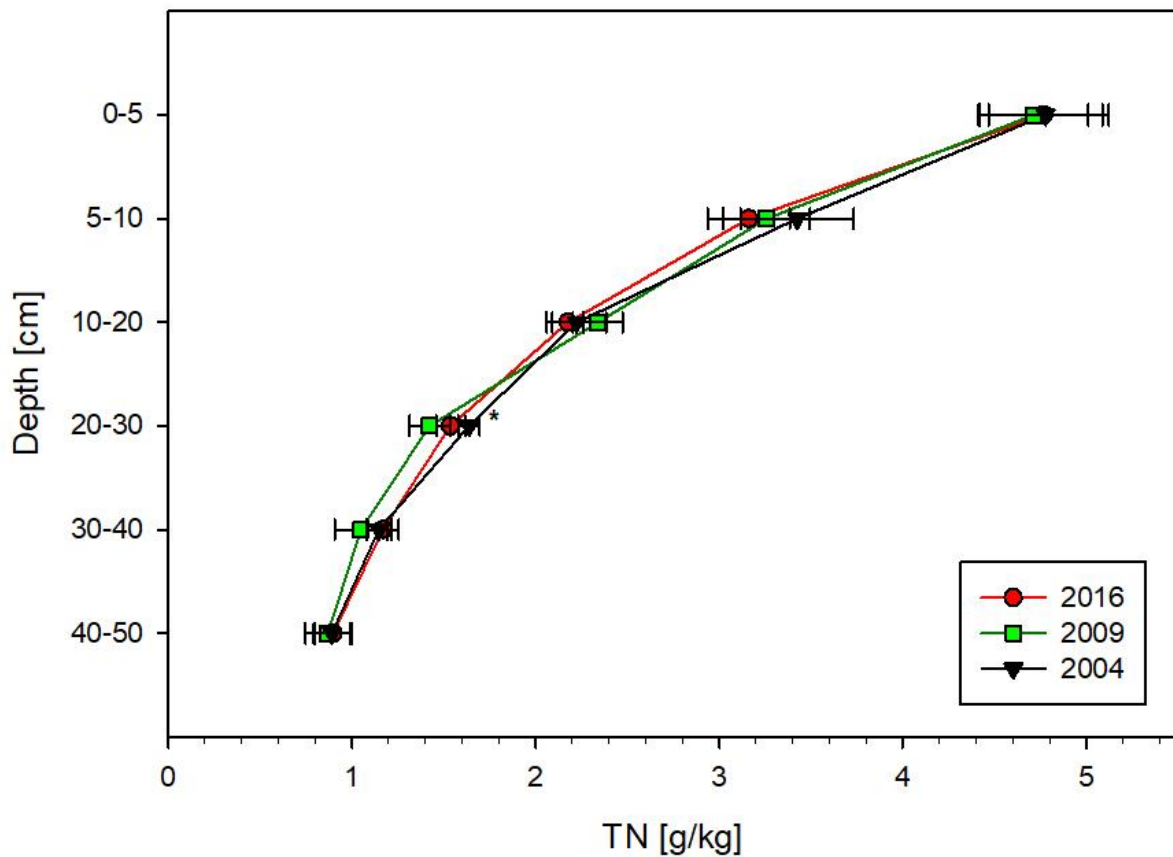
### 4.1 Organic carbon



**Figure 3: Organic carbon values of bulk soil samples; the \* indicates a significant difference of the data between 2016 and 2009 in the marked depth**

The bulk soil OC content, as can be seen in Figure 3, decreases with depth in every year and ranges from a maximum of  $63.99 \pm 5.28 \text{ g kg}^{-1}$  at 0-5 cm in 2004 to a minimum of  $7.53 \pm 1.19 \text{ g kg}^{-1}$  at 40-50 cm in 2016. Though it can be held, that while in the upper soil from 0-20 cm a depletion of OC takes place from 2004 to 2009 and also from 2009 to 2016, in the soil from 20 cm downwards the OC content stays rather unaffected between the years. The only exception to this is to be found in 20-30 cm depth where the OC diminishes from  $15.80 \pm 0.65 \text{ g kg}^{-1}$  in 2004 to  $13.64 \pm 1.23 \text{ g kg}^{-1}$  in 2009 but then increases by  $1.29 \text{ g kg}^{-1}$  to  $14.93 \pm 0.89 \text{ g kg}^{-1}$  in 2016. It has but to be noted, that the mentioned increase of  $1.29 \text{ g kg}^{-1}$  in 2016 and its similarity to the standard error of  $\pm 1.23 \text{ g kg}^{-1}$  in 2009 probably are statistically insignificant, since a significant change in OC was only to be found in 5-10 cm depth from 2009 to 2016.

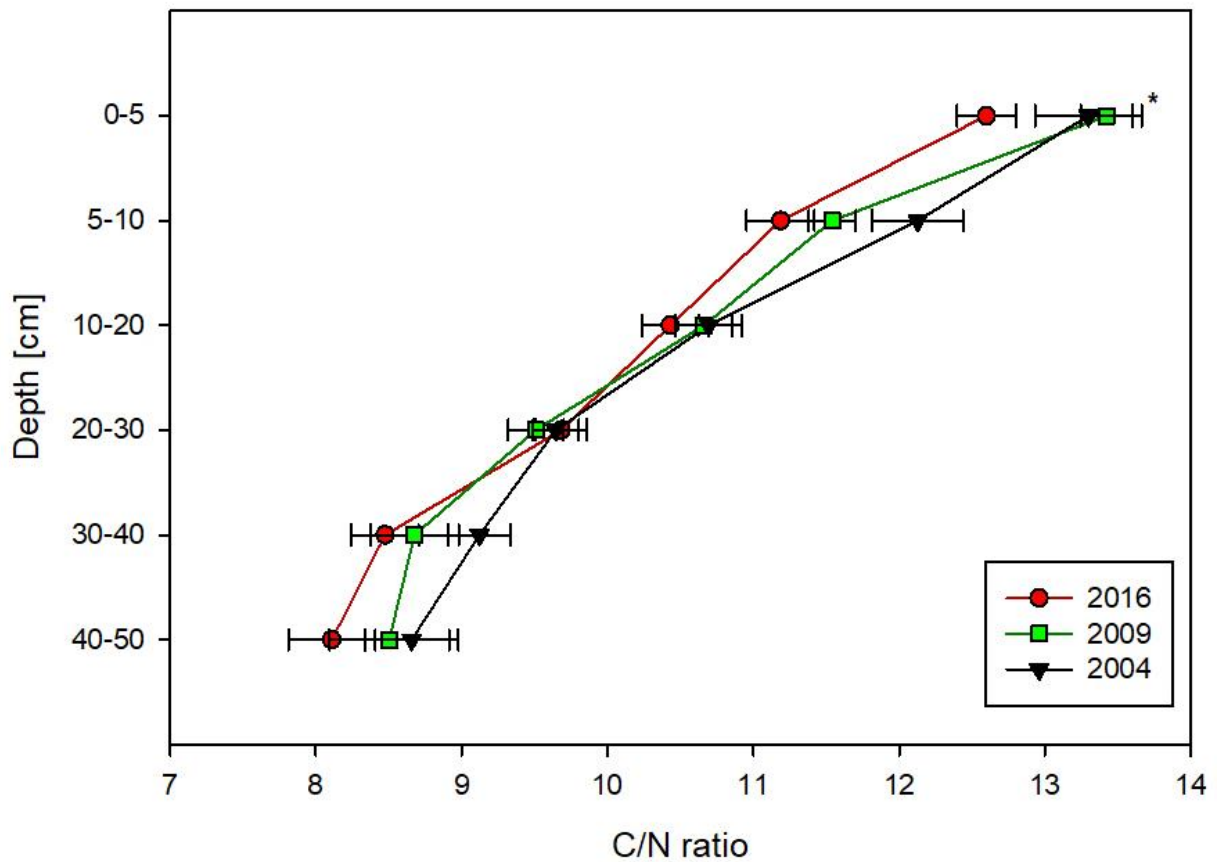
## 4.2 Total Nitrogen



**Figure 4: total nitrogen values of bulk soil samples; the \* indicates a significant difference of the data between 2016 and 2004 in the marked depth**

Looking upon the TN content of the bulk soil samples in Figure 4, a decrease with depth can be seen. TN values range between a maximum of  $4.78 \pm 0.31 \text{ g kg}^{-1}$  in 0-5 cm of 2004 and a minimum of  $0.87 \pm 0.12 \text{ g kg}^{-1}$  in 40-50 cm depth in 2009. In the top soil of 0-5 cm and in the bottom soil of 40-50 cm depth the TN values of the years are closely grouped between  $4.78 \pm 0.31 \text{ g kg}^{-1}$  to  $4.71 \pm 0.30 \text{ g kg}^{-1}$  and  $0.90 \pm 0.10 \text{ g kg}^{-1}$  to  $0.87 \pm 0.12 \text{ g kg}^{-1}$ . Looking but into the depths in between, a decrease in TN from 2009 to 2016 can be seen for 5-10 cm and 10-20 cm as well as an increase of TN from 2009 to 2016 in 20-30 cm and in 30-40 cm depth. After application of statistical tests, the only significant change was but to be found between 2016 and 2004 in the depth of 20-30 cm.

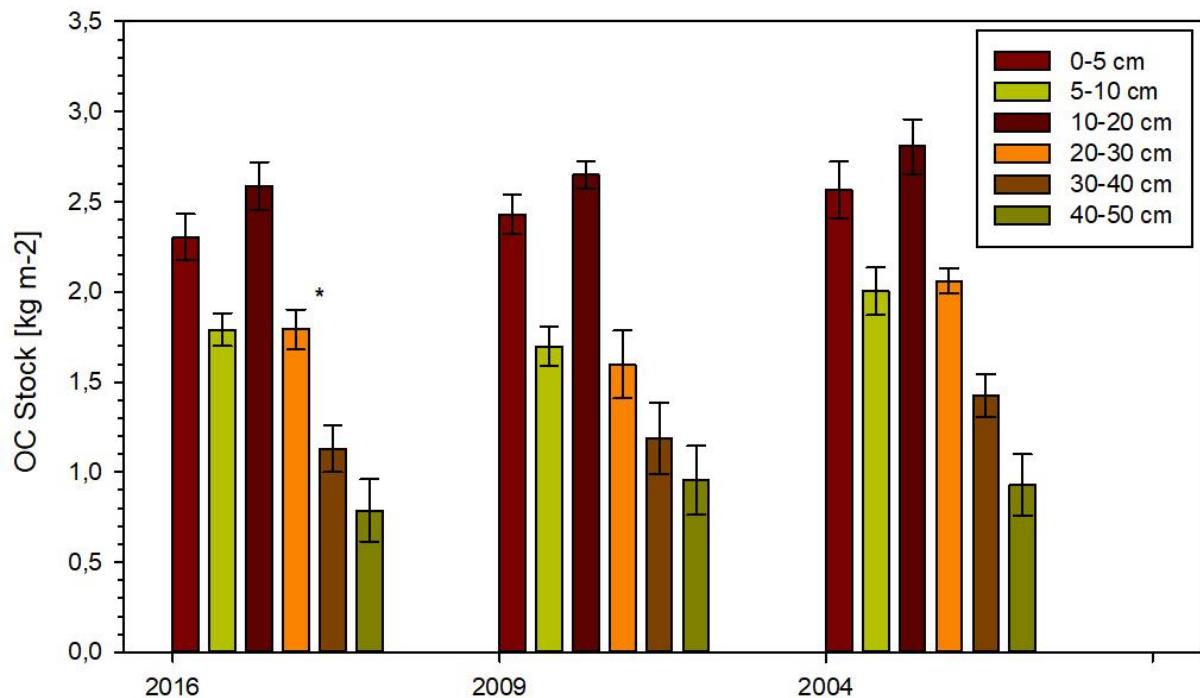
### 4.3 C/N analysis



**Figure 5: calculated CN-ratio of OC to TN of bulk soil samples; the \* indicates a significant difference of the data between 2016 and 2009 in the marked depth**

The in Figure 5 presented C/N-ratios range from  $8.11 \pm 0.30 \text{ g kg}^{-1}$  to  $13.42 \pm 0.30 \text{ g kg}^{-1}$  and decline with depth. Comparing the graphs of the years a decline from 2004 to 2009 and also 2009 to 2016 can be seen in all depths. However, at 10-20 cm and 20-30 m the CN-ratios of the years are grouped close to each other with  $10.43 \pm 0.19 \text{ g kg}^{-1}$  to  $10.70 \pm 0.23 \text{ g kg}^{-1}$  for 10-20 cm and  $9.51 \pm 0.19 \text{ g kg}^{-1}$  to  $9.68 \pm 0.18 \text{ g kg}^{-1}$  for 20-30 cm. Though the error bars of the data might suggest otherwise, the only significant difference was to be found in 0-5 cm depth from 2009 to 2016.

#### 4.4 Density fractionation



**Figure 6: OC stocks for sampled bulk soil; the \* indicates a significant difference of the data between 2016 and 2004**

Depicted in Figure 6 are the calculated stocks for OC which are derived from the OC results in Figure XX. For the upper soil from 0-20 cm OC stocks are declining each year, with the only exception in 5-10 cm soil depth. An increase in OC was to be found from 2009 with  $1.70 \pm 0.11 \text{ kg m}^{-2}$  to  $1.79 \pm 0.09 \text{ kg m}^{-2}$  in 2016. For the lower soil, stocks decreased with depth and for every year with the exception of 40-50 cm from 2004 to 2009 and 20-30 cm from 2009 to 2016. In 40-50 cm and 20-30 cm, slight increased stocks were to be found. Almost all changes in OC from 2009 to 2016, as visible in the graph, are statistically insignificant and were not great enough to exclude the possibility that they were due to chance. The only statistic significant difference was to be found in 20-30 cm depth of 2016 compared to 2004. Here the OC stock decreased from  $2.06 \pm 0.07 \text{ kg m}^{-2}$  in 2004 to  $1.79 \pm 0.11 \text{ kg m}^{-2}$  in 2016.



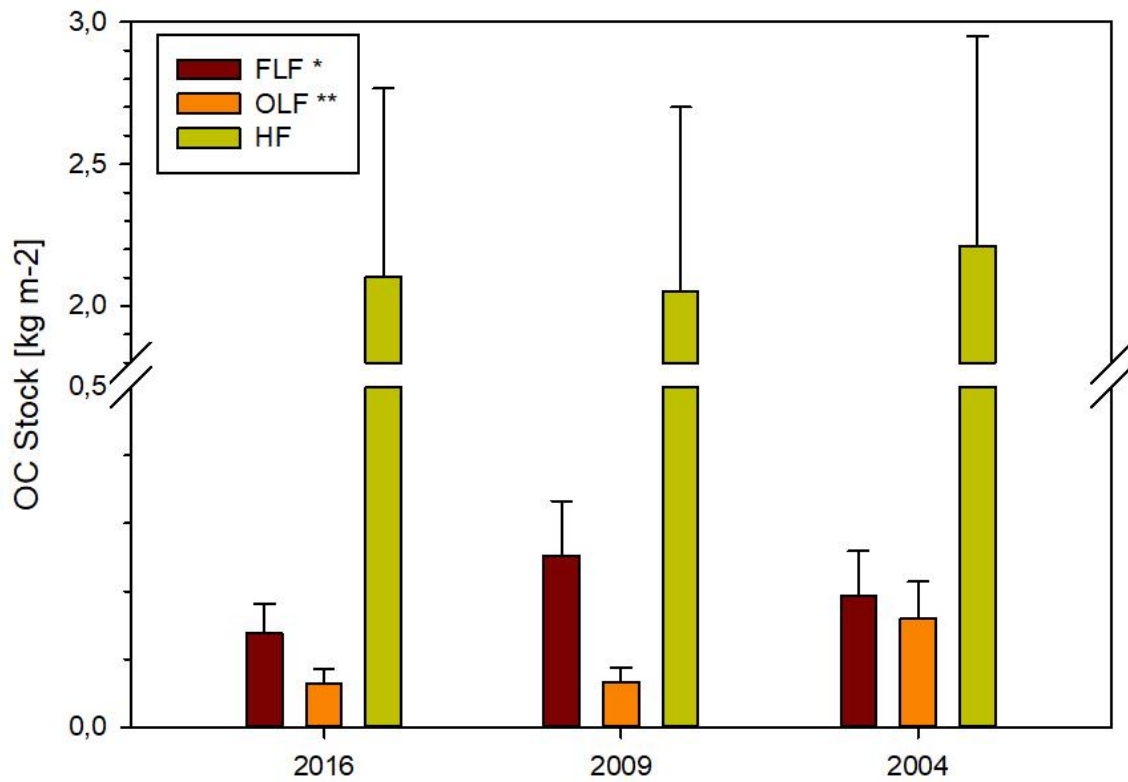


Figure 7: OC stocks for density fractionated samples of 0-5 cm soil depth; \* indicates a significant difference between the data of 2016 and 2009, \*\* indicates a significant difference of the data between 2016 and 2004

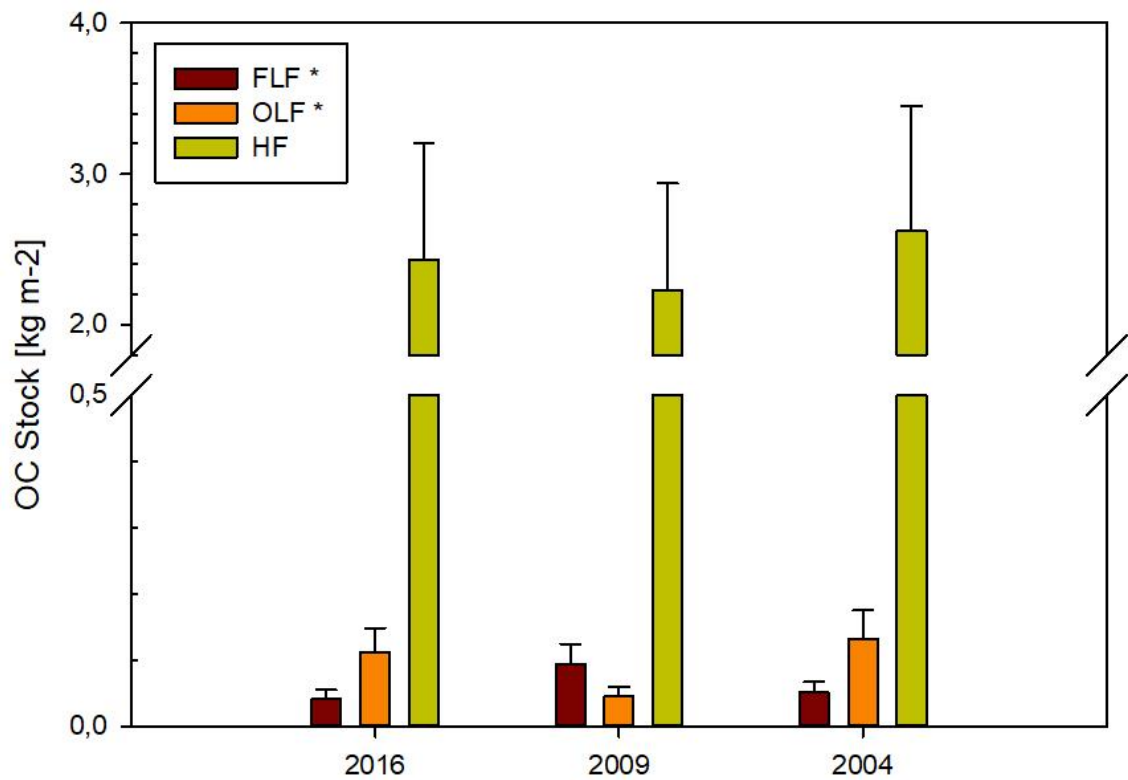


Figure 8: OC stocks for density fractionated samples of 10-20 cm soil depth; \* indicates a significant difference between the data of 2016 and 2009

As can be seen in Figure 7, the stocks of the HF did not alter much from 2004 to 2009 and from 2009 to 2016, as the slight changes were of insignificant difference. While the stocks of the OLF decreased from 2004 with  $0.16 \pm 0.05 \text{ kg m}^{-2}$  to 2009 with  $0.07 \pm 0.29 \text{ kg m}^{-2}$ , the change from 2009 to 2016 with  $0.06 \pm 0.02 \text{ kg m}^{-2}$  was insignificant. The OC stocks of the FLF however, increased from 2004 with  $0.19 \pm 0.06 \text{ kg m}^{-2}$  to 2009 with  $0.25 \pm 0.08 \text{ kg m}^{-2}$  but showed a significant decrease from 2009 to 2016 with  $0.14 \pm 0.04 \text{ kg m}^{-2}$ .

In the soil depth of 10-20 cm, as it is shown in Figure 8, the stocks of the HF also did not change a lot. Comparing but the HF of 0-5 cm with 10-20 cm, the HF in 10-20 cm is with for example  $2.43 \pm 0.77 \text{ kg m}^{-2}$  in 2016 bigger than  $2.10 \pm 0.66 \text{ kg m}^{-2}$  in 0-5 cm of the same year. The OC stocks of the OLF however decreased from 2004 with  $0.13 \pm 0.04 \text{ kg m}^{-2}$  to  $0.05 \pm 0.01 \text{ kg m}^{-2}$  in 2009 but increased significantly again from 2009 to 2016 with  $0.11 \pm 0.04 \text{ kg m}^{-2}$ . Contrary to the OLF, the FLF increased from 2004 to 2009 but decreased significantly from 2009 with  $0.09 \pm 0.03 \text{ kg m}^{-2}$  to  $0.04 \pm 0.01 \text{ kg m}^{-2}$  in 2016. Respective to the difference between the depths, both depths show the same pattern for all fractions.

#### 4.5 $\delta^{13}\text{C}$ Measurements

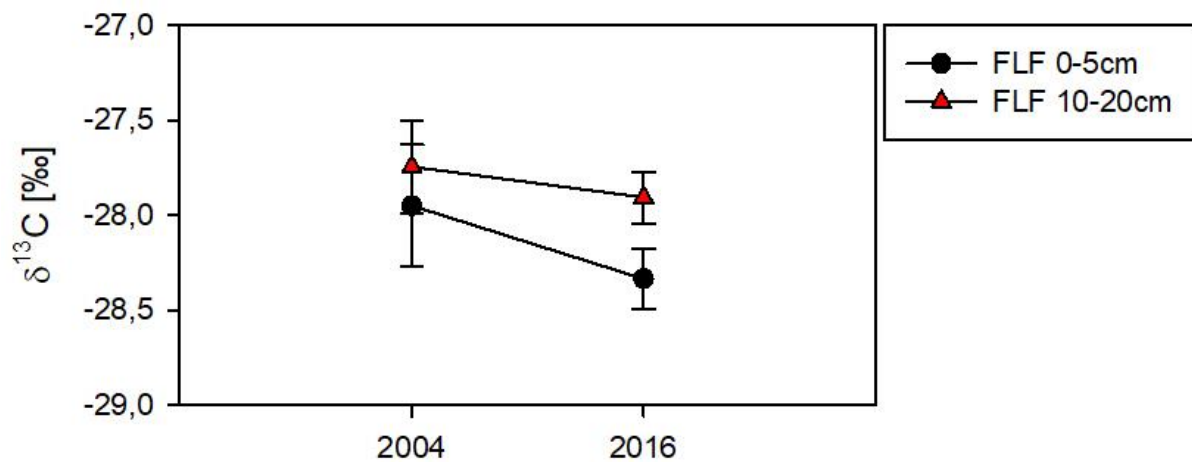


Figure 9:  $\delta^{13}\text{C}$  values of the density-fractionated free light fraction

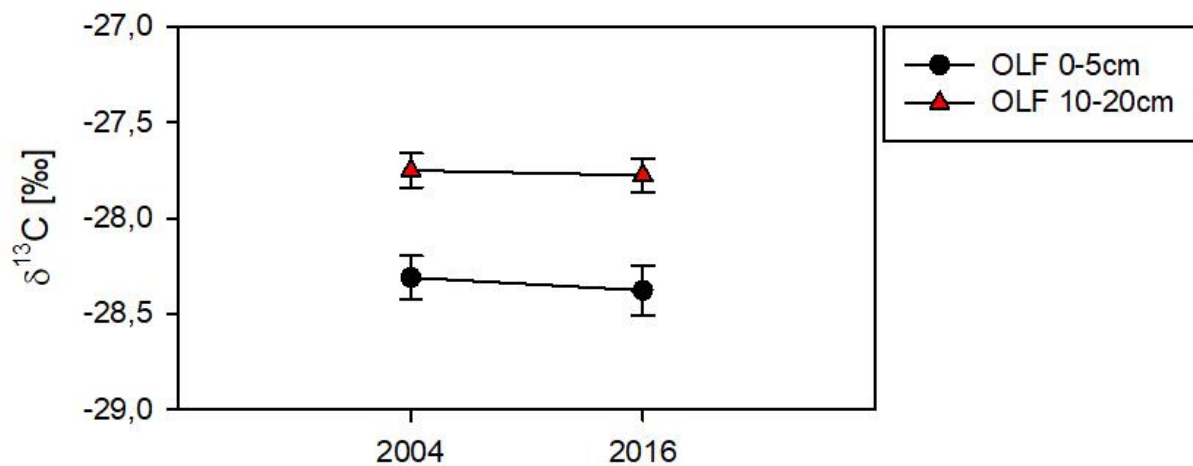


Figure 10:  $\delta^{13}\text{C}$  values of the density-fractionated occluded light fraction

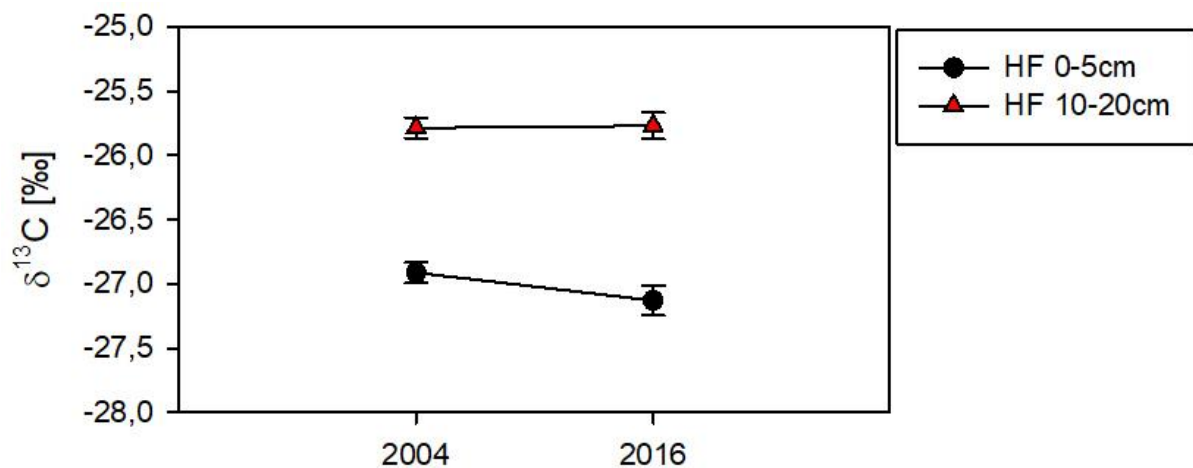
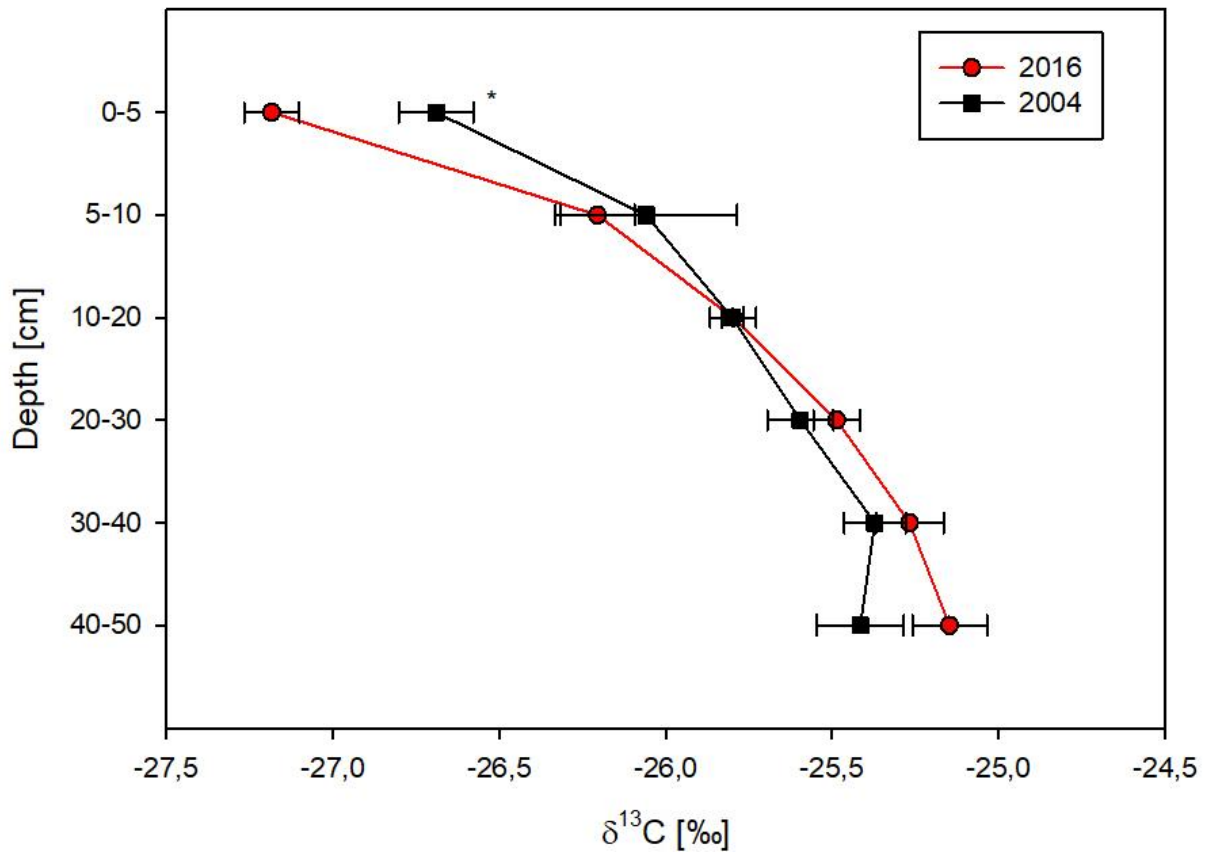


Figure 11:  $\delta^{13}\text{C}$  values of the density-fractionated heavy fraction

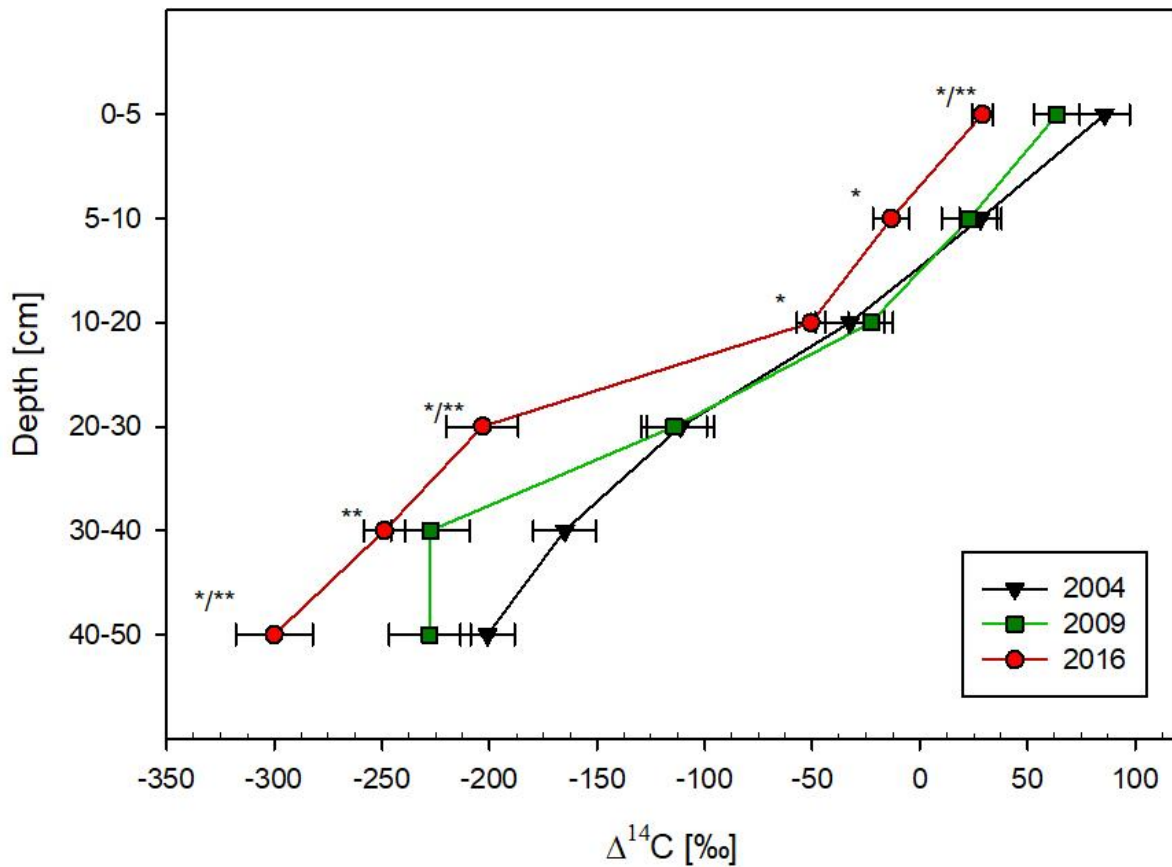
Regarding Figures 9 to 11, for all Fractions, it is shown that the deeper soil of 10-20 cm is more enriched in  $\delta^{13}\text{C}$  than the upper soil of 0-5 cm and that the HF is more enriched in  $\delta^{13}\text{C}$  than the two light fractions FLF and OLF. The  $\delta^{13}\text{C}$  content of the heavy fraction shows almost no change from 2004 to 2016 with just  $-25.79 \pm 0.08 \text{ ‰}$  to  $-25.77 \pm 0.10 \text{ ‰}$  in 10-20 cm depth and only little change with  $-26.91 \pm 0.08 \text{ ‰}$  to  $-27.13 \pm 0.12 \text{ ‰}$  in 0-5 cm depth. Little change can also be seen at the occluded light fraction with only  $-27.75 \pm 0.09 \text{ ‰}$  to  $-27.78 \pm 0.09 \text{ ‰}$  in 10-20 cm and  $-28.31 \pm 0.11 \text{ ‰}$  to  $-28.38 \pm 0.13 \text{ ‰}$  in 0-5 cm depth. Solely the free light fraction of 0-5 cm depth varies in  $\delta^{13}\text{C}$  content from 2004 to 2016 with but a slightly higher error compared to other fractions. Though the difference for the FLF of 0-5 cm from 2004 to 2016 is still minor with just  $-0.39 \text{ ‰}$  from  $-27.95 \pm 0.32 \text{ ‰}$  to  $-28.34 \pm 0.16 \text{ ‰}$ , it can be concluded that  $\delta^{13}\text{C}$  of OLF and HF did not alter much during the years and that only the FLF shows a slight depletion. In turn, none of the examined fractions showed a statistically significant difference from 2004 to 2016.



**Figure 12:  $\delta^{13}\text{C}$  values of bulk soil correlated with soil depth; the \* indicates a significant difference of the data between 2016 and 2004 in the marked depth**

Depicted in Figure 12 are the graphs of the  $\delta^{13}\text{C}$  contents of 2004 and 2016 ranging from  $-27.18 \pm 0.08 \text{ ‰}$  to  $-25.15 \pm 0.11 \text{ ‰}$ . With increasing depth, an enrichment of  $\delta^{13}\text{C}$  is shown in both graphs whereat the line of 2016 held also the most depleted and most enriched  $\delta^{13}\text{C}$  value. As the graph suggests a visible difference between both years, a statistically significant difference from 2004 to 2016 was only proven for the upper soil in 0-5 cm depth, where a depletion in  $\delta^{13}\text{C}$ . For the year 2009 no isotopic values have been measured.

#### 4.6 $\Delta^{14}\text{C}$ Measurements



**Figure 13:  $\Delta^{14}\text{C}$  values of bulk soil samples; the \* indicates a significant difference of the data between 2016 and 2009; \*\* indicates a significant difference of the data between 2016 and 2004**

The  $\Delta^{14}\text{C}$  content of the bulk soil, as can be seen in Figure 13 shows a depletion with ongoing depth for each year. While the lines for the graphs of 2004 and 2009 behave quite similar, except for 30-40 cm depth in 2009 with  $-227.51 \pm 18.28$  ‰ compared to  $-165.06 \pm 14.53$  ‰ in 2004, a stronger depletion for all depths can be seen for the samples of 2016. Additionally, for 2016 a noticeable shift in  $\Delta^{14}\text{C}$  depletion takes place between 10-20 cm with  $-32.60 \pm 16.05$  ‰ and 20-30 cm with  $-111.23 \pm 15.73$  ‰ resulting in a difference of  $-78,63$  ‰. Taking the shift into account, also the line of 2016 resembles the lines of 2009 and 2004 with a similar pattern. Exceptional is but the steep difference between 10-20 cm and 20-30 cm in 2016. The undertaken statistical tests reveal a significant difference for almost all depths in all years marked with a \* in Figure 13.

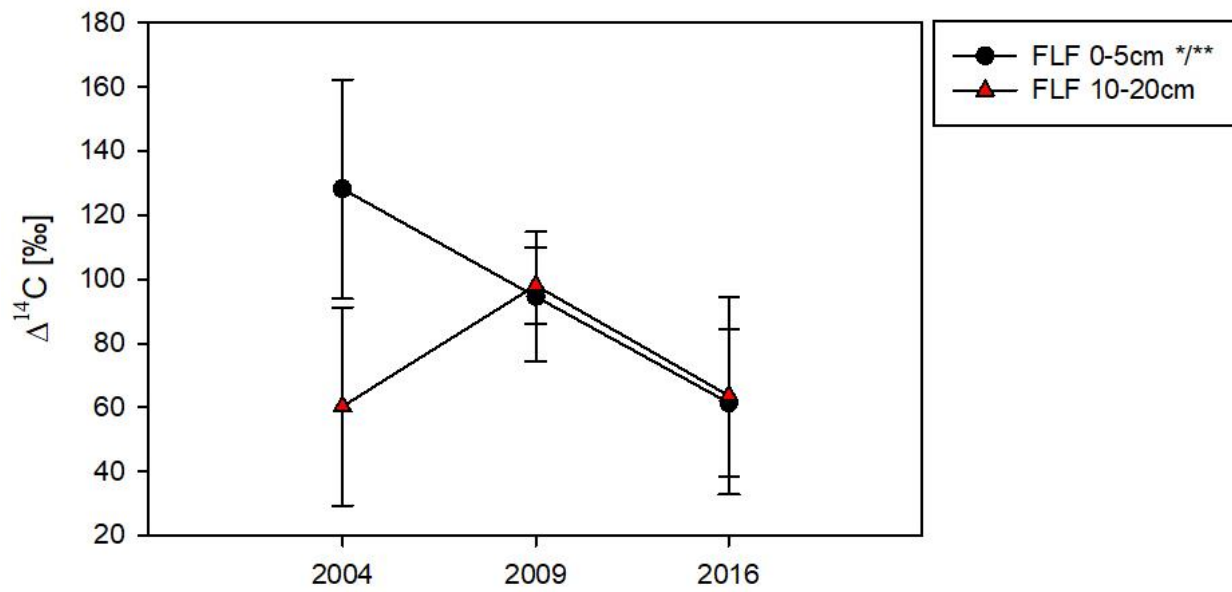


Figure 14:  $\Delta^{14}\text{C}$  values of the density-fractionated free light fraction

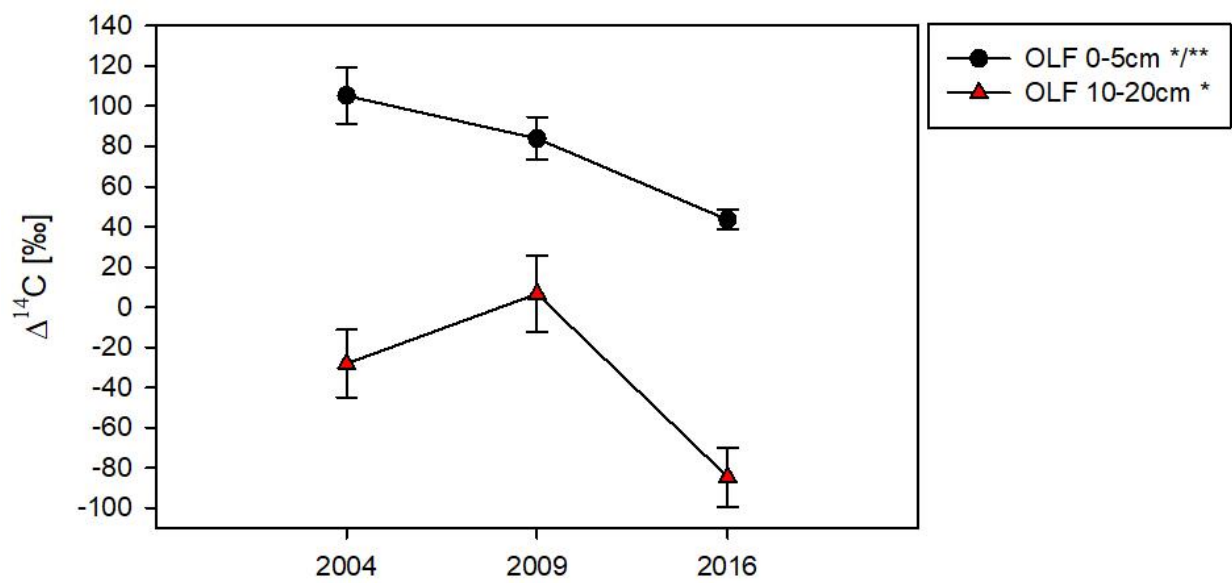
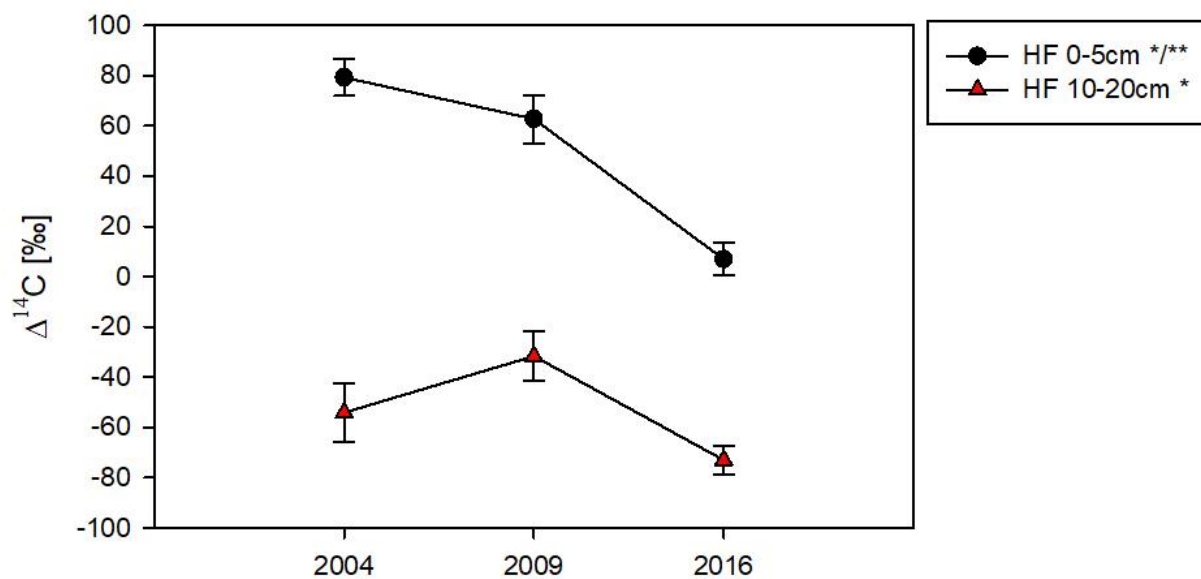


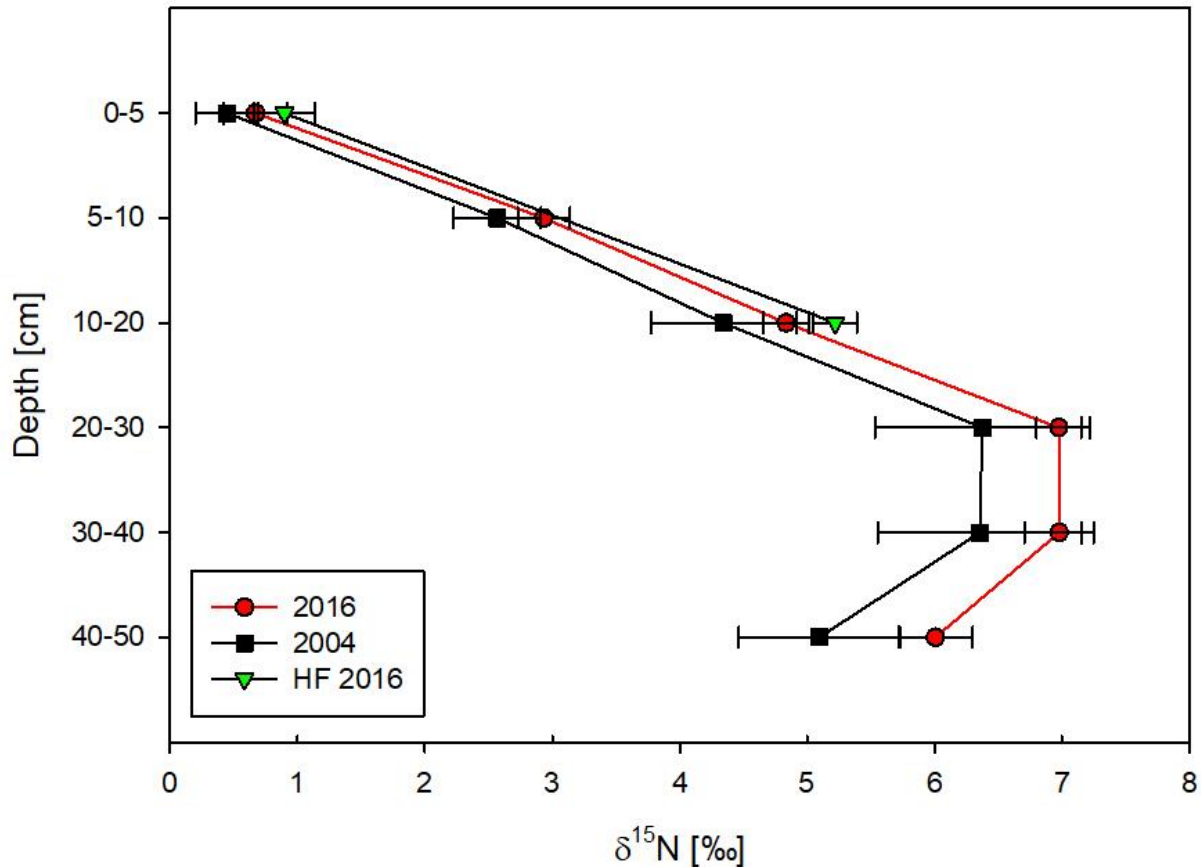
Figure 15:  $\Delta^{14}\text{C}$  values of the density-fractionated occluded light fraction



**Figure 16:  $\Delta^{14}\text{C}$  values of the density-fractionated heavy fraction**

Regarding Figures 14 to 16 for all fractions, it is shown that in the upper soil of 0-5 cm a steady depletion takes place from year to year. While the depletion of the FLF seems almost linear with around -30 ‰ from  $128.17 \pm 12.06$  ‰ in 2004 to  $94.56 \pm 7.16$  ‰ in 2009 and to  $61.41 \pm 7.24$  ‰ in 2016, the depletion for the OLF is slightly increased from 2009 to 2016 and highest in the HF. It has but to be taken into account that the elapsed time with five years from 2004 to 2009 and seven years from 2009 to 2016 differs in each sampling campaign, thus eliminating the idea of linear decrease for the FLF. For but the OLF and the HF, a linear decrease is much more. From 2009 to 2016 the  $\Delta^{14}\text{C}$  content of the HF was depleted from  $62.71 \pm 9.59$  ‰ to  $6.95 \pm 6.40$  ‰ by -55.76 ‰. The deeper soil of 10-20 cm however doesn't act like the upper soil of 0-5 cm. In 10-20 cm, first an enrichment of  $\Delta^{14}\text{C}$  from 2004 to 2009 and then a depletion from 2009 to 2016 is shown for all fractions. While the high standard error of the mean values of the FLF is striking, the error decreases for the OLF and HF, compared to the FLF. Interestingly, the  $\Delta^{14}\text{C}$  content of the FLF of 2009 in 0-5 cm is with  $94.56 \pm 7.16$  ‰ almost similar to the FLF in 10-20 cm with  $98.08 \pm 11.93$  ‰. The same behaviour is visible in 2016 with  $61.41 \pm 7.24$  ‰ for 0-5 cm and  $63.66 \pm 30.85$  ‰ for 10-20 cm.

## 4.7 $\delta^{15}\text{N}$ Measurements



**Figure 17:  $\delta^{15}\text{N}$  bulk soil values of 2004 and 2016 and of the heavy fraction of 2016**

Figure 17 shows the graphs of the  $\delta^{15}\text{N}$  bulk soil values of the years 2004 and 2016. In 2004,  $\delta^{15}\text{N}$  ranged from  $0.45 \pm 0.25$  ‰ in the top soil to  $5.10 \pm 0.63$  ‰ in the depth of 40-50 cm and in 2016 from  $0.67 \pm 0.25$  ‰ in the top soil to  $6.01 \pm 0.29$  ‰ at 40-50 cm. In both years an increase in  $\delta^{15}\text{N}$  contents can be seen from top soil to 20-30 cm, whereas from 20-40 cm the  $\delta^{15}\text{N}$  values stagnate. In the lowest sampled soil depth,  $\delta^{15}\text{N}$  contents are then decreasing. Comparing the two years,  $\delta^{15}\text{N}$  increased from 2004 to 2016, respective to the error, in all depths with the same pattern of 2004. The separated heavy fraction in 10-20 cm depth is slightly more enriched in  $\delta^{15}\text{N}$  than the bulk soil of 2016, with  $5.22 \pm 0.17$  ‰ compared to  $4.83 \pm 0.18$  ‰ of bulk soil resulting in a difference of 0.39 ‰. For the free light fraction and the occluded fraction, no  $\delta^{15}\text{N}$  measurements were undertaken due to the little amounts of processed material. Comparing but the  $\delta^{15}\text{N}$  measurements with the TN content of the bulk soil, the general loss in TN is contrasted by the continued enrichment of the isotope over the years. After statistical testing for significant differences from 2004 to 2016, no significance was found.



## 5 Discussion

### 5.1 Possible Error Sources

Possible contamination during sampling, preparation and analysis cannot be ruled out completely but is unlikely. Admittedly, during preparation for  $^{14}\text{C}$  measurements of the fractionated samples, a contamination from previous foreign samples occurred but was detected and all affected samples were remeasured. During density fractionation, a loss of sampling material occurred. Despite cautious centrifugation and the use of magnesium chloride as a coagulant, clayey parts of the fractionated samples of 2016 were lost in the process. The recovery rate for the mass of the fractions of 0-5 cm is at 95.65 % and at 91.97 % for the fractions of 10-20 cm. This results in an underestimation of carbon content and thus OC stocks especially for the HF as OC is adsorbed to the surface of clay minerals (Wäldchen, 2012). Due to working with a solution at fractionation, the possibility of DOC loss also cannot be ruled out. For the FLF and OLF, an underestimation cannot be excluded completely. Since these fractions are particulate they should not be affected as much as the HF but loss of very fine parts of OLF during filtration is possible although should only be of minor effect.

### 5.2 Changes in Organic Carbon Stocks and C/N Ratio

As for bulk soil OC stocks derived from fine earth masses, with one exception in 20-30 cm depth, no changes could be determined. Opposing to this, slight decreases of OC were found in the upper soil and lower soil from 2009 to 2016. This decrease in OC content, with only little changes in TN content, results in a decreasing or shortening C/N ratio.

A decreasing OC content, with no changes in TN and OC stocks hints to increased respiration of SOC by microbes as an increase in biomass due to microbial activity would come along with an increase in OC stocks and in TN. Since there are small changes to TN, especially in 20-30 cm where a significant increase was observed, the  $\delta^{15}\text{N}$  isotope might provide insight into source and fate of TN.

In a deciduous old growth forest such as the Hainich, an artificial fertilization should not take place and can thus be excluded. This narrows down N deposition and biologic fixation as possible inputs. According to Vitousek et al (1991), terrestrial forests show high C/N ratios except where they are dominated by symbiotic N fixers or where they receive large inputs of anthropogenic fixed N from the atmosphere. This given, an increased N uptake should increase net primary production and thus C fixation of plants, resulting in a widening or increasing C/N ratio and increasing OC stocks. As this is not the case, according to Churkina

et al (2009), with sufficient C and N supply, a synergetic effect of C and N might fail to appear because of colimiting factors such as climate, CO<sub>2</sub>, N availability or a shortage of other nutrients.

Considering the fractions, a significant increase of the FLF of both, 0-5 cm and 10-20 cm depth, was found from 2004 to 2009 followed by a significant decrease from 2009 to 2016. In the OLF of 10-20 cm, a significant increase in OC stocks from 2009 to 2016 hints to either a heightened microbial activity with biomass production or to an accumulation of decomposed FLF material. Isotopic data might provide more insight on this later.

### 5.3 $\delta^{13}\text{C}$ Analysis

According to Dawson (2002) the atmospheric level of  $\delta^{13}\text{C}$  is about -8 ‰ while plant material or plant derived C shows  $\delta^{13}\text{C}$  values of about -26 ‰ to -27 ‰. This is due to the photosynthetic enzyme RuBisCo (abbreviated) in C3 plants, such as beeches for example, which discriminates against the <sup>13</sup>C isotope in. Root OM shows  $\delta^{13}\text{C}$  values of -26 ‰ and Soil OM, characterized by root exudates and litter inputs, shows  $\delta^{13}\text{C}$  values of -25 ‰ (Dawson, 2002).

The  $\delta^{13}\text{C}$  data for the bulk soil samples fits conveniently to this, representing that SOC is derived from plant material in the upper soil while with increasing depth the  $\delta^{13}\text{C}$  ratio shifts towards root derived C. The significant depletion of  $\delta^{13}\text{C}$  from 2009 to 2016 by about -1 ‰, which takes place in 0-5 cm, fits as well in the idea of photosynthetic fractionation and resembles plant material. On the other hand, it could also be explained with the Suess effect influencing photosynthesis, where atmospheric CO<sub>2</sub> becomes more depleted in  $\delta^{13}\text{C}$  with continued emissions CO<sub>2</sub> from combustion of fossil fuels which are naturally depleted in  $\delta^{13}\text{C}$  (Köhler, 2016).

While statistically not deemed significant and also still fitting to the numbers of Dawson (2002), the enrichment of  $\delta^{13}\text{C}$  in the deeper soil as well as the depletion in the top soil could eventually be explained otherwise. According to Werth (2010), heavy C tends to accumulate in soil trophic chains, meaning that depletion in top and enrichment in deep soil might be due to microbial or fungal activity where a fractionation takes place, lighter C is respired and  $\delta^{13}\text{C}$  thus is enriched in biomass and SOM.

None of the fractions of bulk soil has shown any significant changes from 2009 to 2016. Solely visible is the difference in  $\delta^{13}\text{C}$  of top and lower soil, especially in the HF with ~1 ‰. FLF and OLF resemble isotopic ratios of plant material.

## 5.4 $\Delta^{14}\text{C}$ Analysis

Atmospheric  $\text{CO}_2$  was enriched in  $^{14}\text{C}$  isotopes due to atomic bomb testing in the 1950s and onward. As  $^{14}\text{C}$  or radiocarbon decays and is diluted with  $^{14}\text{C}$  free  $\text{CO}_2$  due to combustion of fossil fuels (Suess effect), an annual decline of  $^{14}\text{C}$  in the atmosphere for about 8 ‰ takes place (Trumbore, 2000). C pools as in SOM receive constantly new C inputs from plants and lose C through decomposition, thus the  $\Delta^{14}\text{C}$  in a given organic matter reflects both the rate of decomposition and radioactive decay (Trumbore, 2009). The  $\Delta^{14}\text{C}$  values of the upper bulk soil of 0-20 cm fit with this annual decline with a decrease of ~56 ‰ from 2009 to 2016, meaning that the SOC in this depth is influenced by, in exchange with or produced by fixation of atmospheric  $\text{CO}_2$ . The positive  $\Delta^{14}\text{C}$  values of SOM in the top soil indicate a presence of bomb produced C while negative  $\Delta^{14}\text{C}$  values indicate that the SOM is older, relative to the  $\Delta^{14}\text{C}$  bomb peak, and not influenced by atmosphere. An atmospheric influence would have resulted in an increase of  $\Delta^{14}\text{C}$ . The low  $\Delta^{14}\text{C}$  values in the lower bulk soil from 20-50 cm exclude an atmospheric influence and indicate a high age of SOC while the massive depletion in  $\Delta^{14}\text{C}$  from 2009 to 2016 in 20-50 cm indicates a change in turnover times.

Regarding the fractions, FLF showed younger,  $\Delta^{14}\text{C}$  enriched SOM, whereas OLF and HF were older due to depleted  $\Delta^{14}\text{C}$  values with HF as the oldest and presumably most stable fraction. The enrichment of  $\Delta^{14}\text{C}$  in 10-20 cm in all fractions of 2009 suggests incorporation of post bomb C into these pools. Root growth and decomposition could be a possible explanation. According to Schmidt et al (2011) root derived C is retained much more efficiently than aboveground inputs of leaves. Furthermore, root and also mycorrhizal inputs have more opportunities for physicochemical interactions with soil particles. The transport of light fraction material via desiccation cracks or bioturbation is unlikely but Ahrens et al (2015) reported a rejuvenation (enrichment  $^{14}\text{C}$ ) of SOC by transport of DOC into subsoil, which could serve as explanation for the  $^{14}\text{C}$  enrichment of 2009.

The decline of  $\Delta^{14}\text{C}$  indicates a shortening turnover time of SOM, influencing especially the fractions of 10-20 cm depth. As SOC persistence is controlled by mineral surface stabilization and cyclic microbial assimilation and release (Ahrens et al, 2015), the change in  $\Delta^{14}\text{C}$  and thus the age of SOC and also SOM indicates a change in one of these domains.

Schmidt et al (2011) suggest fresh root inputs to have a priming effect on microbial activity, leading to heightened decomposition of older SOM. This could, with further research, prove as an explanation.

## 5.5 $\delta^{15}\text{N}$ Analysis

The interpretation of  $^{15}\text{N}$  isotope patterns is difficult since many processes fractionate and thus alter  $\delta^{15}\text{N}$  simultaneously and differently (Hobbie & Ouimette, 2009) and since SOM includes a mixture of matter derived from plants, microbial and faunal organic tissues from different pools (Dawson, 2002).

The  $\delta^{15}\text{N}$  measured in 2016, increased from  $\sim 0.7\text{‰}$  at surface to  $\sim 7\text{‰}$  at 20-40 cm, followed by a decline to  $\sim 6\text{‰}$  40-50 cm. Patterns from 2016 compared to 2004 were the same with slight but not significant enrichment of  $\delta^{15}\text{N}$  with depth by up to  $1\text{‰}$ . The HF  $\delta^{15}\text{N}$  showed the same behaviour as the bulk soil but was slightly more enriched with depth. Hobbie & Högberg (2012) report average bulk soil  $\delta^{15}\text{N}$  values between 2 to 6 ‰ and that  $\delta^{15}\text{N}$  increases with soil depth.

A possible explanation could be, according to Dawson (2002),  $\text{N}_2$  fixation from atmospheric N ranging from 0 to 6 ‰ but also mineralization of ammonium from SOM, which ranges from 0 to 5 ‰. Hobbie & Ouimette (2009) lead mycorrhizal fungi as a possible source for enrichment with  $\sim 9\text{‰}$  for ectomycorrhizal (ECM) and  $\sim 4\text{‰}$  for arbuscular mycorrhiza (AM). If so, ECM would seem to contribute more to soil N than AM, due to a broader network of hyphae (Coleman, 2004). Without further research, it is but impossible to determine if mycorrhiza actually contribute to soil N in this case.

The depletion of  $\delta^{15}\text{N}$  in 40-50 cm depth could be explained with leachate assimilation from root or aboveground inputs whereas the latter would require pathways for transport, for example, bioturbation (Hobbie & Ouimette, 2009).

## 5.6 Stability of Soil Organic Matter

As we could determine changes in OC Stocks and  $^{14}\text{C}$  of fractions as well as changes of  $^{14}\text{C}$  in bulk soil, it remains unclear from where these changes originate.

Schmidt et al (2011) reported pulsed wetting and drying events to lead to respiration of relatively old SOM, indicating that old SOM might not be as inert as thought regarding its association with mineral surfaces. OC or SOM as well as N adsorb to mineral surfaces as clay rich minerals, organo minerals and aluminium and iron rich minerals (Kögel-Knabner et al, 2008; Dwivedi et al, 2017). A disturbance of these bonds might release fixated and immobile C and N, making them available for respiration and biomass. For both C and N there are different pools, labile and stabile, resulting in different availability and thus in varying residence times, controlled by mineral surface stabilization and cyclic microbial assimilation and release (Ahrens et al, 2015). Said residence time increases with soil depth

and is only partially influenced by the complexity of OC but more by factors as chemical composition of the substrate, texture, mineralogy, temperature, pH, soil aggregates and hydrologic transport (Dwivedi et al, 2017). Thus, persistence of SOC is a property of the respective ecosystem (Schmidt et al, 2011).

## 6 Conclusions

With this study, we observed changes in carbon and nitrogen content and stocks of bulk soil as well as fractions from 2009 to 2016. Several of these changes have proven to be of statistical significance.  $\Delta^{14}\text{C}$  isotopic analysis of bulk soil and fractions indicated shortened turnover times for SOM in fractions and a massive alteration of bulk soil  $\Delta^{14}\text{C}$  age. This has proven that SOC and SOM in lower soil depths are more dynamic than assumed. It remains unknown though, what caused these changes. To refine the estimated role of soils and the development of their contribution to carbon and nitrogen sequestration and storage, further investigation is necessary. A continuation of soil inventories and SOC monitoring is recommended to figure out if the observed changes are of unique nature or indicate a trend.

Also necessary is, to gain further insight into which processes lead to the results at hand. Conceivable for this are incubation or priming experiments of the soil samples at hand to inquire if and how soil microbial community reacts to climate induced ecosystem changes. Another option is the analysis of PLFA combined with stable isotopes to determine which microbes are metabolically active in soil. To improve the results at hand, measuring  $\delta^{15}\text{N}$  of the fractions as well as evaluation of nitrogen stocks of fractions and bulk soil are useful options.

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## 8 Appendix

### 8.1 OC Bulk Soil [g/kg]

**Table 1: OC Bulk Soil [g/kg]**

Depth [cm]	OC 2016	SE OC 2016	OC 2009	SE OC 2009	OC 2004	SE OC 2004
0-5	60,39	5,05	63,41	4,27	63,99	5,28
5-10	35,64	3,07	37,85	3,25	41,97	4,51
10-20	22,79	1,21	25,10	1,88	23,88	1,95
20-30	14,93	0,89	13,64	1,23	15,80	0,65
30-40	10,02	0,96	9,41	1,55	10,54	0,84
40-50	7,53	1,19	7,79	1,44	7,91	1,12

### 8.2 TN [g/kg]

**Table 2: TN of Bulk Soil [g/kg]**

Depth [cm]	TN 2016	SE TN 2016	TN 2009	SE TN 2009	TN 2004	SE TN 2004
0-5	4,77	0,35	4,71	0,30	4,78	0,31
5-10	3,16	0,22	3,26	0,24	3,43	0,31
10-20	2,18	0,09	2,34	0,13	2,22	0,16
20-30	1,54	0,08	1,42	0,11	1,64	0,06
30-40	1,17	0,09	1,05	0,14	1,15	0,07
40-50	0,90	0,10	0,87	0,12	0,89	0,10

### 8.3 C/N

**Table 3: C/N Ratio of Bulk Soil**

Depth [cm]	CN 2016	SE CN 2016	CN 2009	SE CN 2009	CN 2004	SE CN 2004
0-5	12,60	0,20	13,42	0,18	13,30	0,37
5-10	11,19	0,23	11,54	0,16	12,13	0,31
10-20	10,43	0,19	10,66	0,20	10,70	0,23
20-30	9,68	0,18	9,51	0,19	9,65	0,16
30-40	8,47	0,23	8,68	0,30	9,12	0,21
40-50	8,11	0,30	8,50	0,41	8,66	0,32

## 8.4 $\delta^{13}\text{C}$ [‰]

**Table 4:  $\delta^{13}\text{C}$  of Bulk Soil [‰]**

Depth [cm]	$\delta^{13}\text{C}$ Mean	$\delta^{13}\text{C}$ StD	$\delta^{13}\text{C}$ SE	$\delta^{13}\text{C}$ Mean	$\delta^{13}\text{C}$ StD	$\delta^{13}\text{C}$ SE
	2016	2016	2016	2004	2004	2004
0-5	-27,18	0,26	0,08	-26,69	0,32	0,11
5-10	-26,21	0,36	0,11	-31,74	17,06	5,69
10-20	-25,80	0,22	0,07	-25,80	0,11	0,03
20-30	-25,49	0,22	0,07	-25,60	0,31	0,10
30-40	-25,27	0,32	0,10	-25,37	0,28	0,09
40-50	-25,15	0,35	0,11	-25,42	0,37	0,13

**Table 5:  $\delta^{13}\text{C}$  of Fractions [‰]**

Depth [cm]	$\delta^{13}\text{C}$ Mean			$\delta^{13}\text{C}$ SE			Year
	FLF	OLF	HF	FLF	OLF	HF	
0-5	-27,95	-28,31	-26,91	0,32	0,11	0,08	2004
0-5	-28,34	-28,38	-27,13	0,16	0,13	0,12	2016
10-20	-27,74	-27,75	-25,79	0,24	0,09	0,08	2004
10-20	-27,91	-27,78	-25,77	0,14	0,09	0,1	2016

## 8.5 $\Delta^{14}\text{C}$ [‰]

**Table 6:  $\Delta^{14}\text{C}$  of Bulk Soil [‰]**

Depth	$\Delta^{14}\text{C}$	$\Delta^{14}\text{C}$ 2004	$\Delta^{14}\text{C}$ 2009	$\Delta^{14}\text{C}$ 2009	$\Delta^{14}\text{C}$ 2016	$\Delta^{14}\text{C}$ 2016
	2004Mean	SE	Mean	SE	Mean	SE
0-5	85,84	11,54	63,79	10,53	29,04	4,82
5-10	28,16	9,73	23,04	12,86	-13,32	8,47
10-20	-32,60	16,05	-22,77	10,34	-50,50	6,52
20-30	-111,23	15,73	-114,16	15,17	-203,26	16,67
30-40	-165,06	14,53	-227,51	18,28	-248,82	9,58
40-50	-200,96	12,55	-227,75	18,93	-299,96	17,80

**Table 7:  $\Delta^{14}\text{C}$  of Fractions [‰]**

Depth [cm]	$\Delta^{14}\text{C}$ Mean	$\Delta^{14}\text{C}$ StD	$\Delta^{14}\text{C}$ SE	Year
FLF 0-5	128,17	34,12	12,06	2004
FLF 0-5	94,56	20,26	7,16	2009
FLF 0-5	61,41	22,9	7,24	2016
OLF 0-5	105,21	36,64	13,85	2004
OLF 0-5	83,98	30,34	10,73	2009
OLF 0-5	43,52	15,21	4,81	2016
HF 0-5	79,22	22,12	7,37	2004
HF 0-5	62,71	27,13	9,59	2009
HF 0-5	6,95	20,23	6,4	2016
FLF 10-20	60,32	69,09	30,9	2004
FLF 10-20	98,08	33,74	11,93	2009
FLF 10-20	63,66	97,57	30,85	2016
OLF 10-20	-28,07	44,43	16,79	2004
OLF 10-20	6,68	41,96	18,77	2009
OLF 10-20	-84,57	46,39	14,67	2016
HF 10-20	-54,13	35,29	11,76	2004
HF 10-20	-31,65	27,76	9,82	2009
HF 10-20	-72,99	18,23	5,76	2016

## 8.6 $\delta^{15}\text{N}$ [‰]

**Table 8:  $\delta^{15}\text{N}$  of Bulk Soil [‰]**

Depth [cm]	$\delta^{15}\text{N}$ 2016			$\delta^{15}\text{N}$ 2004		
	Mean [‰]	StD [‰]	SE [‰]	Mean [‰]	StD [‰]	SE [‰]
0-5	0,67	0,80	0,25	0,45	0,70	0,25
5-10	2,93	0,65	0,20	2,57	0,97	0,34
10-20	4,83	0,57	0,18	4,35	1,61	0,57
20-30	6,98	0,55	0,18	6,38	2,38	0,84
30-40	6,98	0,85	0,27	6,35	2,26	0,80
40-50	6,01	0,92	0,29	5,10	1,79	0,63
MOM 0-5	0,90	0,75	0,24			
MOM 10-20	5,22	0,53	0,17			

## 8.7 OC Stocks

**Table 9: OC Stocks of Fractions [kg/m<sup>2</sup>]**

Depth [cm]	FLF Mean	OLF Mean	HF Mean	FLF SE	OLF SE	HF SE	Year
0-5	0,14	0,06	2,1	0,04	0,02	0,66	2016
0-5	0,25	0,07	2,05	0,08	0,02	0,65	2009
0-5	0,19	0,16	2,21	0,06	0,05	0,74	2004
10-20	0,04	0,11	2,43	0,01	0,04	0,77	2016
10-20	0,09	0,05	2,23	0,03	0,01	0,71	2009
10-20	0,05	0,13	2,62	0,02	0,04	0,83	2004

**Table 10: OC Stocks of Bulk Soil [kg/m<sup>2</sup>]**

Depth [cm]	2016 Mean	2009 Mean	2004 Mean	2016 SE	2009 SE	2004 SE
0-5	2,30	2,43	2,57	0,13	0,11	0,16
5-10	1,79	1,70	2,00	0,09	0,11	0,13
10-20	2,59	2,65	2,81	0,13	0,08	0,15
20-30	1,79	1,60	2,06	0,11	0,19	0,07
30-40	1,13	1,19	1,43	0,13	0,2	0,12
40-50	0,79	0,95	0,93	0,17	0,19	0,17