agronomy

MDPI

Article

Carbon Isotope Measurements to Determine the Turnover of Soil Organic Matter Fractions in a Temperate Forest Soil

Dóra Zacháry [1,2,*], Tibor Filep [1,3], Gergely Jakab [1,4], Mihály Molnár [2], Titanilla Kertész [2], Csilla Király [1], István Hegyi [5], Lilla Gáspár [1] and Zoltán Szalai [1,6,*]

1 Geographical Institute, Research Centre for Astronomy and Earth Sciences, Budaörsi út 45, H-1112 Budapest, Hungary; filep.tibor@csfk.mta.hu (T.F.); jakab.gergely@csfk.mta.hu (G.J.); kiraly.csilla@csfk.mta.hu (C.K.); gsprlilla@gmx.com (L.G.)
2 Hertelendi Laboratory of Environmental Studies, Institute for Nuclear Research, Bem tér 18/c, H-4026 Debrecen, Hungary; molnar.mihaly@atomki.mta.hu (M.M.); kertesz.titanilla@atomki.mta.hu (T.K.)
3 Department of Meteorology, Eötvös Loránd University, Pázmány Péter sétány 1/A, H-1117 Budapest, Hungary
4 Institute of Geography and Geoinformatics, University of Miskolc, Building A4, Miskolc-Egyetemváros, H-3515 Miskolc, Hungary
5 Institute for Geological and Geochemical Research, Research Centre for Astronomy and Earth Sciences, Budaörsi út 45, H-1112 Budapest, Hungary; hegyi.istvan@csfk.mta.hu
6 Department of Environmental and Landscape Geography, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary
* Correspondence: zachary.dora@csfk.mta.hu (D.Z.); szalai.zoltan@csfk.mta.hu (Z.S.)

Received: 21 November 2020; Accepted: 8 December 2020; Published: 10 December 2020

Abstract: Soil organic matter (SOM) is a combination of materials having different origin and with different stabilization and decomposition processes. To determine the different SOM pools and their turnover rates, a silt loam-textured Luvisol from West Hungary was taken from the 0–20 cm soil depth and incubated for 163 days. Maize residues were added to the soil in order to obtain natural ^{13}C enrichment. Four different SOM fractions—particulate organic matter (POM), sand and stable aggregate (S + A), silt- plus clay-sized (s + c) and chemically resistant soil organic carbon (rSOC) fractions—were separated and analyzed using FT-IR, $\delta^{13}C$, and ^{14}C measurements. The mean residence time (MRT) of the new C and the proportion of maize-derived C in the fractions were calculated. The POM fraction was found to be the most labile C pool, as shown by the easily decomposable chemical structures (e.g., aliphatic, O-alkyl, and polysaccharides), the highest proportion (11.7 ± 2.5%) of maize-derived C, and an MRT of 3.6 years. The results revealed that the most stable fraction was the rSOC fraction which had the smallest proportion of maize-derived C (0.18 ± 2.5%) and the highest MRT (250 years), while it was the only fraction with a negative value of $\Delta^{14}C$ (−75.0 ± 2.4‰). Overall, the study confirmed the hypothesis that the SOM associated with finer-sized soil particles decomposes the least, highlighting the significance of the fractionation process for more accurate determination of the decomposition processes of SOM pools.

Keywords: carbon stabilization; ^{13}C labeling; fractionation; FT-IR spectroscopy; radiocarbon

1. Introduction

Soil organic matter (SOM) plays an important role in the functioning of land ecosystems. It regulates many physical, chemical, and biological functions and processes in the soil. It maintains the soil structure and porosity by aggregate formation and stabilization, increasing the water and air supply

and retention in soils, and thereby enhancing the biological activity in soil environments. At the same time SOM provides a habitat and nutrient source for microorganisms. It also enhances the buffer capacity of the soil over a wide range of pH values due to the wide variety of functional groups and organic compounds (such as carboxylic, hydroxy, amine, and amide) in SOM [1].

Litter and root deposition are the two natural sources of C in the soil. Once C enters into the soil, it decomposes or stabilizes in different ways at different rates. The turnover of SOM, and thus its stabilization, is determined by (i) the variable chemical structure of SOM components and (ii) the physical protection against microbial attacks provided by aggregation and/or mineral bonding (by clay minerals and pedogenic oxides) [2]. As a consequence, the various materials making up SOM have different levels of resistance to microbial degradation. The residence time (the time the C atom spends in the soil system) of root exudates is a few hours, whereas plant residues decompose within a few weeks or months, while certain organic compounds may remain untouched for even ten thousand years [3–5].

The physical fractionation of soils allows the separation of these chemically and physically diverse pools. Soil fractionation results in SOM pools with different size, density, molecular, and aggregate composition, thus having various physical and chemical properties to stabilize C [6]. In general, sand-associated SOM (20 µm–2 mm) represents the active pool, whereas the silt and clay-sized fractions typifies the relatively stable C domains [7–9], though, several studies have produced contradictory results [10–12]. For example, Leifeld and Fuhrer [13] found that the mean residence time (MRT) of the S + A (sand and stable aggregate) fraction was more than 100 years, whereas the MRT of the POM (particulate organic matter) fraction was 5−7 years in meadow and pasture topsoils. In contrast, Dondini et al. [14] reported a smaller proportion of fresh carbon in the POM fraction than in the S + A fraction.

A better understanding of the properties and dynamics of SOM decomposition and sequestration is critical for predicting ecosystem responses to global change. The MRTs of the SOM fractions derived from fractionation are not just important parameters of soil ecosystem functioning, but are also significant input parameters for coupled climate-carbon models (e.g., Earth System Models), with which the size of terrestrial carbon sinks can be predicted more precisely [15,16]. In addition, the appropriate SOM management is crucial for agroecosystem health due to the above mentioned positive effects of SOM (e.g., water retention, nutrient supply, and aggregation). Nevertheless, as pointed out by Lehmann and Kleber [17] the investigation of SOM turnover is more important to gain the fertility of the soils then just to increase the number of passive carbon stocks.

The aim of this study was to quantify the stability of different SOM fractions based on the turnover characteristics derived from the ^{13}C natural abundance and ^{14}C methods. The C/N ratio, aromaticity, $\delta^{13}C$ value, proportion of new maize-derived C, proportion of bomb-derived ^{14}C, and MRT of the SOM fractions were used to test the hypothesis that the clay-sized (s + c) and the chemically resistant soil organic carbon (rSOC) fractions are the most stabile fractions. In addition, FT-IR spectroscopy was applied to characterize differences in the chemical structure of the organic matter in the separated soil fractions.

2. Materials and Methods

2.1. Sampling and Laboratory Measurements Before Incubation

A soil sample was taken from a Haplic Luvisol [18] with loamy soil texture (of the A horizon) (upper 0−20 cm) in the Sopron Mountains, Hungary (N 47°40′14.6″, E 16°33′53.6″) in November 2015 with a spade from a 0.5 × 0.5 m^2 area. The vegetation cover at the study site is dominated by deciduous, mainly beech (*Fagus sylvatica*) forest. The mean annual temperature in the area is 9.2 °C and the annual precipitation is 700–750 mm.

In the laboratory, the soil sample was air-dried, homogenized, passed through a 2-mm sieve, and stored at room temperature.

The soil texture was determined with the sieve-pipette method [19] and was classified as silt loam (with 29% sand, 54% silt, and 17% clay content). The pH was 4.31 in distilled water and 3.27 in 1 M KCl solution.

2.2. Incubation Experiment

Sieved, air-dried soil samples weighing 200 g were placed in 1 L Duran® glass bottles. After preincubation at 50% field capacity at 20 °C for two weeks, the soil was amended with maize residues ($\delta^{13}C = -13.33 \pm 0.04‰$). One gram of the air-dried, cut into pieces, and sieved (2-mm) maize residues was thoroughly mixed with 200 g preincubated soil. There were three replicates of amended soil and one subsample for control with no residue addition. The samples were kept in an incubator (KBW400 E5.1, Binder, Tuttlingen, Germany) at 20 °C for 163 days at 70% field capacity, as this moisture content allowed the handling and compaction of the soil without the collapse of small soil aggregates [20].

2.3. Soil Fractionation

After incubation, 30 g was weighted from the amended, control, and native (without incubation and maize addition) samples and fractionated following the recommendations of Zimmermann et al. [21]. Figure 1 shows the steps of the fractionation procedure applied. The 30 g sieved soil samples were added to 150 mL distilled water and dispersed in an ultrasonic shaker with 22 J mL^{-1} energy for 10 s. After dispersion the suspension was wet-sieved over a 63-µm sieve until the rinsing water was clear. Of the two fractions separated by wet sieving, the >63 µm portion was dried at 40 °C and separated by NaI at a density of 1.6 g cm^{-3}. The centrifugation (at 2275 rpm for 15 min) of the suspension resulted in a light fraction represented the POM fraction (with fresh plant residues having visible plant structure), whereas the heavy fraction contained the S + A (sand and stable aggregate) fraction. The POM and S + A parts were cleaned with distilled water to remove the NaI, dried, weighed, and subjected to further analysis.

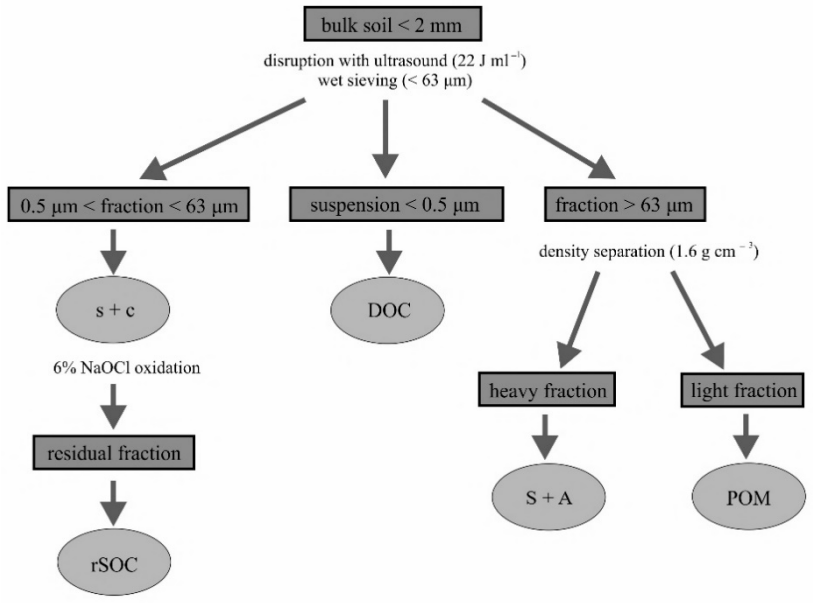

Figure 1. Schematic diagram of the fractionation procedure applied after Zimmermann et al. [21]. POM = particulate organic matter; S + A = sand and stable aggregate fraction; DOC = dissolved organic carbon; s + c = silt- plus clay-sized fraction; rSOC = chemically resistant SOC fraction.

A 0.5-µm aperture glass filter was used to filter the <63 µm fraction. The fraction retained on the filter representing the s + c (silt- and clay-associated) fraction was dried at 40 °C and weighted. The filtered suspension, which represents the dissolved organic carbon (DOC), was frozen for use

in other measurements, which are not included in this paper. One gram of the s + c fraction was added to 50 mL of 6% NaOCl and kept undisturbed for 18 h at 25 °C to separate the rSOC (chemically resistant soil organic carbon) fraction from the s + c fraction. The oxidation residue was centrifuged at 2275 rpm for 15 min, decanted, washed with distilled water and centrifuged. The oxidation step was repeated twice and the residue was dried to obtain the rSOC fraction. The s + c and rSOC fractions were subjected to further analysis.

2.4. Total C, N, and $\delta^{13}C$ Measurements

The SOM fractions were analyzed with a Flash 2000 Elemental Analyzer (Thermo Scientific, Rhodano, Italy) using an internal TCD detector to determine the elemental percentages of C and N. The SOM fractions were also analyzed with the Flash 2000 Organic Elemental Analyzer (Thermo Scientific, Rhodano, Italy) coupled to a continuous flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Finnigan, Bremen, Germany) to measure the stable carbon isotopes. The stable carbon isotope data are presented using the $\delta^{13}C$ notation [22], where differences in the $^{13}C/^{12}C$ isotope ratio are expressed as per mil (‰) relative to the Vienna-PDB international standard. The IAEA-CH-7 ($\delta^{13}C = -32.51$‰) and IAEA-CH-6 ($\delta^{13}C = -10.45$‰) reference standards [23] were used for calibration. Samples were measured in three replicates.

The proportion of new added carbon (maize residues) to the total C content was calculated using $\delta^{13}C$ values in a mixing model for the separated SOM fractions as follows [24]:

$$F = (\delta_{AB} - \delta_A)/\delta_B - \delta_A), \quad (1)$$

where F is the proportion of C derived from maize residues, δ_A and δ_{AB} is the $\delta^{13}C$ value of the control and maize-amended soils, respectively, and δ_B is the $\delta^{13}C$ value of maize residues.

The mean residence time *(MRT)* of C in the separated fractions was calculated from F as follows [25]:

$$MRT = -t/\ln(1 - F), \quad (2)$$

where t is the incubation time.

2.5. ^{14}C Measurements

^{14}C analysis was carried out on the native samples (bulk soil and SOM fractions) before incubation. Since the pH of the bulk soil was 4.31 (distilled water) and 3.27 (KCl solution), no other carbonate removal was applied, but the pH of the samples was adjusted to 3 with 4% HCl prior to analysis. A two-step combustion method was applied for the bulk soil sample: first, combustion at low temperature (at 400 °C for 40 min, "L" fraction), followed by high temperature combustion on the same sample (at 800 °C for 20 min, "H" fraction) in the presence of high-purity oxygen gas in a quartz tube [26,27]. Because of the low organic content of the sample (2.97%), 1–2 g of sample had to be combusted in the on-line combustion system to provide at least 0.2 mg C/sample, allowing the production of graphite targets and normal accelerator mass spectrometry (AMS) analysis. The resulting CO_2 gas was then collected and purified separately to form "L" and "H" fractions using an on-line combustion system line and then converted to graphite using the sealed tube Zn-graphitization method [28]. IAEA–C9 (fossil wood) standards were treated and measured parallel to the sample to check the quality of sample preparation.

The separated SOM fractions were dried at 50 °C. Then, CO_2 was generated from the samples by combustion at 550 °C for at least 12 h in the presence of MnO_2 [29]. A specially designed vacuum line was used for the extraction and purification of the resulting CO_2 gas. The pure CO_2 gas was sealed by flame in glass tubes (with Zn and TiH catalysts and iron powder) and graphitized [28]. Finally, the graphitized samples were pressed into aluminum targets and subjected to EnvironMicadas AMS analysis to measure the carbon isotopic composition of the samples [30]. The "Bats" data reduction

software was used [31] for data evaluation, and percent Modern Carbon (pMC) units were used in the calculations [32]. The radiocarbon data were expressed in $\Delta^{14}C$ units (‰) according to Stuiver and Polach [33]:

$$\Delta^{14}C = (A_{SN}/A_{ABS} - 1) \times 1000, \quad (3)$$

where A_{SN} is the activity of ^{14}C in the sample, normalized for $\delta^{13}C$ isotopic fractionation and decay-corrected between 1950 and the time of analysis (2018) and A_{ABS} is the absolute international standard activity of oxalic acid.

2.6. FT-IR Measurements

All the fractionated samples were powdered and dried at 40 °C directly before FT-IR measurement to remove the absorbed water which can significantly affect the vibration of OH groups and H_2O molecules [34]. The FT-IR measurements were carried out on a Bruker Vertex 70 spectrometer with an RT-DLaTGS detector. For each sample a spectral range of 4000–400 cm^{-1}, a resolution of 4 cm^{-1}, 16 scans, and three replicates were recorded. The spectra were corrected (atmospheric water and CO2 correction), normalized, subjected to 17-point smoothing, and baseline corrected.

For the detection of relative changes in the spectra and spectral comparison, an aromaticity index (I_{AR}) and relative absorbances (rA) were calculated. The aromaticity index derived from Inbar et al. [35] was calculated by dividing the intensity of absorption at 1640 cm^{-1} (aromatic C = C) by the intensity of absorption at 2920 cm^{-1} (aliphatic C–H) as follows:

$$I_{AR} = A_{1640\ cm^{-1}}/A_{2920\ cm^{-1}}. \quad (4)$$

Relative absorbances [36–38] were calculated by dividing the peak height of each of the peak of the six peaks (2920, 2850, 1730, 1640, 1515, or 1420 cm^{-1}) by the sum of the absorbances of all six peaks and multiplying it by 100. For example:

$$rA_{1420} = (A_{1420}/\sum A_{2920\text{-}1420\ cm^{-1}}) \times 100. \quad (5)$$

2.7. Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics 22.0 (Armonk, NY, USA). One-way analysis of variance (ANOVA) with post hoc Tukey's HSD test was used to evaluate differences among the treatment categories—(i) native soil without incubation and maize addition; (ii) maize-amended soil with incubation; and (iii) control soil with incubation but without maize residue addition—within the SOM fractions (POM, S + A, s + c, and rSOC).

3. Results and Discussion

3.1. Total C and N Concentration in the Soil Fractions

The total C and N concentrations in the different fractions are presented in Table 1, which shows that the POM fraction had the highest and the rSOC fraction the smallest C and N concentrations in the native, amended, and control samples. The S + A and s + c fractions had similar C and N values in all the samples with concentrations between those of the POM and rSOC fractions.

Incubation and maize addition had the least influence on the total C and N concentrations of the POM fraction, whereas the other SOM fractions had significant total C and N concentration differences ($p < 0.05$) between the native and the incubated samples (Table 1). The decrease in the C and N concentrations of POM in the amended sample compared to the control sample (Table 1, $p < 0.05$) can be attributed to the increased mineralization of C caused by the maize residues [39–41].

The fact that C/N ratios decreased from the POM to the s + c fraction (Table 1) is consistent with the literature [42–44] showing that the C/N ratio decreased as the density of the fractions increased. The C/N ratio was found to be the widest in the rSOC fraction in the present study. Leifeld and

Fuhrer [13] also recorded the widest C/N ratios (17.5–22.2) for the rSOC fractions at almost all soil depths of two soils, which they explained by the presence of black carbon in the samples. Similar to black carbon, the rSOC fraction consists high carbon content organic compounds most resistant to oxidation, whereas N-containing carbohydrates and amino acids decompose most rapidly during NaOCl oxidation [45], resulting in a C enrichment and a N depletion of this fraction.

Table 1. Total C and N content and C/N ratio of the different soil fractions in the Luvisol studied.

Fraction [1]	Treatment [2]	TOC (g kg^{-1})	TN (g kg^{-1})	C/N Ratio
POM	Native	245 ± 25.4 [3] a [4]	9.84 ± 1.28 ab	25.2 ± 1.31 a
	Amended	229 ± 16.4 a	8.02 ± 0.552 a	28.5 ± 0.43 b
	Control	341 ± 3.39 b	11.9 ± 0.099 b	28.7 ± 0.21 b
S + A	Native	21.6 ± 0.13 b	1.53 ± 0.009 b	14.1 ± 0.03 a
	Amended	17.5 ± 0.36 a	1.12 ± 0.023 a	15.7 ± 0.05 c
	Control	17.6 ± 0.11 a	1.15 ± 0.006 a	15.3 ± 0.11 b
s + c	Native	23.0 ± 0.18 c	1.69 ± 0.002 c	13.6 ± 0.10 a
	Amended	19.4 ± 0.16 a	1.44 ± 0.011 a	13.5 ± 0.05 a
	Control	20.3 ± 0.03 b	1.49 ± 0.008 b	13.7 ± 0.06 a
rSOC	Native	7.72 ± 0.07 c	0.21 ± 0.001 b	36.5 ± 0.49 b
	Amended	5.05 ± 0.17 a	0.17 ± 0.004 a	30.2 ± 0.65 a
	Control	6.56 ± 0.09 b	0.20 ± 0.006 b	33.2 ± 1.43 a
Bulk recovery (mass %)	Native	80.5 ± 0.39 a	113.6 ± 0.33 b	
	Amended	134.3 ± 8.14 b	101.6 ± 3.28 a	
	Control	93.9 ± 0.20 a	98.0 ± 0.31 a	

[1] POM = particulate organic matter fraction; S + A = sand plus stable aggregate fraction, s + c = silt plus clay fraction; rSOC = chemically resistant soil organic carbon fraction. [2] Native = native soil without incubation and maize addition; amended = maize-amended soil with incubation; control = control soil with incubation but without maize residue addition. [3] Mean ± standard error. [4] Means with different letters indicate significant differences (ANOVA, $p < 0.05$) among the treatment categories within an SOM fraction.

3.2. Quality Assessment of SOM Fractions Using FT-IR Spectroscopy

The FT-IR absorbance of the soil fractions (Figures S1–S3) was evaluated by establishing the ratios between the main absorbance peaks of the spectra and calculating relative absorbance values (Table 2). The bands at 2920 and 2850 cm^{-1}, associated with aliphatic C−H stretching [36,46], had the highest relative absorbance in the POM fraction and the lowest in the s + c and rSOC fractions in all the soils.

The POM fraction also had by far the highest relative absorbance for the carboxylic substituents of aromatic esters in the band at 1730 cm^{-1} [46] in all the soil samples regardless of the treatment (Table 2), indicating a high proportion of aromatic compounds. The relative absorbance of the band at 1640 cm^{-1}, characteristic of aromatic C and carboxylic acid [47], was also high in the POM fraction. The relative absorbance of the band at around 1515 cm^{-1}, which is assigned to aromatic C = C vibrations [46], was found to be the highest in the POM fraction and the lowest in the rSOC fraction in the native soil spectrum (Table 2). The high proportion of aromatic compounds can be attributed to the fact that the POM sample is composed of relatively undecomposed or partly degraded plant residues that contain both aliphatic and aromatic (lignin-derived) structures.

The levels of carboxyl and aromatic carbon was found to be low in the NaOCl-resistant rSOC fraction at 1660–1620 cm^{-1} [45], in agreement with our results (Table 2). As shown in Table 2, there were significant differences ($p < 0.05$) between the incubated samples and the native sample in all SOM fractions (except for the rSOC fraction) in the relative absorbance at 1420 cm^{-1}, which can be attributed to the aromatic ring stretching of COO^- [35]. The rA_{1420} of the rSOC fraction showed high values and no difference ($p < 0.05$) between the treatments, which can be attributed to the fact that NaOCl has high pH (~10), which may cause the deprotonation of most of the carboxylic groups in organic matter in every treatment.

Table 2. Relative absorbance as a % of the sum of all selected peak heights, and aromaticity index (I_{AR}) derived from the FT-IR spectra of the soil fractions in the Luvisol studied.

Fraction [1]	Treatment	rA_{2920}	rA_{2850}	rA_{1730}	rA_{1640}	rA_{1515}	rA_{1420}	I_{AR}
POM	Native	12.7 ± 0.89 [2] a [3]	8.7 ± 0.62 a	24.7 ± 0.87 ab	23.1 ± 0.33 b	11.2 ± 0.93 b	10.9 ± 1.6 b	1.9 ± 0.15 b
	Amended	13.6 ± 0.15 a	8.8 ± 0.07 a	24.1 ± 0.54 a	23.5 ± 0.22 b	7.0 ± 0.30 a	6.8 ± 0.41 a	1.7 ± 0.02 b
	Control	17.8 ± 0.18 b	12.6 ± 0.21 b	26.4 ± 0.33 b	20.7 ± 0.36 a	8.5 ± 0.18 a	5.4 ± 0.51 a	1.2 ± 0.03 a
S + A	Native	10.8 ± 0.11 b	6.7 ± 0.09 b	7.1 ± 0.31 a	21.6 ± 0.22 a	5.7 ± 0.30 a	12.7 ± 0.57 a	2.0 ± 0.01 a
	Amended	8.9 ± 0.37 a	5.5 ± 0.26 a	6.5 ± 0.31 a	22.2 ± 0.22 ab	8.5 ± 0.70 ab	19.8 ± 1.6 b	2.5 ± 0.10 b
	Control	8.1 ± 0.40 a	4.9 ± 0.24 a	6.2 ± 0.32 a	23.5 ± 0.72 b	9.0 ± 1.2 b	20.7 ± 1.7 b	2.9 ± 0.14 c
s + c	Native	7.9 ± 0.19 ab	4.5 ± 0.13 ab	7.0 ± 0.18 a	24.7 ± 0.29 a	7.5 ± 0.74 b	20.7 ± 1.3 b	3.1 ± 0.08 a
	Amended	8.4 ± 0.23 b	5.0 ± 0.16 b	6.4 ± 0.19 a	24.4 ± 0.48 a	5.1 ± 0.29 a	13.0 ± 0.78 a	2.9 ± 0.12 a
	Control	7.4 ± 0.29 a	4.4 ± 0.18 a	6.9 ± 0.26 a	24.5 ± 0.67 a	5.4 ± 0.43 a	15.5 ± 1.5 a	3.3 ± 0.16 a
rSOC	Native	8.3 ± 0.21 b	5.3 ± 0.13 b	4.0 ± 0.40 c	18.0 ± 0.28 a	11.3 ± 0.37 a	21.9 ± 1.0 a	2.2 ± 0.03 a
	Amended	3.5 ± 0.14 a	2.5 ± 0.09 a	2.1 ± 0.13 a	20.0 ± 0.74 a	15.3 ± 0.37 b	18.7 ± 2.1 a	5.8 ± 0.30 b
	Control	7.4 ± 0.52 b	4.9 ± 0.36 b	3.2 ± 0.16 b	20.2 ± 0.75 a	11.1 ± 0.49 a	18.2 ± 1.9 a	2.8 ± 0.24 a

[1] POM = particulate organic matter fraction; S + A = sand plus stable aggregate fraction, s + c = silt plus clay fraction; rSOC = chemically resistant soil organic carbon fraction. [2] Mean ± standard error. [3] Means with different letters indicate significant differences (ANOVA, $p < 0.05$) among the treatment categories within an SOM fraction.

The aromaticity index of the soil fractions differed among the fractions (Table 2). The POM fractions had the lowest aromaticities, while the s + c and rSOC fractions had high values. Baldock et al. [48] also found that the content of aromatic carbon was highest in the intermediate-sized fractions (2–53 µm). Incubation had a great effect on the aromaticity of the soil fractions: the aromaticity of rSOC increased ($p < 0.05$) during incubation in the amended soil, probably due to the enhanced biological activity, which may have caused a decrease in aliphatic compounds.

3.3. Evaluation of C Isotope Changes in SOM Fractions and Turnover Time of the SOM Fractions

Based on the results of stable C isotope measurements, it can be seen that the $\delta^{13}C$ values of the S + A fraction (−26.32 ± 0.04‰ for the native soil, −26.48 ± 0.03‰ for the control soil, and −25.98 ± 0.09‰ for the amended soil) were closest to that of the unfractionated bulk soil (−26.28 ± 0.06‰ for the native soil, −26.50 ± 0.09‰ for the control soil, and −25.94 ± 0.08‰ for the amended soil) in all the soils (Figure 2). The s + c fraction was slightly enriched in ^{13}C compared to the bulk soil in all the soils. In contrast, ^{13}C was depleted in the POM and rSOC fractions compared to the bulk soil, regardless of incubation or maize addition. The negative $\delta^{13}C$ values of the POM fractions in the native (−27.47 ± 0.01‰) and control (−27.76 ± 0.04‰) soils could be attributed to the fact that the organic matter of fresh plant residues entering the soil under forest vegetation is depleted in ^{13}C [49]. Since *Fagus sylvatica* is the main vegetation cover in the study site, the C isotopic signature of this tree species may be reflected in the POM fraction. Keitel et al. [50] reported that the $\delta^{13}C$ values of the total leaf organic matter of *Fagus sylvatica* varied from −26.5 to −32‰.

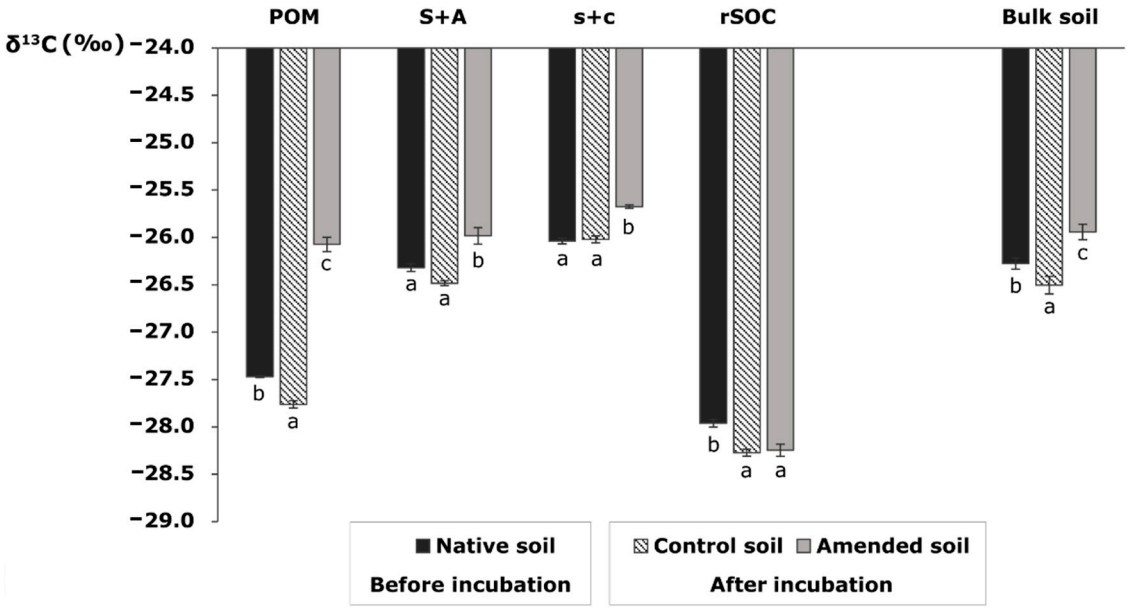

Figure 2. $\delta^{13}C$ values of the bulk forest soil and of the soil fractions (POM = particulate organic matter; S + A = sand and stable aggregate fraction; s +c = silt plus clay-sized fraction; rSOC = chemically resistant SOC fraction) separated from the native soil before incubation and from the control and maize-amended samples after the 163-day incubation. Line bars indicate standard error. Different letters indicate significant differences (ANOVA, $p < 0.05$) among the treatment categories within an SOM fraction.

In general, $\delta^{13}C$ values increase with an increasing degree of mineral–organic association in soil fractions [43,51–53]. This trend could be detected for the POM, S + A, and s + c fractions of native, control, and amended soils in the present study. The most negative $\delta^{13}C$ value recorded for the rSOC fraction in all the soils (Figure 2) could be the consequence of chemical oxidation. Zimmermann et al. [45] also revealed that NaOCl-resistant fractions were depleted in $\delta^{13}C$, which they explained by the fact that oxidation with NaOCl dissolves ^{13}C-enriched compounds (cellulose, hemi-cellulose, carboxyl, and amino acids) and leaves the rSOC fraction depleted in ^{13}C. After incubation, the fractions of the

control samples had more negative $\delta^{13}C$ values than the native samples, except for the s + c fraction, where the values were similar. This can be explained by the preferential use of ^{13}C-enriched substrates (including sugars, starch, cellulose, etc.) by microorganisms during decomposition, resulting in the depletion of ^{13}C in the remaining SOM [54]. Microbial metabolism, taking place during the incubation, results ^{13}C-depleted CO_2 and ^{13}C-enriched SOM [55], which would cause more positive $\delta^{13}C$ value of the SOM fractions. Thus the fractionation effect of the preferential use of ^{13}C-enriched substrates and the microbial metabolism are contradictory, but according to Šantrůčková et al. [56] the effect of preferential substrate utilization is stronger than that of the microbial metabolism, which causes the more negative $\delta^{13}C$ value of the SOM fractions of the control sample compared to the SOM fractions of the native sample.

The $\Delta^{14}C$ values of the fractions in the studied soil (Table 3) showed the highest values for the bulk soil (78.2 ± 1.7‰) and the POM fraction (75.2 ± 1.9‰), similar values for the S + A (51.0 ± 2.1‰) and s + c (49.7 ± 1.9‰) fractions, and the lowest value for the rSOC fraction (−75.0 ± 2.4‰), thus showing the highest proportion of bomb-derived ^{14}C in the bulk soil and the POM fraction, as positive values of $\Delta^{14}C$ indicate the presence of bomb-derived ^{14}C [43,57]. The >100 pMC values of the bulk soil and the POM, S + A, and s + c fractions (Table 3) also suggested a significant amount of bomb-derived ^{14}C [58]. The lowest and only negative $\Delta^{14}C$ value measured in the rSOC fraction indicated that the ^{14}C in the sample was not in contact with the atmosphere, so significant radioactive decay could have occurred [57]. This is in accordance with the findings of Kögel-Knabner et al. [59] who also reported the smallest ^{14}C activities (99.3 and 90.7 pMC) for the heavy (>1.6 g cm^{-3}), NaOCl-resistant fractions in the "A" horizons of a Cambisol and a Podzol, respectively.

Table 3. Proportion and mean residence time of C derived from the C4–C (maize residues) input, ^{14}C activity and $\Delta^{14}C$ for the soil fractions separated from the Luvisol studied.

Fraction [1]	F_{maize} [2] (%)	MRT [3] (Years)	^{14}C activity (pMC)	$\Delta^{14}C$ (‰)
POM	11.7 ± 2.5 [4]	3.6	108.4 ± 0.19	75.2 ± 1.9
S + A	3.8 ± 2.8	11.5	106.0 ± 0.21	51.0 ± 2.1
s + c	2.7 ± 3.0	16.1	105.8 ± 0.19	49.7 ± 1.9
rSOC	0.18 ± 2.5	249.7	93.3 ± 0.24	−75.0 ± 2.4
Bulk soil	4.3 ± 2.9	10.2	108.7 ± 0.17	78.2 ± 1.7

[1] POM = particulate organic matter fraction; S + A = sand plus stable aggregate fraction; s + c = silt plus clay fraction; rSOC = chemically resistant soil organic carbon fraction. [2] F_{maize} = proportion of C derived from the C4–C (maize residues) input. [3] MRT = mean residence time of C derived from the C4–C (maize residues) input. [4] Means with ± standard error.

The fraction of C derived from the maize residues was the highest (11.7 ± 2.5%) for the POM fraction, followed by 3.8 ± 2.8 and 2.7 ± 3.0% for the S + A and s + c fractions, respectively, and 0.18 ± 2.5% for the rSOC fraction (Table 3). The fact that the POM fraction had the highest proportion of maize-derived C and the highest ^{14}C activity/$\Delta^{14}C$ value with the fastest turnover (Table 3) confirmed that this fraction was the most labile C pool in the Luvisol investigated in the present study. This is consistent with the statement that this fraction is predominantly plant-derived and sensitive to soil disturbances such as land use and management [21]. Further evidences are the following: (1) the aliphatic and aromatic (lignin-derived) structures of the POM fractions, determined by FT-IR spectroscopy, showed that these fractions were composed of partly degraded plant residues; (2) the $\delta^{13}C$ value of POM was similar to that of plant litter; and (3) the MRT determined for POM in the present study was (3.6 years) in the MRT range (2–5 years) reported by Gaudinski et al. [60] for recognizable leaf litter.

The MRTs of the S + A (11.5 years) and s + c (16.1 years) fractions were similar, as were the proportions of maize-derived C and the ^{14}C values (Table 3). The fast turnover of the S + A fraction can be explained by the fact that this fraction also contains POM particles attached to sand grains [61]. In addition, fresh C imbedded in aggregates represents an active pool with short turnover, while the size of the aggregates plays a significant role in C stabilization. For example, Yamashita et al. [62]

reported that occluded POM with a density of 1.6–2.0 g cm^{-3} within the aggregates had considerably shorter MRTs (20–34 years) for the mega- and large macroaggregates than for the microaggregates (51 years) or <53 μm aggregates (166 years).

The oxidation using NaOCl (resulted in the rSOC fraction) changed the C isotope values of the s + c fraction in the present study. Although Poeplau and Don [61] found that the rSOC fraction was almost as sensitive to land use changes as the total SOC, the highest MRT of 249.7 years was found for the rSOC fraction in the present study. It could be explained by the fact that this fraction was free of plant residues, POM, and aggregates [21]. Many studies [11,63] demonstrated that NaOCl oxidation can be used to obtain a stable C pool with high MRTs and radiocarbon ages.

4. Conclusions

The results from the use of combined physical and chemical fractionation, in combination with FT-IR data and stable and radioactive isotope measurements, agree with the consensus about SOM fractions representing different stabilities. The results revealed that the POM fraction in this Luvisol was the most labile C pool, with easily degradable chemical structures (e.g., aliphatic, O-alkyl, and polysaccharides), depleted ^{13}C values, the highest proportion of new C, and the fastest turnover rate, confirming that this fraction contained non-stabilized organic matter which was easily available for decomposition. The analysis of the POM, S + A, and s + c fractions showed that, in general, with decreasing grain size distribution: (1) the C/N ratio decreases; (2) the aromaticity increases; (3) the $\delta^{13}C$ value increases; (4) the proportion of new maize-derived C decreases; (5) the proportion of bomb-derived ^{14}C decreases; and (6) the MRT of C increases, suggesting an increasing degree of mineral–organic association, leading to decreased degradability and greater resistance to decomposition. Therefore, the hypothesis that the smallest fraction is the most stabile was confirmed. In addition, the fact that the rSOC fraction represents a relatively inert C pool was also demonstrated by the much smaller proportion of maize-derived C and bomb-derived ^{14}C and by far the longest MRT, confirming the ability of NaOCl oxidation to separate a C fraction which is less transformed by microorganisms in the native forest soil investigated. Nevertheless, the fact that the rSOC fractions unexpectedly had the widest C/N ratio, a decrease in relative aromaticity and the most negative $\delta^{13}C$ value, regardless of incubation or maize addition, highlighted the effect of chemical oxidation by NaOCl.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1944/s1, Figure S1: The FT-IR spectra of the native samples, Figure S2: The FT-IR spectra of the control samples, Figure S3: The FT-IR spectra of the amended samples.

Author Contributions: Conceptualization, D.Z., T.F., G.J., and Z.S.; data curation, D.Z and T.F.; methodology, M.M., T.K., C.K., I.H., and L.G.; resources, G.J. and M.M.; supervision, T.F., G.J., and Z.S.; visualization, D.Z.; writing—original draft, D.Z.; writing—review & editing, T.F., G.J., and Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the European Union and the State of Hungary, co-financed by the European Regional Development Fund in the project of GINOP-2.3.2-15-2016-00009 'ICER' and by the ÚNKP-17-3 New National Excellence Program of the Ministry of Human Capacities, Hungary, and by the Development and Innovation Fund of Hungary [No. NKFIH 123953].

Acknowledgments: The authors are grateful for the help of Dóra Kesjár in the FT-IR measurements.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kögel-Knabner, I.; Amelung, W. Dynamics, Chemistry, and Preservation of Organic Matter in Soils. In *Treatise on Geochemistry*; Holland, H., Turekian, K., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 157–215. ISBN 9780080983004.
2. von Lützow, M.; Kögel-Knabner, I.; Ludwig, B.; Matzner, E.; Flessa, H.; Ekschmitt, K.; Guggenberger, G.; Marschner, B.; Kalbitz, K. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 111–124. [CrossRef]

3. Trumbore, S.E. Age of soil organic matter and soil respiration. *Ecol. Appl.* **2000**, *10*, 399–411. [CrossRef]
4. Kuzyakov, Y. Sources of CO_2 efflux from soil and review of partitioning methods. *Soil Biol. Biochem.* **2006**, *38*, 425–448. [CrossRef]
5. Schmidt, M.W.I.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; Kögel-Knabner, I.; Lehmann, J.; Manning, D.A.C.; et al. Persistence of soil organic matter as an ecosystem property. *Nature* **2011**, *478*, 49–56. [CrossRef]
6. Creamer, C.A.; Filley, T.R.; Boutton, T.W. Long-term incubations of size and density separated soil fractions to inform soil organic carbon decay dynamics. *Soil Biol. Biochem.* **2013**, *57*, 496–503. [CrossRef]
7. Trumbore, S.E.; Chadwick, O.A.; Amundson, R. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science* **1996**, *272*, 393–396. [CrossRef]
8. Balesdent, J.; Wagner, G.H.; Mariotti, A. Soil organic matter turnover in long-term field experiments as revealed by Carbon-13 natural bbundance. *Soil Sci. Soc. Am. J.* **1988**, *52*, 118–124. [CrossRef]
9. Saviozzi, A.; Vanni, G.; Cardelli, R. Carbon mineralization kinetics in soils under urban environment. *Appl. Soil Ecol.* **2014**, *73*, 64–69. [CrossRef]
10. Percival, H.J.; Parfitt, R.L.; Scott, N.A. Factors controlling soil carbon levels in New Zealand grasslands. *Soil Sci. Soc. Am. J.* **2000**, *64*, 1623–1630. [CrossRef]
11. Kleber, M.; Mikutta, R.; Torn, M.S.; Jahn, R. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. *Eur. J. Soil Sci.* **2005**, *56*, 717–725. [CrossRef]
12. Wei, H.; Guenet, B.; Vicca, S.; Nunan, N.; Asard, H.; AbdElgawad, H.; Shen, W.; Janssens, I.A. High clay content accelerates the decomposition of fresh organic matter in artificial soils. *Soil Biol. Biochem.* **2014**, *77*, 100–108. [CrossRef]
13. Leifeld, J.; Fuhrer, J. Long-term management effects on soil organic matter in two cold, high-elevation grasslands: Clues from fractionation and radiocarbon dating. *Eur. J. Soil Sci.* **2009**, *60*, 230–239. [CrossRef]
14. Dondini, M.; Hastings, A.; Saiz, G.; Jones, M.B.; Smith, P. The potential of Miscanthus to sequester carbon in soils: Comparing field measurements in Carlow, Ireland to model predictions. *GCB Bioenergy* **2009**, *1*, 413–425. [CrossRef]
15. Carvalhais, N.; Forkel, M.; Khomik, M.; Bellarby, J.; Jung, M.; Migliavacca, M.; Mu, M.; Saatchi, S.; Santoro, M.; Thurner, M.; et al. Global covariation of carbon turnover times with climate in terrestrial ecosystems. *Nature* **2014**, *514*, 213–217. [CrossRef]
16. He, Y.; Trumbore, S.E.; Torn, M.S.; Harden, J.W.; Vaughn, L.J.S.; Allison, S.D.; Randerson, J.T. Radiocarbon constraints imply reduced carbon uptake by soils during the 21st century. *Science* **2016**, *353*, 1419–1424. [CrossRef]
17. Lehmann, J.; Kleber, M. The contentious nature of soil organic matter. *Nature* **2015**, *528*, 60–68. [CrossRef]
18. IUSS Working Group WRB. *World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015.
19. Gee, G.W.; Bauder, J.W. Particle-size analysis. In *Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods*; Klute, A., Ed.; Soil Science Society of America: Madison, WI, USA, 1986; pp. 383–411.
20. Frøseth, R.B.; Bleken, M.A. Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect. *Soil Biol. Biochem.* **2015**, *80*, 156–166. [CrossRef]
21. Zimmermann, M.; Leifeld, J.; Schmidt, M.W.I.; Smith, P.; Fuhrer, J. Measured soil organic matter fractions can be related to pools in the RothC model. *Eur. J. Soil Sci.* **2007**, *58*, 658–667. [CrossRef]
22. Craig, H. The geochemistry of the stable carbon isotopes. *Geochim. Cosmochim. Acta* **1953**, *3*, 53–92. [CrossRef]
23. Coplen, T.B.; Brand, W.A.; Gehre, M.; Gröning, M.; Meijer, H.A.J.; Toman, B.; Verkouteren, R.M. New Guidelines for δ^{13} C Measurements. *Anal. Chem.* **2006**, *78*, 2439–2441. [CrossRef]
24. Balesdent, J.; Mariotti, A. Measurement of soil organic matter turnover using ^{13}C natural abundance. In *Mass Spectrometry of Soils*; Boutton, T.W., Yamasaki, S.I., Eds.; Marcel Dekker Inc.: New York, NY, USA, 1996; pp. 83–111. ISBN 0-8247-9699-3.
25. Amelung, W.; Brodowski, S.; Sandhage-Hofmann, A.; Bol, R. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. *Adv. Agron.* **2008**, *100*, 155–250. [CrossRef]

26. Jull, A.J.T.; Burr, G.S.; Beck, J.W.; Hodgins, G.W.L.; Biddulph, D.L.; Gann, J.; Hatheway, A.L.; Lange, T.E.; Lifton, N.A. Application of accelerator mass spectrometry to environmental and paleoclimate studies at the University of Arizona. *Radioact. Environ.* **2006**, *8*, 3–23.
27. Molnár, M.; Janovics, R.; Major, I.; Orsovszki, J.; Gönczi, R.; Veres, M.; Leonard, A.G.; Castle, S.M.; Lange, T.E.; Wacker, L.; et al. Status Report of the New AMS ^{14}C Sample Preparation Lab of the Hertelendi Laboratory of Environmental Studies (Debrecen, Hungary). *Radiocarbon* **2013**, *55*, 665–676. [CrossRef]
28. Rinyu, L.; Molnár, M.; Major, I.; Nagy, T.; Veres, M.; Kimák, Á.; Wacker, L.; Synal, H.A. Optimization of sealed tube graphitization method for environmental C-14 studies using MICADAS. *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. At.* **2013**, *294*, 270–275. [CrossRef]
29. Janovics, R.; Futó, I.; Molnár, M. Sealed tube combustion method with MnO2 for AMS ^{14}C Measurement. *Radiocarbon* **2018**, *60*, 1347–1355. [CrossRef]
30. Molnár, M.; Rinyu, L.; Veres, M.; Seiler, M.; Wacker, L.; Synal, H.-A. EnvironMICADAS: A Mini ^{14}C AMS with Enhanced Gas Ion Source Interface in the Hertelendi Laboratory of Environmental Studies (HEKAL), Hungary. *Radiocarbon* **2013**, *55*, 338–344. [CrossRef]
31. Wacker, L.; Christl, M.; Synal, H.A. Bats: A new tool for AMS data reduction. *Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms* **2010**, *268*, 976–979. [CrossRef]
32. Stenström, K.E.; Skog, G.; Georgiadou, E.; Genberg, J.; Johansson, A. *A Guide to Radiocarbon Units and Calculations*; Lund University: Lund, Sweden, 2011.
33. Stuiver, M.; Polach, H.A. Discussion Reporting of ^{14}C Data. *Radiocarbon* **1977**, *19*, 355–363. [CrossRef]
34. Udvardi, B.; Kovács, I.J.; Kónya, P.; Földvári, M.; Füri, J.; Budai, F.; Falus, G.; Fancsik, T.; Szabó, C.; Szalai, Z.; et al. Application of attenuated total reflectance Fourier transform infrared spectroscopy in the mineralogical study of a landslide area, Hungary. *Sediment. Geol.* **2014**, *313*, 1–14. [CrossRef]
35. Inbar, Y.; Chen, Y.; Hadar, Y. Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. *Soil Sci. Soc. Am. J.* **1989**, *53*, 1695–1701. [CrossRef]
36. Haberhauer, G.; Rafferty, B.; Strebl, F.; Gerzabek, M.H. Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. *Geoderma* **1998**, *83*, 331–342. [CrossRef]
37. Haberhauer, G.; Gerzabek, M. Drift and transmission FT-IR spectroscopy of forest soils: An approach to determine decomposition processes of forest litter. *Vib. Spectrosc.* **1999**, *19*, 413–417. [CrossRef]
38. Gerzabek, M.H.; Antil, R.S.; Kogel-Knabner, I.; Knicker, H.; Kirchmann, H.; Haberhauer, G. How are soil use and management reflected by soil organic matter characteristics: A spectroscopic approach. *Eur. J. Soil Sci.* **2006**, *57*, 485–494. [CrossRef]
39. Martín, J.V.; Miralles De Imperial, R.; Calvo, R.; Garcia, M.C.; Leon-Cófreces, C.; Delgado, M.M. Carbon mineralisation kinetics of poultry manure in two soils. *Soil Res.* **2012**, *50*, 222–228. [CrossRef]
40. Thiessen, S.; Gleixner, G.; Wutzler, T.; Reichstein, M. Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass-An incubation study. *Soil Biol. Biochem.* **2013**, *57*, 739–748. [CrossRef]
41. Shahbaz, M.; Kuzyakov, Y.; Heitkamp, F. Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls. *Geoderma* **2017**, *304*, 76–82. [CrossRef]
42. Dou, X.; He, P.; Zhu, P.; Zhou, W. Soil organic carbon dynamics under long-term fertilization in a black soil of China: Evidence from stable C isotopes. *Sci. Rep.* **2016**, *6*, 21488. [CrossRef]
43. Baisden, W.T.; Amundson, R.; Cook, A.C.; Brenner, D.L. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Glob. Biogeochem. Cycles* **2002**, *16*, 64-1–64-16. [CrossRef]
44. Sollins, P.; Swanston, C.; Kleber, M.; Filley, T.; Kramer, M.; Crow, S.; Caldwell, B.A.; Lajtha, K.; Bowden, R. Organic C and N stabilization in a forest soil: Evidence from sequential density fractionation. *Soil Biol. Biochem.* **2006**, *38*, 3313–3324. [CrossRef]
45. Zimmermann, M.; Leifeld, J.; Abiven, S.; Schmidt, M.W.I.; Fuhrer, J. Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. *Geoderma* **2007**, *139*, 171–179. [CrossRef]
46. Niemeyer, J.; Chen, Y.; Bollag, J.-M. Characterization of Humic Acids, Composts, and Peat by Diffuse Reflectance Fourier-Transform Infrared Spectroscopy. *Soil Sci. Soc. Am. J.* **1992**, *56*, 135–140. [CrossRef]
47. Piccolo, A.; Zaccheo, P.; Genevini, P.G. Chemical characterization of humic substances extracted from organic-waste-amended soils. *Bioresour. Technol.* **1992**, *40*, 275–282. [CrossRef]

48. Baldock, J.A.; Oades, J.M.; Waters, A.G.; Peng, X.; Vassallo, A.M.; Wilson, M.A. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. *Biogeochemistry* **1992**, *16*, 1–42. [CrossRef]
49. Boutton, T.W. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In *Mass Spectrometry of Soils*; Boutton, T.W., Yamasaki, S.I., Eds.; Marcel Dekker Inc.: New York, NY, USA, 1996; pp. 47–82.
50. Keitel, C.; Matzarakis, A.; Rennenberg, H.; Gessler, A. Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. *Plant Cell Environ.* **2006**, *29*, 1492–1507. [CrossRef]
51. Shang, C.; Tiessen, H. Carbon turnover and carbon-13 natural abundance in organo-mineral fractions of a tropical dry forest soil under cultivation. *Soil Sci. Soc. Am. J.* **2000**, *64*, 2149–2155. [CrossRef]
52. Gerzabek, M.H.; Haberhauer, G.; Kirchmann, H. Soil organic matter pools and carbon-13 natural abundances in particle-size fractions of a long-term agricultural field experiment receiving organic amendments. *Sci. Soc. Am. J.* **2001**, *65*, 352–358. [CrossRef]
53. Gunina, A.; Kuzyakov, Y. Pathways of litter C by formation of aggregates and SOM density fractions: Implications from ^{13}C natural abundance. *Soil Biol. Biochem.* **2014**, *71*, 95–104. [CrossRef]
54. Ågren, G.I.; Bosatta, E.; Balesdent, J. Isotope discrimination during decomposition of organic matter: A theoretical analysis. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1121–1126. [CrossRef]
55. Blair, N.; Leu, A.; Muñoz, E.; Olsen, J.; Kwong, E.; Des Marais, D. Carbon isotopic fractionation in heterotrophic microbial metabolism. *Appl. Environ. Microbiol.* **1985**, *50*, 996–1001. [CrossRef]
56. Šantrůčková, H.; Bird, M.I.; Frouz, J.; Šustr, V.; Tajovský, K. Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. *Soil Biol. Biochem.* **2000**, *32*, 1793–1797. [CrossRef]
57. Trumbore, S.E.; Sierra, C.A.; Hicks Pries, C.E. Radiocarbon nomenclature, theory, models, and interpretation: Measuring age, determining cycling rates, and tracing source pools. In *Radiocarbon and Climate Change*; Schuur, E.A.G., Druffel, E.R.M., Trumbore, S.E., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 45–82.
58. Leifeld, J.; Zimmermann, M.; Fuhrer, J.; Conen, F. Storage and turnover of carbon in grassland soils along an elevation gradient in the Swiss Alps. *Glob. Chang. Biol.* **2009**, *15*, 668–679. [CrossRef]
59. Kögel-Knabner, I.; Guggenberger, G.; Kleber, M.; Kandeler, E.; Kalbitz, K.; Scheu, S.; Eusterhues, K.; Leinweber, P. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 61–82. [CrossRef]
60. Gaudinski, J.B.; Trumbore, S.E.; Eric, A.; Zheng, S. Soil carbon cycling in a temperate forest: Radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry* **2000**, *51*, 33–69. [CrossRef]
61. Poeplau, C.; Don, A. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. *Geoderma* **2013**, *192*, 189–201. [CrossRef]
62. Yamashita, T.; Flessa, H.; John, B.; Helfrich, M.; Ludwig, B. Organic matter in density fractions of water-stable aggregates in silty soils: Effect of land use. *Soil Biol. Biochem.* **2006**, *38*, 3222–3234. [CrossRef]
63. Mikutta, R.; Kleber, M.; Torn, M.S.; Jahn, R. Stabilization of soil organic matter: Association with minerals or chemical recalcitrance? *Biogeochemistry* **2006**, *77*, 25–56. [CrossRef]