

Changes of carbon-isotope ratios in soil organic matter relative to parent vegetation and site specificity

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Abstract

Investigating the correlation between biodiversity and ecosystem function in natural environments using carbon-isotope composition ($\delta^{13}\text{C}$) allows distinguishing the nutrient cycling pattern and anthropogenic effects incorporation in plants and soil processes. The mechanisms behind the isotopic composition of soil organic matter (SOM) and parent vegetation in relation to the context of site-specificity was approached in this work. Formation of SOM can be affected by the presence of a high concentration of heavy metals in soils. Still, no systematic studies were performed in most of the industrial sites to support this hypothesis. In order to explore this incomplete understood influence, investigation of carbon isotope signatures ($\delta^{13}\text{C}$) variations in soil organic matter were performed in two industrial areas from Romania (Copșa Mică industrial platform and Baia Sprie mining zone). The current study, also, investigated the C:N ratio variation, as well as the influence of N speciation regarding $\delta^{13}\text{C}$ values of SOM. The decrease in C:N ratio indicated an increasing effect of the microbial products on SOM matter at increasing depth, for both regions, while an increase of the denitrification processes with depth was found for both areas. For the most appropriate depth (20–40 cm), the soil from Baia Sprie region was more enriched in ^{13}C comparing with the soil from Copsa Mica region, and this higher isotope fractionation of SOM might be due to a higher carbon content, respectively a higher nitrogen content of Baia Sprie soil. It was concluded that the SOM of the surface soil in the two investigated regions has an ^{13}C isotopic composition similar to the plant remains from which it was formed, offering an integrated value of plant material, time and the local origin and providing useful markers of tree isotopic composition.

Keywords: C:N ratio; ^{13}C ; heavy metals; microbial products; isotope fractionation; soil organic matter

Introduction

Over the past three decades, analytical techniques for stable isotopes have been extensively used to investigate the formation of soil organic matter from plant debris, as well as the processes that govern its

transformation (Gleixner, 2013). Isotopic signatures of soil carbon were applied to quantify the rotation rates of soil organic carbon (SOC), to determine sources of SOC, and even to reconstruct the history of the plant community (Balesdent *et al.*, 1987; Choi *et al.*, 2001; Krull *et al.*, 2007; Boutton *et al.*, 2015; Metcalfe and Mead, 2019).

The organic carbon migrates from plant to soil system via root turnover and especially through litterfall (van Kessel *et al.*, 1994). The plant waste entering the soil system may suffer isotope fractionation associated with microbial decay processes, and therefore its carbon-isotope composition ($\delta^{13}\text{C}$ values) might be slightly modified from their initial values. More specifically, during the above-mentioned fractionation processes, a differential decay of isotopically unique biochemical compounds that comprise soil organic matter might appear. Therefore, firstly the carbon inputs from plant residues and secondly the biological decay processes are controlling the $\delta^{13}\text{C}$ values of SOC and their evolution over time (Bai *et al.*, 2012).

Besides the natural processes, the isotope composition of SOC can be affected by fossil fuel combustion residues (Metcalfe and Mead 2019), wet and dry deposition of particulate matter (PM), and other anthropogenic sources (Bai *et al.*, 2012). Generally, it was shown both for C3 and C4 plants that the $\delta^{13}\text{C}$ values of the soil organic matter (SOM) are with about 1-3 ‰ more enriched comparing the originating plants (Staddon, 2004), ecosystems, but the reasons for this increase are not yet fully elucidated. Some mechanisms regarding the fractionation of SOM includes differential mobility and sorption of dissolved organic C with variable isotopic values; microbes as precursors of stable organic matter; kinetic discrimination against ^{13}C during respiration; and preferential decomposition of specific components (Boström *et al.*, 2007). The kinetic discrimination is probably the most relevant explanation for fractionation of SOM as the microorganisms respire in most of the cases ^{13}C -depleted CO_2 (Fernandez and Cadisch, 2003), while in the case of buried litter, the preferential decomposition of individual components (lignin, polysaccharides) is becoming relevant with the soil depth.

Formation of SOM can be affected by the presence of a high concentration of heavy metals in soils, elements originating mostly from mining and industrial production (Morkunas *et al.*, 2018; Li *et al.*, 2018). The chemical processes that are influencing the effects of metals in soils are ion-exchange adsorption-desorption, oxidation-reduction, and complexation-dissociation. These processes, together with the presence of transporters, can influence the complex impact of metals in soils. The oxidation-reduction can change the metal valence, while the adsorption-desorption and complexation-dissociation reactions can affect metal activity in soil (Swartjes *et al.*, 2007). On the other hand, the pH can significantly influence the bioavailability and toxicity of metals in soils (Badawy *et al.*, 2002). The presence of heavy metals in very high concentrations might inhibit physiological processes such as N-metabolism and mineral nutrition, cell elongation, photosynthesis, respiration and transpiration rates, and leads to decrease in biomass and ultimately plant death (Zornoza *et al.*, 2002). The excessively content of heavy metals in soils can also affect the soil microbial community by inhibiting the growth of some microorganisms (Chu, 2018), which influences the isotope fractionation of SOM.

Given these aspects, an investigation of the change in carbon-isotope ratios ($\delta^{13}\text{C}$) in soil organic matter from two industrial areas from Romania (Copsa Mica industrial platform and Baia Sprie mining zone), was conducted in this study. A correlation of the obtained $\delta^{13}\text{C}$ values was done with those of plants from which SOM was formed while also studying the influence of C and N content on the formation of SOM.

Materials and Methods

Sampling of the soil and plants samples

Two fields impacted by ore processing or smelting operations experiments were considered (Figure 1). The first one was conducted to Sibiu county, in a highly polluted area namely Copsa Mica, at 800 m away from the source of contamination, in forest land, during the second one to Baia Mare county, near the Baia Sprie ore

deposit. Soil sampling focused on two different levels, at a depth of 0-20 and 20-40 cm, within the selected areas, where the soils were characterized as freely draining podzols. The chosen sites differed in terms of vegetation coverage and appearance. To Copsa Mica, common oak (*Quercus robur* L.) forest is the dominant vegetation. To Baia Sprie, a mixed forest is present with a vegetational transition between the coniferous forest of Scots Pine (*Pinus sylvestris* L.) and firs (*Abies* sp.) and broad-leaved deciduous forest of oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.). Different other plant materials were also collected from the two sites (Figure 1).

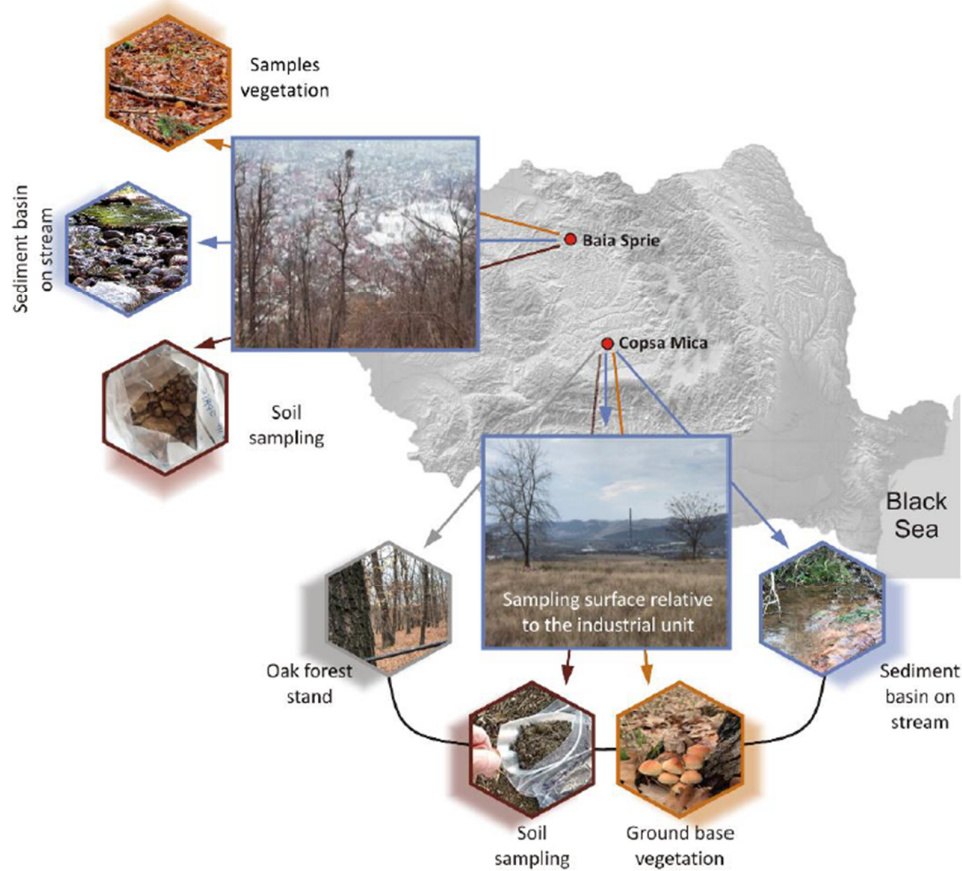


Figure 1. Schematics of the experimental work

Table 1. Description of the samples collection

Matrix	Sampling location/ Sample and code			
	Copsa Mica		Baia Sprie	
Vegetation samples	<i>Quercus robur</i> acorn core - AC _{CM}	White fungi WF _{CM}	<i>Abies</i> sp. leaves AL _{BS}	<i>Quercus robur</i> leaves QL _{BS}
	<i>Quercus robur</i> acorn peel AP _{CM}	Red fungi RF _{CM}	<i>Pinus sylvestris</i> leaves - PL _{BS}	<i>Fagus sylvatica</i> leaves - FL _{BS}
	<i>Quercus robur</i> vine - QV _{CM}	<i>Quercus robur</i> leaves QL _{CM}	<i>Cupressus sempervirens</i> L. leaves - CF _{BS}	
Soil	S ₁ -I _{CM} at 0-20 cm	S ₁ -II _{CM} at 20-40 cm	S ₁ -I _{BS} at 0-20 cm	S ₁ -II _{BS} at 20-40 cm
Sediment	S _{2CM}		S _{2BS}	

Basic physicochemical indicators

For the anion measurements (NO_3^- , NO_2^-), the soil samples were extracted into the water as follows: air-dried soil samples (10 g) were weighed into 100 ml specimen containers and 50 ml H_2O was added. The samples were stirred for 15 min and filtered through filter paper into a vial. The nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in soil and sediment samples were determined using the colorimetric method, by UV-VIS spectrophotometer type SPECORD 250 (Analytic Jena, Germany). The quality control analysis has been tested using Certified Reference Material, namely nitrite and nitrate standard solutions traceable to SRM from NIST from Certipur Merck. All chemicals and reagents used were of spectroscopic grade; Ultrapure water, having a maximum resistivity of 18.2 $\text{M}\Omega/\text{cm}$, was used for sample treatment and dilution. All the investigated calibration curves were characterized by a high correlation coefficient ($r > 0.995$).

Chemical analysis of SOM by elemental analysis (EA)

The soil and sediment samples were analysed for chemical composition (C, N, H) using an elemental analyser (EA Flash HT 2000 - ThermoScientific, UK) equipped with a thermal conductivity detector. Prior to the analyses, soil samples were picked free of macroscopic roots and after removing water by a drying pre-treatment step, were grinded to 0.2 mm in a ball mill and brought to a mass of 2.5 - 3.0 mg. Further, the samples were inserted in a tin capsule, neutral carbon material, and weighted using an ultra-microbalance (Mettler Toledo XP6, USA). For the determination of carbon, hydrogen and nitrogen (CHN), an EA Flash2000 (ThermoScientific, UK) coupled with a thermal conductivity detector (TCD), at $T = 120^\circ\text{C}$ and $I = 1000\mu\text{V}$, was used and operated according to the dynamic flash combustion. Each determination was based on the mean of three replicate analysis. For the quality control of the analysis, during each series of determinations, at the beginning and the end of each sequence, a quality control (QC) was used, namely Cystine, an amino acid traceable to NIST Standard reference material, with the following composition: N:11.66 (%), C:29.99 (%), H:5.03 (%) and S:26.69 (%).

SOM Isotope composition determination by EA-IRMS

The carbon-isotope ratio ($\delta^{13}\text{C}$) of vegetation and soil samples was determined using an elemental analyzer (EA) coupled with isotope ratio mass spectrometer (Thermo Flash2000 EA interfaced with a Thermo Delta V Plus IRMS, Thermo Scientific, Germany) operated at the Stable Isotope Laboratory of National Institute for Cryogenics and Isotopic Technologies - ICSI Rm. Vâlcea. Before the sample analysis, about 1g of each soil and sediment samples were weighed in 5 mL plexiglass capped tubes and acidified with 2 mL HCl 37 % then shaken at vortex device for 3 minutes. After 48 h, the HCl was removed by evaporation at 110°C for 3 h using a silicone oil bath heated by a hot-plate device. Afterwards, the samples were freeze-dried firstly by immersing into liquid nitrogen and then at about -80°C .

For carbon isotopes measurements were done in triplicate and the results were reported in per mil units (‰) with respect to the international standards Vienna Pee Dee Belemnite (VPDB), (Craig, 1957; Coplen, 1995):

$$\delta(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} * 1000, \quad (1)$$

where R is the ratio between the heavier isotope and the lighter one.

The $\delta^{13}\text{C}$ isotopic values were calibrated against an international reference material, sugar IAEA-CH-6 (IAEA - International Atomic Energy Agency, Vienna, Austria), with a certified value of $\delta^{13}\text{C}_{\text{VPDB}} = -10.45 \pm 0.05\text{‰}$, and three other laboratory standards provided by Iso-Analytical Laboratory Standard, namely, L-Alanine IA-R041 ($-23.33 \pm 0.10\text{‰}$), Beet Sugar IA-R005 ($-26.03 \pm 0.11\text{‰}$), and Cane Sugar IA-R006 ($-11.64 \pm 0.03\text{‰}$). The analytical precision for $\delta^{13}\text{C}$ measurements was $\pm 0.2\text{‰}$.

Results and Discussions

Shift in the chemical composition of soil and sediments

The results of the chemical composition of soil organic matter from the soil and sediment samples investigated are highlighted in Table 2. The highest carbon content was determined in soil from the top 0 - 20 cm (19.45%) layer, originated from Baia Sprie area, while the lowest carbon content was in the sediment S2_{CM} (0.54%) from Copsa Mica. The N content showed a similar pattern as observed for carbon. The highest nitrogen content (1.57%) was recorded for the 0-20 cm (litter) soil sample S1-I_{BS} from Baia Sprie, while the lowest nitrogen content (0.15%) was recorded for the sediment sampled from Copsa Mica area (S2_{CM} sample).

To both investigated areas, the highest organic carbon concentration was recorded on the surface soil and is consistent with the mixing of the organic layer, which has a high carbon concentration into the shallow mineral soil and decreases with the depth as a result of the loss of labile soil organic matter, typical of early stages of post-harvest regeneration. Baia Sprie region presented higher concentrations, this particularity being strongly related to the forest stand age, litter input and decomposition rates, the chemical behaviour being the same for both stands. In addition to these factors, decomposition rate and litter input are influenced by tree composition, anthropogenic activities and climatic factors such as temperature, precipitation and latitude (Giweta, 2020). The litter decay process is mainly conditioned by the soil microbial activity, that is regulated by climate and litter quality, all together determining the decomposition rate.

Table 2. Soil and sediment samples determined by elemental analysis

Origin	Sample code	N (%)	C (%)	H (%)	C:N
Copsa Mica	S1-I _{CM}	0.75	10.12	1.26	13.49
	S1-II _{CM}	0.26	0.95	0.48	3.65
	S2 _{CM}	0.15	0.54	0.27	3.60
Baia Sprie	S1-I _{BS}	1.57	19.45	2.74	12.39
	S1-II _{BS}	0.36	2.1	1.36	5.83
	S2 _{BS}	0.21	0.58	0.64	2.76

An additional and important factor linked to carbon-isotope discrimination regarding microbial activity, including soil organic matter (SOM) decomposition, is represented by the C/N ratio. To Baia Sprie area, the C:N ratio is decreasing from 12.39, for the 0-20 cm soil (S1-I_{BS}), to 5.83, for the 20-40 cm depth soil (S1-II_{BS}), while to Copsa Mica from 13.49, for the 0-20 cm soil, to 3.65, for the 20-40 cm depth soil (S1-II_{CM}). This decrease in C:N ratio indicates an increasing influence of the microbial products on SOM matter at increasing depth, for both heavy polluted regions. This is an interesting observation, also recorded by (Boström *et al.*, 2007) in a study regarding the SOM of the soil profiles from a Norway spruce (*Picea abies* (L.) Karst) forest planted 68 years before the actual sampling of soil. Therefore, the variation of C:N ratio might be correlated with the microbial activity and with the degree of pollution with heavy metals contents from Copsa Mica area (for example the content in one soil profile taken from 0 to 0.2 m were 182.5 mg/kg for Pb and 12.50 mg/kg for Cd, respectively) (Blaj *et al.*, 2013) and from Baia Sprie area (3074.29 ± 201.65 for Pb and 14.13 ± 1.36 mg/kg for Cd respectively, soil sampled from 0 to 0.2 m depth (Bora *et al.*, 2020).

Regarding the speciation of N, the 10-20 cm soil from Baia Sprie has a NO₃⁻ of 14.32 mgN/kg and a NO₂⁻ of 0.106 mgN/kg, while the 20-40 cm depth soil has a NO₃⁻ of 6.35 mgN/kg and of NO₂⁻ of 0.12 mgN/kg, clearly showing an increase of the denitrification processes with depth. An interesting observation that might appear to decrease of heavy metals content with depth, that reduce the denitrification processes in near-surface soil. Regarding the sediment sample from Baia Sprie, the content in NO₃⁻ was 0.90 mgN/kg and the NO₂⁻ was below the detection limit of the method (<0.03 mgN/kg) indicating that the denitrification processes are negligible in sediments. To Copsa Mica area, the NO₃⁻ content of the superior soil layer (0-20 cm) registered a value of 4.51 mgN/kg and NO₂⁻ of 4.98 mgN/kg, while the depth soil layer (at 20-40 cm) has 8.47 mgN/kg NO₃⁻ and 0.148 mgN/kg NO₂⁻, reflecting that denitrification processes are decreasing with depth. For

the sediment sample from Copsa Mica, the NO_3^- content was 12.41 mgN/kg, while NO_2^- registered a value of 1.48 mgN/kg, highlighting significant denitrification processes in sediments.

Change in carbon-isotope ratios

Soil $\delta^{13}\text{C}$ is predetermined by the $\delta^{13}\text{C}$ of vegetation and by carbon isotopic fractionation during organic matter decomposition, and to further integrate the effects of soil and vegetation type on $\delta^{13}\text{C}_{\text{SOM}}$ the values of organic matter of soil and sediment samples were compared with the $\delta^{13}\text{C}$ values of leaves originating from the same geographic areas. The analyzed vegetation for the Baia Sprie mixed forest consisted of *Abies sp.* leaves - AL_{BS}, *Quercus robur* leaves - QL_{BS}, *Pinus sylvestris* leaves - PL_{BS}, *Fagus sylvatica* leaves, and *Cupressus sempervirens* L. leaves - CF_{BS} leaves. The leaf $\delta^{13}\text{C}$ content of the five genera we sampled ranged from -30.8‰ to -27.8‰, with an average of -28.9‰, which was 1.5‰ more ^{13}C -depleted than surface soil $\delta^{13}\text{C}$. The observed difference, between the $\delta^{13}\text{C}$ values of a mean bulk sample of forest litter component and surface soil, is indicating a fractionation process that occurs during the plant litter decomposition to soil organic matter, causing ^{13}C -enrichment in soil organic matter compared to the plant sources (Staddon, 2004). Was observed that $\delta^{13}\text{C}$ increased along with soil depth profile (Figure 2A), the results being consistent with other studies (Balesdent *et al.*, 1993; Martin and Yakov, 2010; Wang *et al.*, 2017; Camino-Serrano *et al.*, 2019). This difference in the enrichment of soil $\delta^{13}\text{C}$ with depth may be attributed to the differences relative to the bulk leaf $\delta^{13}\text{C}$, which contains lignin and lipids that are ^{13}C depleted.

In contrast, other plant metabolites such as cellulose, sugars, protein and organic acids are ^{13}C enriched. Consequently, fresh litter and recent organic matter with high concentrations of sugars, starch, and cellulose present an enriched ^{13}C isotopic composition. Moreover, the decomposition rates of sugars, starch, cellulose and protein are more accessible and faster than lignin (Dijkstra *et al.*, 2006; Du *et al.*, 2014), leading to a depleted $\delta^{13}\text{C}$ value in the topsoil layer (0 - 20 cm) related to the depth soil (20 - 40 cm).

For Copsa Mica experimental site, the $\delta^{13}\text{C}$ values of a mean bulk sample of forest litter components (*Quercus robur* species) ranged from -30.1 ‰ for whole leaves to -28.0 ‰ in the surface soil recording a 2.1‰ enrichment, suggesting a fractionation process during the decomposition process (Figure 2B). However, no significant difference (less than 1‰) was identified between the surface and depth soil, this being probably due to the Suess effect that could have caused the pattern of increasing $\delta^{13}\text{C}$ with depth because deeper soil is older and less affected by changing $\delta^{13}\text{C}$ of atmospheric CO_2 (Wynn *et al.*, 2006). However, considering the high soil organic matter C/N ratio, we can conclude that this is indicating a strong ^{13}C discrimination by SOM formation.

Evaluating the $\delta^{13}\text{C}$ specific values for each region, for the surface soil, no significant difference was observed even if the type of vegetation is different, while the deep soil recorded a higher value for Baia Sprie experimental site. This higher isotope fractionation of SOM might be due to a higher carbon content (2.1% vs. 0.95%), respectively, a higher nitrogen content (0.36% vs. 0.26%) of Baia Sprie soil. Besides, this could be explained by the mixed tree population in Baia Sprie that leads to the occurrence of mixtures materials from different plants and different times inputs, all together influencing the carbon isotopic dynamics in soils. Decay processes are also governed by plant species composition and diversity. Mixed species decompose faster compared to monoculture (Melo *et al.*, 2013). The insignificant variation with the depth of $\delta^{13}\text{C}$ value in Copsa Mica soils could be triggered by the fact that in some monoculture species, the strongly lignified leaf tissue (as is the oak type) could prevent further decomposition of leaf litter due to the high structural stability (Giweta, 2020). The differences between the two regions are also generated by the microbial diversity involved in decomposition processes that increase with tree species community variation, which in turn increases the rate of leaf decomposition.

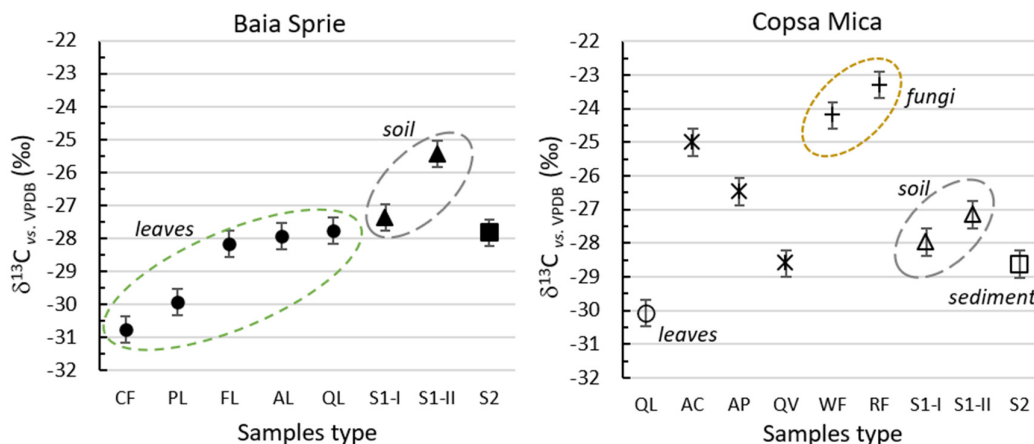


Figure 2. The isotope composition of the soil, sediment, and plant samples from Baia Sprie and Copsa Mica regions

Another element that affects the decomposition rates, targeting the microbial activity is represented by the climate (Djukic *et al.*, 2018). Warmer and humid weather generates an increased decomposition rate compared to colder and dryer ones, primarily affecting the microbial activity and the biomass growth, which results in increased litter input to the forest floor. The complexity of such variation explains the $\delta^{13}\text{C}$ specific deep soil value from Baia Sprie, a region with a mean annual temperature between 6.1 and 8 °C and an average annual rainfall of 801-1000 mm. At the same time, Copsa Mica is warmer with an average annual temperature of 10.1-12 °C, and the average annual precipitation is around 550 mm (ANM, 2019). This is why the decomposition rates are slower in Baia Sprie forest and concurrent it can be influenced by a microbial preferential behaviour for a certain type of substrate that is ^{13}C enriched (Werth and Kuzyakov, 2010). However, providing a mechanistic explanation for these results requires more detailed studies of the interaction of microbial diversity and litter chemical diversity, mainly because the two studied regions are also affected by historical pollution which significantly contributes to the environment changing.

The lowest C and N content was found in the sediment samples, both regions presenting approximately the same values, assuming that the primary source of the organic materials stored in sediments was represented by rich litter material and topsoil, and indicating that differences in precipitation type may not be an essential driver. While no significant difference was observed in the amount of C, the isotopic compositions were distinct for the two regions. Still, their values are similar to the bulk terrestrial plant matter, indicating that the sediments are almost entirely composed of this source with small variations induced by the microbial community structure. Our results show that it is possible to explain specific soil $\delta^{13}\text{C}$ observed patterns in different forest ecosystems and establish that substrate and decomposers interactions are a significant influence on the pattern.

Conclusions

The processes influencing the formation of SOM and the isotope signatures in both Baia Sprie and Copsa Mica are involved, and they can be correlated with the type of vegetation that grows in the respective area. Also, other parameters like C:N ratio, NO_2^- and NO_3^- content can indicate the effects of heavy metals on the formation of SOM in the respective regions. It is often observed that the difference in the enrichment of soil $\delta^{13}\text{C}$ with depth is dependent on the quality and quantity of aboveground plant C inputs and the consequences of C cycling processes in soil depth profiles. By comparing the two types of ecosystems (Baia Sprie vs. Copsa Mica) it is highlighted that the formation of organic matter in the soil is accentuated in the regions

where there is the vegetation of deciduous plants (oak type). If we are considering the 20-40 cm depth as the most relevant, the soil from Baia Sprie region is more enriched in ^{13}C compared with the soil from Copsa Mica region, and this higher isotope fractionation of SOM might be due to a higher carbon content, respectively a higher nitrogen content of Baia Sprie soil. It can be concluded that the SOM of the surface soil in the two investigated regions has an isotopic composition in ^{13}C similar to the plant remains from which it was formed, a higher isotope fractionation occurring in Baia Sprie soils. To elucidate the isotope fractionation processes of the SOM from these areas, a variety of samples (litter, soil, sediment) must be analysed for a longer period of time.

Authors' Contributions

Conceptualization: SLB and ORB; Data curation: SLB, REI and ORB; Formal analysis: DC; Funding acquisition: ORB; Investigation: CN and ORB; Methodology: REI, MB and ORB; Project administration: ORB; Resources: REI and ORB; Software: REI and ORB; Supervision: ORB; Validation: REI and ORB; Visualization: MB, REI and ORB; Writing - original draft: SLB; Writing - review and editing: REI and ORB. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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