

Article

Qualitative and Quantitative Changes in Soil Organic Compounds in Central European Oak Forests with Different Annual Average Precipitation

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Abstract: The various climate scenarios consistently predict warming and drying of forests in Hungary. Soils play a significant role in the long-term sequestration of atmospheric CO₂, while in other cases they can also become net carbon emitters. Therefore, it is important to know what can be expected regarding future changes in the carbon storage capacity of soils in forests. We used precipitation gradient studies to solve this problem, using a type of “space–time” substitution. In this research, we primarily examined the quality parameters of soil organic matter (SOM) to investigate how climate change transforms the ratio of the main SOM compound groups in soils. For our studies, we applied elemental and ¹³C and ¹⁵N isotopic ratio analysis, NMR analysis, FT-IR spectra analysis, thermogravimetric and differential thermal analyses to measure SOM chemistry in samples from different oak forests with contrasting mean annual precipitation from Central Europe. Our results showed that soil organic carbon (SOC) was lower in soils of humid forests due to the enhanced decomposition processes and the leaching of Ca, which stabilizes SOM; however, in particular, the amount of easily degradable SOM compounds (e.g., thermolabile SOM, O-alkyl carbon, carboxylic and carbonyl carbon) decreased. In dry forest soils, the amount of recalcitrant SOM (e.g., thermostable SOM, alkyl carbon, aromatic and phenolic carbon and organo–mineral complexes stabilized by Ca increased, but the amount of easily degradable SOM increased further. The main conclusion of our study is that SOC can increase in forests that become drier, compensating somewhat for the decrease in forest plant biomass.

Keywords: soil biology; decomposition; forest soil; NMR; FT-IR; carbon; climate change

1. Introduction

Global climate change is altering ecosystem net primary productivity in forests [1], which may alter above- and below-ground litter deposition. Furthermore, it has a significant impact on decomposition processes by changing the microclimate, which affects the amount of soil organic carbon (SOC) stored in soils [2]. Forests in many regions have responded to elevated atmospheric CO₂ with increased growth [3]. However, in some of the drier areas (forests with continental or sub-Mediterranean influences, which are drier and warmer in summer than the more humid mesophytic forests with oceanic influences), these effects are less pronounced or non-existent [4]. Annual average temperatures have

been rising rapidly around the world for the last four decades, and the frequency of extreme droughts has grown in many areas of the world. This trend is expected to intensify in the future [5,6]. The number of droughts and extreme droughts will double in the coming decades, which will subsequently increase the risk of mortality for sensitive tree species [7]. State-of-the-art results have identified the complex effects of drought, fungi, insects and other factors as reasons for oak decline: it has been found that drought weakens the trees, which are then more easily destroyed by pests [8]. The effects of temperature and precipitation on the carbon (C) dynamics of the forest and forest soils have been reported in numerous studies [9,10]. This process can result in increased detritus inputs (for a period of time, then the process goes into decline) due to the dead trees. This phenomenon was observed in our earlier research at the Sikkókút experimental forest site, where in recent decades 70% of the sessile oak stand declined [11] along with many other forest areas in Hungary [5]; this has also affected the C content of the soil [4].

The capacity of terrestrial pools to store carbon and their expected responses to warmer temperatures need to be elucidated to better predict the future impacts of climate change [12,13]. The reaction of SOM decomposition to global environmental changes is of outstanding importance from the point of view of the global carbon cycle [14]. Increasing SOC has the potential to contribute to climate mitigation while promoting soil fertility, health and resilience against drought, soil contamination or erosion. One of the concerns about SOC is the uncertainty about the stability of the newly sequestered C [15]. Decomposition depends on several factors that change simultaneously as a result of global environmental changes [16–18]. It is widely known that climate change could significantly influence the annual distribution and amount of precipitation, as well as the amount of evapotranspiration. All these factors significantly affect the dynamics of SOM. In fact, soil moisture has a strong influence on decomposition processes as it has a direct effect on the availability of substrates for microbes and on the activity of microbes [19,20]. Whether the soil C pool acts as an emitter or a sequester of atmospheric CO₂ depends to a large extent on how climate change affects the moisture content of soils, which can significantly affect the activity of decomposition processes [21].

The plant debris on and in the soil is biophysically degraded to various biochemical products, e.g., low molecular weight compounds (monosaccharides, glucose, etc.), high molecular weight biopolymers (e.g., lignin) or a range of compounds of different chemistries and persistence in soil. [22,23]. Based on its decomposability and availability to soil microorganisms, SOC is usually classified into labile and recalcitrant soil carbon. The recalcitrant carbon generally refers to the components of SOM that are resistant to microbial decomposition or protected by mineral soil particles, while labile carbon mainly consists of dissolved organic matter and easily oxidative organic matter [24].

We have little information about the effect of a drier climate due to climate change and, in this context, the migration of vegetation zones on the soils; within that, there is also little information about the quality parameters of SOM. Precipitation gradient studies help fill this gap by exploiting the “space-for-time” substitution. In our previous study, we investigated the carbon content of the soils of Hungarian oak forests under different types of primary production along different precipitation gradients. We studied the carbon content of soils at 30 forest sites. This study showed that two-and-a-half times more SOC was accumulated in the soils of dry forests than in the soils of humid forests in the upper 30 cm layer [25]. However, at that time, we were only able to examine the parameters indicating the quality of the SOM to a limited extent. Here, we examine the SOM of dry, meso and humid forests with the help of various techniques, including spectroscopic techniques (FTIR and NMR) that characterize the structural composition of SOM, and thermogravimetry and differential thermal analysis which distinguish and quantify labile and recalcitrant substances of the overall SOM. We also used isotopic techniques that can highlight the source and transformations from plant residues to SOM in different climatic zones. Thermal stability of SOM is found to be strongly and positively correlated with its

resistance to biological decomposition. [26,27] since environmental recalcitrance is intimately connected with the thermal stability associated with structural factors (e.g., aromaticity and ring condensation) of plant litter and SOM [28]. The stability of SOM, which represents its resistance to decomposition [26], can play a significant role in mitigating the effects of climate change [29,30]. The techniques used, such as thermogravimetry (TG) and spectroscopic techniques, are increasingly being used to study SOM stability in several soil processes [31–33]. According to our hypothesis, more easily decomposed plant debris can only accumulate to a lesser extent in the soil of humid forests than in the soil of dry forests. On the other hand, according to our other hypothesis, the amount of recalcitrant, or permanently stored organic matter is also greater in drier areas.

2. Materials and Methods

2.1. Sites Description, Experimental Design and Soil Sampling

The sites selected for our study occur along a precipitation gradient in Hungary where the mean annual precipitation (Table 1) differs, but the original parent material, forest stand features and particle size distribution are similar. The texture of the topsoil layer was silty clay loam or silty clay, and there was little difference in the clay content (soils of humid oak forests ($36.2\% \pm 1.51\%$), soils of mesoforests ($39.1\% \pm 2.46\%$) and soils of dry forests ($39.2\% \pm 2.11\%$)) [25]. The climatic data of these sites were compiled from the daily gridded climate dataset of the Hungarian Meteorological Service (HMS, <https://odp.met.hu/> (accessed date 20 November 2022)) with approx. 10 km spatial resolution for the period 1971–2020 (Table 1).

We chose sites with similar characteristics, i.e., with deep zonal soils (where there was no stagnant water at the investigated depths) formed from similar parent materials (loess and weathered dust both settled at the end of the Ice Age) and with similar forest vegetation—zonal oak seedling stands (mainly the *Quercetum petraeae—cerris* and *Quercus-Carpinetum* community). The weight of the mean leaf litter in 2018 was 992 ± 101.4 ^b in humid forests, 681 ± 24.9 ^a in meso forests and 723 ± 46.4 ^a [25] in dry forests.

We excluded wetlands and steep to medium slopes from this analysis to avoid erosion/deposition effects; only well-drained sites with flat or slight slopes (1–2%) were selected. The differences between the soils of the sites (Luvisols, Cambisols, Phaeozems and Chernozems) are explained by macroclimatic factors (for example, annual average precipitation, annual average temperature, the amount of solar radiation, humidity of the overlying regional air mass, temperature of the growing season, precipitation of the growing season and vapor pressure deficit) and associated vegetation in the past (Table 1, Figure 1).

Table 1. Sites in this study (MAT: Mean Annual Temperature, MAP: Mean Annual Precipitation), partly based on [25].

Name of the Site	Coordinates		Forest Types	MAP (mm)	MAT (C)	Type of Soil
	N°	E°				
Zalaegerszeg	46.8180	16.8147	Humid	695	10.3	Luvisols
Bak	46.7551	16.8059	Humid	716	10.1	Luvisols
Szilvágý	46.7397	16.6521	Humid	722	10.1	Luvisols
Bajánysenyé	46.8230	16.4029	Humid	733	10.2	Luvisols
Ispánk	46.8785	16.4400	Humid	726	9.9	Luvisols
Hévízgyörk	47.6170	19.5032	Meso	556	10.4	Cambisols
Kerepes	47.4014	19.1550	Meso	574	10	Cambisols
Sirok	47.9221	20.1718	Meso	565	9	Luvisols
Kerecsend	47.7925	20.3249	Dry	570	10.3	Chernozems
South-Demjén	47.8306	20.3556	Dry	581	10.1	Phaeozems
Aszaló	48.2281	20.9331	Dry	558	9.8	Phaeozems
Füle	47.0281	18.2719	Dry	571	10.7	Chernozems
Galgamácsa	47.5027	19.2608	Dry	550	10.6	Phaeozems

Füzesabony	47.4916	20.2708	Dry	553	10.4	Chernozems
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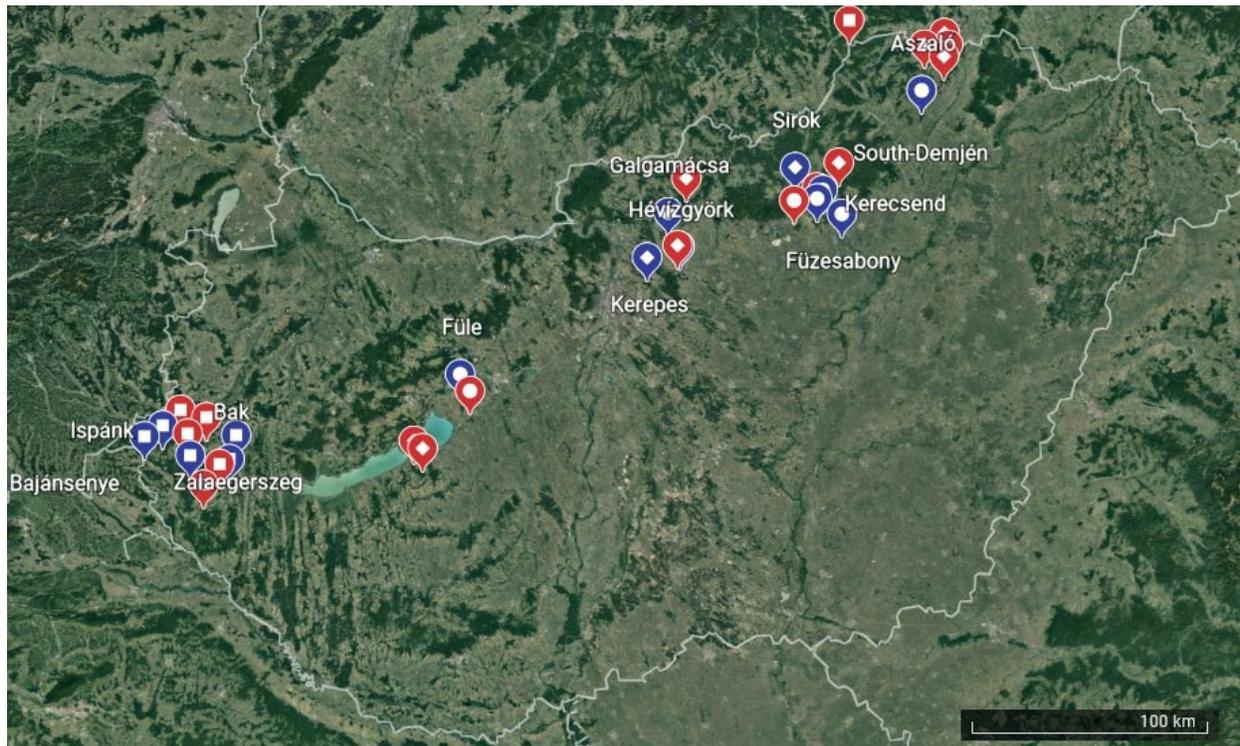


Figure 1. The sites where the instrumental analytical methods were used in this study are marked in blue (the names of the sites are indicated), while the previously examined sites where the C content of the soils and some other parameters were examined are marked in red [25]. (□ = humid; ◇ = meso; ○ = dry).

Forests have been expanding in the studied areas for at least a century, but probably for centuries or perhaps thousands of years in most places. The pH of the soils also developed along this gradient, which varied between 4.71 and 6.95 in parallel with the rainfall conditions. The sites were not affected by direct anthropogenic effects (apart from forestry interventions). Forest management afforested the sessile oak stands within a wide spectrum of climates in Hungary. Other tree species may also appear in the sessile oak stands, which are indicators of the humidity of the climate; however, in all sites, sessile oak is the dominant species. Using these indicator species, we classified the studied forest sites into the three applied forest categories (dry, meso and humid). According to the Map of natural vegetation of Europe [34], the absence of hornbeam (*Carpinus betulus*) indicates the xeric limit of the mesophytic forests. In the thermophilous dry oak forests, there are no European hornbeams (*Carpinus betulus*) [35]. The more frequent appearance of beech (*Fagus sylvatica*) in the understory of the afforested sessile oak stands indicates that the stands are already in the humid part of the mesophytic climate according to the forestry climate categories in Hungary. Hence, in this research, we consider oak forests with the presence of the hornbeams but where beech has not yet appeared as a mesoforest. [5]. Another approach yielded almost identical results. The three forest types were defined by the Broken Stick model using the values of the Ellenberg index and the SOC stock of each site. The breaking points marked the border between forest types [25].

The soil was sampled in December 2017. Soil samples were collected from three layers (0–5, 5–15 and 15–30 cm) in mineral soil with a 20 mm diameter Pürckhauer1175/1000 mm soil corer (Bürkle GmbH) at five randomly selected locations in each plot (randomly located 10 × 10 m square) and were combined to form composite samples for each layer. The soil samples were dried, sieved (remaining plant debris, mainly root pieces, were

carefully removed with tweezers) and ground where necessary. We performed elemental analysis and solid-state ^{13}C nuclear magnetic resonance analysis of three soil layers, while the rest, i.e., the ^{13}C and ^{15}N isotopic ratio analysis, analysis of FT-IR spectra, thermogravimetric (TG) and differential thermal (DTA), were completed only for the upper layer.

2.2. Elemental and ^{13}C and ^{15}N Isotopic Ratio Analysis

Soil organic C, total N and their isotopic ratios were measured by CF-IRMS (continuous-flow isotope ratio mass spectrometry) with an elemental analyzer (Flash 2000 Thermo Fisher Scientific, Massachusetts, USA) coupled with an isotope ratio mass spectrometer (IRMS) (Delta V Advantage Thermo-Fisher Scientific Massachusetts, USA). Soil samples were pre-treated with HCl 6M in order to eliminate inorganic C, as described in [36]. The isotopic composition of the samples is expressed as units of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ using Pee Dee Belemite (PDB) and air atmosphere as standard for C and N. The pH value of the soil was measured from an aqueous suspension. A quantity of 12.5 mL of water was added to 5 g of soil and the pH values of the samples were measured after standing for 24 h using an Adwa AD8000 instrument (Debrecen, Hungary). In this study, we examined the ^{13}C and ^{15}N isotopic ratio of the soils of five humid, three meso and six dry sites at 0–5 cm depths.

Furthermore, total C concentrations in ground and homogenized soil samples at all three depths (0–5, 5–15 and 15–30 cm) were determined using a combustion analyzer (VarioMax CN analyser, ElementarAnalysensysteme GmbH, Hanau, Germany).

2.3. Solid-State ^{13}C Nuclear Magnetic Resonance (NMR) Analysis

The overall structural composition of soil organic matter was measured using solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Soil samples were extracted with hydrogen fluoride (10% *v/v*) repeatedly to concentrate the soil organic matter and remove minerals which may interfere with the analysis [37]. After extraction, samples were repeatedly rinsed with deionized water and freeze dried. Samples were then analyzed by cross polarization-magic angle spinning NMR, using previously published methods [38]. NMR spectra were processed with 50 Hz line broadening and integrated into four general structural regions: alkyl (0–50 ppm), *O*-alkyl (50–110 ppm), aromatic and phenolic (110–165 ppm) and carboxyl and carbonyl (165–200 ppm; [39]). Integrated regions were normalized to the total NMR signal and expressed as a percentage. The ratios of alkyl to *O*-alkyl carbon were calculated by dividing the integrated area of the alkyl region by the integrated area of the *O*-alkyl region. This ratio increases with progressive soil organic matter decomposition due to the preferential use of *O*-alkyl carbon by soil microbiota [40]. In this study, we examined the soils of five humid, three meso and six dry sites at three depths (0–5, 5–15 and 15–30 cm).

2.4. Analysis of FT-IR Spectra

FT-IR spectroscopy can provide insight into SOM quality because it enables identification of the chemical building blocks that make up SOM (i.e., moieties), including labile C pools relevant to nutrient mineralization [41–43].

FT-IR spectra of soils were recorded with a Bruker ALFA FT-IR Spectrophotometer (Bruker, Ettlingen, Germany) equipped with an attenuated total reflection (ATR) diamond crystal sampling device. The spectra were collected from 4000 to 400 cm^{-1} and averaged over 64 scans (resolution 4 cm^{-1}). Soil samples were ball milled and less than 1 mg of sample was used for analyses. Isopropyl alcohol was used to clean the diamond crystal before each analysis. Background spectrum using the diamond crystal was recorded before the collection of each sample spectrum. All spectra were preprocessed using baseline correction and normalized to set the baseline and shift the intensities of the spectra so that the minimum absorbance value was zero. Five replicates were performed. In all spectra, the

region between 1200 and 400 was dominated by strong bands attributed to mineral compounds. Therefore, we analyzed the region between 1800 and 1250 cm^{-1} corresponding to the most important functional groups of SOM by using the curve-fitting analysis with Grams/386 spectroscopic software (version 6.00, Galactic Industries Corporation, Salem, NH, USA).

All spectra were fitted with Gaussian bands; the best fitting parameters were determined by minimization of the reduced Chi square (χ^2). Good agreement between experimental and calculated profiles was obtained, with coefficients of determination (R^2) ranging from 0.999 to 0.988 and the standard error (SE) from 0.0001 to 0.001 [44]. These values indicate the accuracy and efficacy of the fitting process. At identified peaks, an integration was performed to calculate the peak area. Peak area integration of single peaks was processed and converted into peak area percentage of a single peak for each sample. It was used as relative quantity of different functional groups. Five replicates were performed for spectra analysis. Absorbance intensity can be used to fingerprint SOM composition, and specific spectral features (e.g., bands) offer a means to monitor compositional changes induced by the different climatic backgrounds. In this study, we examined the soils of the soils of five humid, three meso and six dry sites at 0–5 cm depths.

2.5. Thermogravimetric (TG) and Differential Thermal (DTA) Analyses

Thermogravimetric (TG) and differential thermal (DTA) analyses were carried out simultaneously using a TG-DTA92B instrument (SETARAM, France). Samples were loaded (about 30 mg) into an alumina crucible with an identical empty crucible used as a reference. Samples were heated from ambient ($\approx 25^\circ\text{C}$) to 750°C at $10^\circ\text{C min}^{-1}$; the furnace atmosphere consisted of Ultra-Zero Grade air ($<1\text{ ppm CO}_2$) flowing at 130 mL min^{-1} . TG-DTA analysis involves continuous and simultaneous measurement of weight loss (TG) and energy change (DTA) during the heating of samples [25]. The thermo-analysis curves (TG-DTA) of the dry, meso and humid soils were characterized by an endothermic peak ranging between 50 and 120°C , related to hygroscopic water loss, and by two exothermic peaks. One peak occurs in the 250 to 400°C region and the second peak occurs in the 400– 550°C interval; this is related to the presence of a thermolabile and a more recalcitrant component, respectively. In this study, we examined the soils of six dry, three meso and five humid sites at 0–5 cm depths.

2.6. Statistical Methods

Statistical analyses were performed using ANOVA and Tukey's HSD test. We compared different parameters of humid, meso and dry soils. The significant differences were considered when $p \leq 0.05$.

3. Results and Discussion

3.1. SOC Content in the Different Forest Types

We found a significant difference in the SOC content of the soils of the humid, meso and dry forests (except in the top layer between meso and dry), as well as in the SOC content of the soil layers of the same forest types (Figure 2).

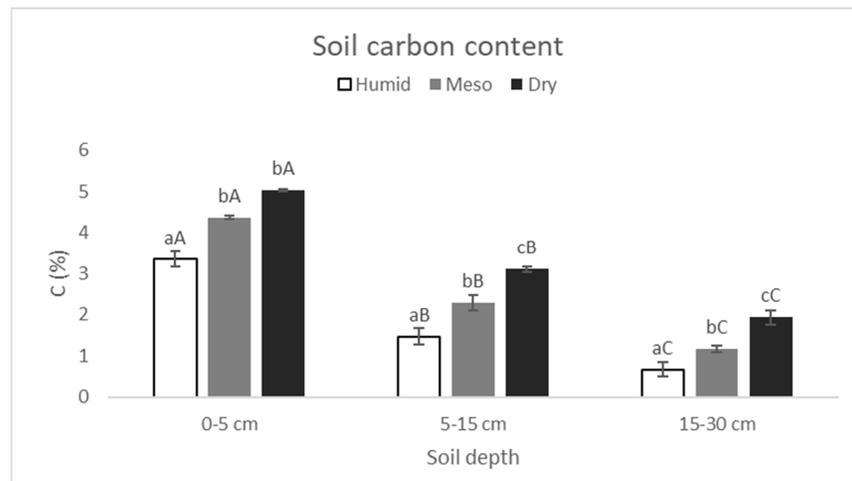


Figure 2. SOC content of humid, meso and dry forest soils in 3 different soil layers. Different lowercase letters indicate differences between SOC of forest types (comparing columns of different colors), while different uppercase letters indicate differences between soil layers (comparing columns of the same color).

3.2. Stable Isotope Composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of SOC

The isotope ratio of the SOC of dry, meso and humid forests can provide valuable data on the history of vegetation (Table 2). The basis of this approach is that each SOM compound inherits a specific isotopic ratio, which results from its natural origin and its transformation path after deposition.

Table 2. SOC, N content of humid, meso and dry soils, as well as stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of SOC in the top 5 cm layer of the soils and standard error values (SE). Number of repetitions in groups (n): dry 6; meso 3; humid 5. Different lowercase letters indicate differences between the soil parameters of forest types (humid, meso, dry). C/N is molar ratio. Different letters indicate differences between forest types.

	Humid	Meso	Dry
SOC%	3.4 ± 0.2 a	4.4 ± 0.2 b	5.0 ± 0.2 b
N%	0.24 ± 0.01 a	0.31 ± 0.01 b	0.39 ± 0.01 c
C/N	12	12	11
$\delta^{13}\text{C}\text{‰}$	-26.3 ± 0.1 a	-25.9 ± 0.1 b	-25.8 ± 0.1 b
$\delta^{15}\text{N}\text{‰}$	-0.57 ± 0.10 a	0.33 ± 0.09 b	0.95 ± 0.26 b

The low $\delta^{13}\text{C}$ values (between -25.40‰ and -26.78‰) we measured in soils indicate that woodlands, groves and shrub clusters are dominated exclusively by C3 plants. The C3 plants also dominated both humid, meso and dry forests. As such, C4 grasses were not widespread in this area before, at least within the time frame that can be observed through this study. These data also show that the differences in the carbon isotopic composition between the investigated regions were caused by the climate and not from changes in vegetation.

We measured lower (more negative) values in the soils of humid forests, which was followed by the meso and then the dry. Carbon isotopic fractionation in C3 plants is strongly linked to water availability, and more negative $\delta^{13}\text{C}$ values in more humid regions are indicative of higher transpiration and lower water use efficiency. In contrast, water deficiency generally led to an increase in $\delta^{13}\text{C}$ values [36–38]. Moreover, plant components differ in their C isotopic composition. Generally, leaves are ^{13}C -depleted when compared to branches, stems and roots [45,46]. In previous work on the same forest sites [25], a greater amount of leaf litterfall was measured in autumn in the humid as compared

to the dry forest; therefore, in these sites (in humid forests), a greater portion of ^{13}C -depleted substrates were decomposed and entered the SOM. Furthermore, a greater quantity of woody detritus relative to leaves provided C substrates more enriched in ^{13}C . The soils of dry, meso and humid forests have different climatic and pedoclimatic conditions (mainly soil moisture) which affect the rate of the decomposition processes. This is also indicated by the amount of fungal biomass measured in the soils of each forest type and the differences in enzyme activities [25]. These can explain the quantitative and qualitative differences in SOM between the three types, which can affect the degrading organisms as well. On the one hand, the root mass ratio is higher when compared to the biomass ratio above the surface [47]; on the other hand, the death of trees due to desiccation has increased in the meso and dry forests within the last four decades due to the drier climate. In the humid forests, this did not occur to the same extent; thus, the proportion of plant inputs with a high lignin content is also more significant in the detritus of meso and dry forests than in humid forests [4,5]. In the dry (and partly the meso) forests, the growth and activity of wood saprotrophs was probably more prevailing than in the humid forests, and this might have further contributed to the ^{13}C enrichment of SOM since this fungal community is characterized by a higher content of ^{13}C when compared to the community of litter saprotrophs [46]. The fractionation of ^{13}C in soil is due to multiple processes such as microbial transformation and leaching following the deposition of the plant material [45,48], but usually these are also affected by the climate of the given area, including humidity, which significantly affects both leaching and microbial processes [49,50].

The $\delta^{15}\text{N}$ values, which show the degree of decomposition of N-containing organic materials in soils [51] reflected the same trend of the $\delta^{13}\text{C}$. In this study, significantly different values were obtained between the different forest areas (for humid forest soils $-0.57 \pm 0.1\text{‰}$, for meso forests $0.33 \pm 0.1\text{‰}$, and for dry forests $0.95 \pm 0.3\text{‰}$). Values between -2‰ and -4‰ are typical for fresh litter and living tissues and are generally higher at sites characterized by higher mean annual temperature and lower mean annual precipitation [52]. These values pass into the positive range over time due to decomposition, microbial utilization cycles and stabilization on organo–mineral complexes in the soil [53]. The progressive ^{15}N enrichment depends on the extent to which the organic N of the litter is decomposed and transformed by the microbial biomass into the N-containing compounds in the soil. Furthermore, ^{15}N enrichment shows a correlation with the degree of stabilization and age of the compounds. In general, one of the important sources of N-containing compounds is chitin, which enters the soil mainly from the cell walls of fungi that are driving decomposition processes [54]. In humid forests, we measured a higher fungal biomass (more 60%) than in dry forests; however, the amount of other recalcitrant compounds and organo–mineral complexes (which are older than chitin) are much lower [25]. The results of our previous study also showed that the SOM from the soils of humid forests is significantly younger (these are about half as old as the SOC of dry forest soils) [25]. This may explain the significantly lower $\delta^{15}\text{N}$ values in the soils of humid forests.

3.3. Thermogravimetric (TG) and Differential Thermal (DTA) Analyses

For dry soils, the first exothermic reaction ($337 \pm 5 \text{ }^\circ\text{C}$) corresponded to a mass loss of 6.8%, while for the second exothermic peak ($506 \pm 5 \text{ }^\circ\text{C}$) the mass loss was 3.4%. In the case of meso soil samples, the two exothermic reactions occurred at $336 \pm 5 \text{ }^\circ\text{C}$ (6.0%) and $510 \pm 5 \text{ }^\circ\text{C}$ (3.0%), respectively. For the humid soils, the first exothermic peak occurred at a lower temperature ($320 \pm 5 \text{ }^\circ\text{C}$) than for the dry or meso soils, with a mass loss of 4.9%. The second exothermic peak appeared at higher temperature ($530 \pm 5 \text{ }^\circ\text{C}$) with a mass loss of 2%. The mass loss at both exothermic peaks was significantly higher in dry forest soils than in humid forest soils. The meso samples showed a significantly higher value than humid only for the second exothermic peak. There was no significant difference between meso and dry for any of the exothermic peaks.

Humid, meso and dry forest soils showed different thermogravimetric patterns; particularly, the dry and meso forest soils had a higher proportion of compounds with greater

thermal stability than humid forest ones [25]. The first exothermic peak at 320–337 °C is considered to be the result of thermal combustion of thermolabile SOM. The second exothermic peak at 506–530 °C is related to the combustion of more thermal resistant compounds.

The humid forest sites have the most intense activity of degrading organisms during the warmer periods of the year, but only when sufficient moisture is available [25]. The climate of the dry forest sites was consistently drier over a longer period of time (centuries, millennia) than the humid forests, especially in the summer, which allowed more organic matter to accumulate in the soils due to slower decomposition. This was supported by our previously conducted radiocarbon (^{14}C) studies on these sites which found that the turnover time of SOC in soils of dry forests is roughly twice as long as the humid forests [25].

The longer turnover time made it possible for the organic matter entering the soil to be transformed in a greater proportion into more recalcitrant SOC. Due to more precipitation, a larger amount of water flows through the soil which increases the dissolution and leaching processes; however, different pH values can also affect these processes. Therefore, the various leaching processes were also lower in the drier areas and are exemplified by the significant quantitative difference in calcium (Ca) between the soils of dry and humid forests. In the soils of humid forests, not only higher precipitation, but also lower pH could increase Ca loss [55].

Ca forms can play an important role in SOC accumulation [56], increasing the stability of SOC compounds through several potential mechanisms [57,58]. In the soils of dry forests, there is more than seven times as much Ca (while the amount of CaCO_3 was negligible in the examined soils) as in the case of the soils of humid forests [25]. This may indicate a different amount of Ca incorporated into organic matter [59]. A higher Ca content can increase the thermostability of SOC [60]. Simultaneously, organic materials from debris are present in greater quantities in dry forest areas because of slower decomposition than in humid forests. Consequently, this may represent a kind of enrichment of particulate organic material (POM). This is supported by the results of solid-state ^{13}C nuclear magnetic resonance (NMR) analysis (Table 3) which also demonstrates a difference in SOC chemistry and overall decomposition.

Table 3. Solid-state ^{13}C nuclear magnetic resonance (NMR) integration results and SE values. Number of repetitions in groups (n) is 3.

Forest Types	Depth (cm)	Alkyl C (0–50 ppm)	O-Alkyl C (50–110 ppm)	Aromatic + Phenolic C (110–165 ppm)	Carboxyl + Carbonyl C (165–220 ppm)	Total Signal	Alkyl/O-Alkyl C Ratio	Potential Resistance to Decomposition Ratio
humid	0–5	33.3 ± 2.02	52.7 ± 0.57	10.3 ± 1.0	3.8 ± 0.9	100	0.63 ± 0.05	0.78 ± 0.04
humid	5–15	34.3 ± 0.29	51.6 ± 1.44	9.5 ± 0.9	4.6 ± 0.6	100	0.66 ± 0.03	0.78 ± 0.03
humid	15–30	36.4 ± 2.60	49.8 ± 1.70	10.1 ± 0.6	3.8 ± 0.0	100	0.74 ± 0.08	0.87 ± 0.06
dry	0–5	27.6 ± 3.0	51.8 ± 1.50	14.0 ± 1.2	6.6 ± 0.9	100	0.54 ± 0.07	0.72 ± 0.05
dry	5–15	26.1 ± 0.60	51.2 ± 0.60	14.6 ± 0.9	8.0 ± 0.0	100	0.51 ± 0.01	0.69 ± 0.01
dry	15–30	24.9 ± 0.30	49.1 ± 1.20	17.0 ± 1.2	9.1 ± 0.3	100	0.51 ± 0.01	0.72 ± 0.02

SOM decomposition is calculated from the ratio of alkyl/O-alkyl carbon and increases with progressive degradation [40]. Potential resistance to decomposition is calculated from the ratio of (alkyl carbon + aromatic and phenolic carbon/O-alkyl carbon + carboxylic and carbonyl carbon) and higher values signify greater potential for persistence based on the SOM chemical composition [61].

3.4. Solid-State ^{13}C Nuclear Magnetic Resonance (NMR) Analysis of the SOM of Dry and Humid Forests

The alkyl/O-alkyl C ratio is a relative indicator of SOM decomposition (as SOC compounds that resonate in the O-alkyl region, such as cellulose, are preferentially used by soil microbiota, the ratio increases) [40]. The progressive decomposition of SOM results in

higher alkyl/O-alkyl C ratios because of the selective preservation and accumulation of SOC compounds that resonate in the alkyl C region [40]. Solid-state ^{13}C data revealed that, overall, soil samples collected from the humid forests contained SOM that was relatively more decomposed (the weighted average of the 0–30 cm layer for humid forest soils is 0.69 ± 0.06) than the drier forest soils (0.52 ± 0.02) (Table 3). At all depths analyzed, the relative SOM decomposition (alkyl/O-alkyl carbon) ratio is higher for humid area soils, indicating that the SOM chemistry is sensitive to moisture conditions, specifically via different turnover processes [40]. Increased organic matter degradation in response to higher precipitation (and soil moisture content) is likely due to increase in microbial biomass and activity [25].

Interestingly, differences in SOM decomposition between humid and dry sites were observed, but the extent of these differences varied. The NMR integration results clearly reflect these differences between humid and dry forest soils. This is further supported by the potential resistance to decomposition ratio where the proportion of more persistent forms of SOM (alkyl and aromatic and phenolic carbon) is compared to shorter-lived SOM components (O-alkyl, carboxyl and carbonyl carbon) [61]. In a drier environment, compounds that typically would decompose faster (O-alkyl C) accumulate relatively more than in a wetter environment where they would decompose faster. Thus, the potential resistance to decomposition ratio in humid forest soils is higher (0.83 ± 0.05 —the weighted average of the 0–30 cm layer) than in dry forest soils (0.71 ± 0.02 —the weighted average of the 0–30 cm layer) and is consistent with the SOM decomposition ratio (alkyl/O-alkyl C ratio). Sampling took place in late autumn, during the leaf fall period, when the fresh litter had not yet decomposed enough, and hardly any organic matter could enter the mineral soils. The previous autumn's litter in the soils of the humid sites had almost completely decomposed and transformed due to the faster decomposition, while in the soils of the drier forests much more of this remains [25].

In the dry forest soils, the resonance between 65–95 ppm is higher and is representative of the easily decomposable substances and molecules that were found in litter (cellulose, simple sugars and amino acids/peptides). The NMR spectrum (Figure 3) shows a more significant peak (between 70–80 ppm) in the dry forest soils, indicating a greater accumulation likely due to slower decomposition at these sites. The chemical shift range between 110 and 165 ppm was attributed to the accumulation of lignin and other aromatic compounds. The soils of dry forests show higher values by 36% in the 0–5 cm layer, by 54% in the 5–15 cm layer and by 69% in the 15–30 cm layer as compared to the humid forests. Moving towards the deeper layers, the SOC difference between the soils of dry and humid forests also increases in favor of dry soils.

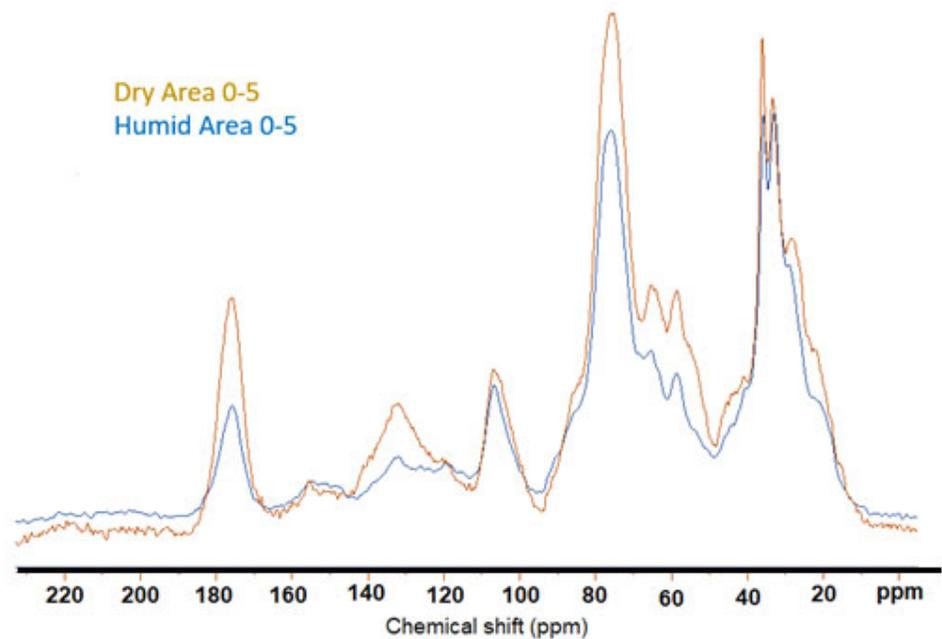


Figure 3. Solid-state ^{13}C NMR spectra of dry and humid forest soils.

In summary, relative to the dry forest soils, the higher soil moisture content resulted in enhanced soil organic matter degradation (increased ratio of alkyl/*O*-alkyl C) in the humid forest soils (under aerobic conditions). Increased organic matter degradation in response to higher precipitation (and soil moisture content) is likely due to shifts in microbial biomass and activity. Hence, the significantly higher SOC content of the soils of the dry forests is due both to the larger amount of faster decomposing POM and the accumulation of recalcitrant substances and organo–mineral complexes that are much more resistant to decomposition (potential resistance to decomposition ratio; Table 3). This was also supported by the results of infrared spectroscopy, which showed that aromatic, e.g., the proportion of lignin and other recalcitrant compounds in samples of dry forests, is higher than in humid forest soil samples. The selective preservation of lignin is only relevant during the early stages of litter decomposition, and latter lignin degradation occurs at the same or even higher rate as the overall litter decomposition [62,63]. Recent studies using solid-state ^{13}C NMR and pyrolysis techniques have confirmed that lignin is decomposed relatively quickly and does not appear to be stabilized in the long term in any soil fraction [64]. Thus, the accumulation of lignin compounds in the soils of dry forests with lower litter production (and lower lignin production) clearly shows the lower extent of the decomposition processes compared to the soils of humid forests [25].

3.5. FT-IR Spectra and Curve-Fitting Analysis

A curve-fitting process was performed to overcome the problem of overlapping bands in the region from 1800 to 1250 cm^{-1} . This region is particularly relevant because it contains several bands assigned to the SOM [65]. The deconvoluted spectra of dry, meso and humid forest soils are shown (Figure 4). The resolved bands (dotted line) were considerably different from the original spectra (solid line). A series of functional groups related to carboxylic acid (1724–1632 cm^{-1}), amide I (1669–1632 cm^{-1}) and amide II (1545 cm^{-1}), aromatic rings (1591, 1524 and 1500 cm^{-1}) and lignin (1524 cm^{-1}) derivatives and aliphatic chains (1460–1375 cm^{-1}) were found in deconvoluted spectra. In Table 4, tentative assignments of the deconvoluted bands were listed.

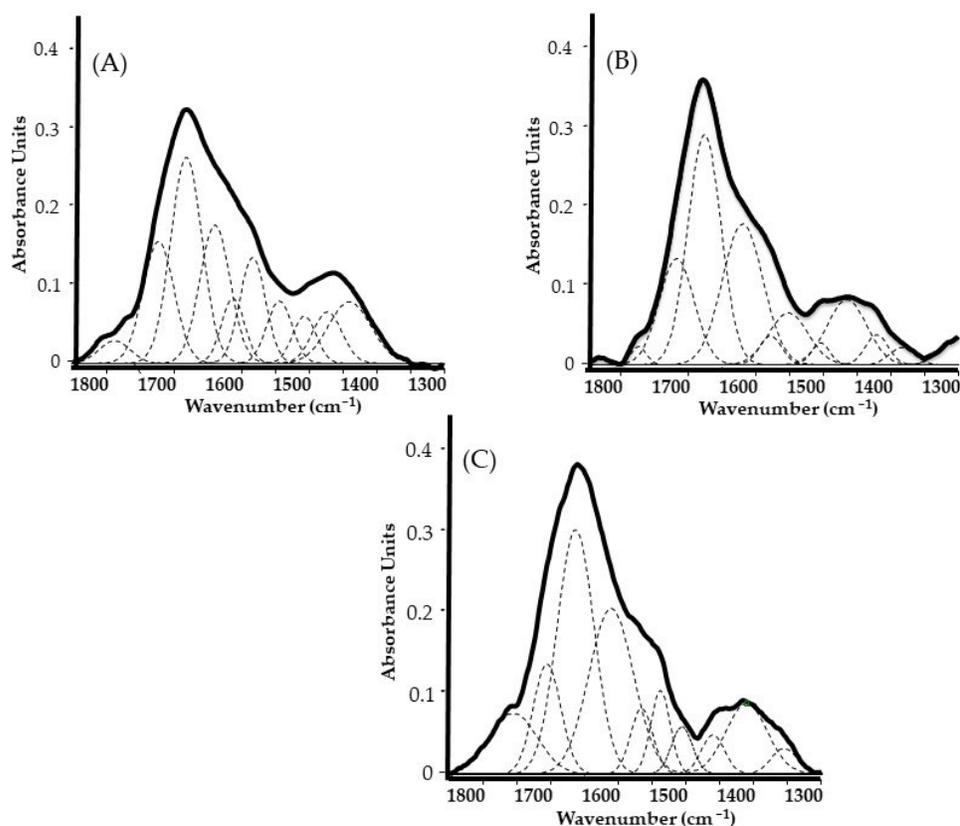


Figure 4. Original spectra (solid line) in the range from 1800 to 1250 cm^{-1} of humid (A), meso (B) and dry (C) soils and their deconvoluted spectra resolved using the curve-fitting process (dotted line).

Table 4. Tentative attributions of deconvoluted bands by using curve-fitting process of set samples soils according to [41,65–68].

Wave Number (cm^{-1})	Assignments	Humid (Area \pm SD%)	Meso (Area \pm SD%)	Dry (Area \pm SD%)
1724–1727	C = O stretching in carboxyl acids	7.4 \pm 1.7	0.5 \pm 0.01	5.0 \pm 0.7
1679–1669	Amide I; C = O stretching; C = C stretching in linear olefins;	19.0 \pm 1.3	12.0 \pm 2.2	11.7 \pm 1.9
1635–1632	C = O stretching in aryl acids Hydration water; C = C stretching in linear olefins and aromatic ring, (oleic acid, waxes, cutin)	25.9 \pm 2.4	29.9 \pm 3.1	28.6 \pm 3.0
1591–1583	Benzene derivatives C = C skeletal vibration	25.5 \pm 2.0	23.4 \pm 1.3	27.9 \pm 2.4
1561–1542	Amide II; COO ⁻ asymmetric stretching	7.4 \pm 3.1	2.8 \pm 1.1	8.6 \pm 1.2
1530–1524	Lignin	7.1 \pm 2.3	8.5 \pm 2.0	9.9 \pm 1.6
1508–1495	Benzene derivatives C = C skeletal vibration	nd	6.1 \pm 2.4	5.6 \pm 1.3

1460–1452	CH ₂ scissoring vibration and CH ₃ asymmetrical deformation	2.2 ± 1.7	5.1 ± 1.0	4.1 ± 0.9
1420–1395	CH ₂ deformation (e.g., fatty acid)	7.7 ± 2.2	5.6 ± 1.6	4.3 ± 1.4
1386–1250	CH ₃ symmetrical deformation; COO ⁻ symmetric stretching; C-O stretch in phenolic OH	4.8 ± 0.9	3.9 ± 1.2	8.1 ± 2.6

To provide more information on the semi-quantitative distribution of SOM functional groups within each soil sample, the integral values of the deconvoluted bands (% of total area) were used (Figure 4). Carboxyl groups (1724 cm⁻¹) accounted for 7.4% in humid and only 5% in dry. Amide I (~1669 cm⁻¹) accounted for 19% in humid and 12% in dry and meso (Table 4). These differences might be partially due to the low pH found in humid (5.1, while the average of the soils of meso forests is 6.6, and that of dry forests is 6.7). The vegetal residues rich in organic acids can affect soil acidity through decomposition processes facilitated by high precipitation [69]. The more intense leaching of alkaline earth metals (e.g., Ca) and alkaline metals (e.g., K) in the soils of humid forests reduces the pH of the soils, just as a larger amount of CO₂ formed due to more intensive soil respiration. A more acidic environment further increases the leaching of these nutrients [55]. The higher precipitation values resulted in a significantly lower Ca content in humid forest soils, which reduces the possibility of interaction between Ca and SOM compounds, and thus, the permanent formation of SOM as explained in Section 3.3 [25]. This might mean a considerable increase in the mineralization of organic matter. Aromatic structures (~1632 cm⁻¹; ~1583 cm⁻¹; ~1524 cm⁻¹; ~1495 cm⁻¹) accounted for 30–29%, 23–28%, 8–10% and 6% in meso and dry, and 26%, 25% and 7% in humid, respectively.

The last peak was missing in humid. The dry climate might contribute to an accumulation of undecomposed vegetal residues or lignin derivatives. This result is also corroborated by the accumulation of lignin and other aromatic compounds detected by solid-state ¹³C NMR (110–165 ppm) and the higher weight loss of the second exothermic peak of organic matter in dry soil. The aromaticity of SOM has been considered a key structural factor affecting environmental recalcitrance, so the number of aromatic structures significantly affects the turnover time of SOM [70,71].

Methyl terminal chains (~1450 cm⁻¹) accounted for 5%, 4% and 2% in meso, dry and humid, respectively. In contrast, methylene chains (~1420 cm⁻¹) accounted for 4% in dry and 8% in humid. This is also supported by the higher percentage of alkylic C found in this area (Table 3). The ratio of these compounds in soils significantly affects the rate of degradation processes (see what was described in the previous section).

4. Conclusions

Anthropogenic carbon emissions can be partially balanced by carbon (C) pools in vegetation and soil; however, this can only happen under certain conditions. We analyzed the SOM of the soils of different forest areas using several study methods. Our study showed that climate (and especially the differences in humidity) had a significant effect on the concentrations of SOC in the soils and the quality parameters of the SOM compounds in the humid, meso and dry sites in Hungary. These processes are strongly regulated by the climatic, edaphic and biotic backgrounds. Therefore, a large number of regional field studies is needed in order to obtain a more accurate global picture of the impact of climate change on SOC accumulation or reduction. In the drier forest areas we examined, both the more easily degradable and recalcitrant SOC accumulated in larger amounts than in the soils of humid forests. However, some more resistant compounds (e.g., aromatic + phenolic C) were more concentrated than the more easily degradable

compounds in the soils of dry forests. At the same time, the amount of easily decomposable organic compounds in dry forests also increased significantly; the breakdown of these compounds can take place quickly if the area becomes wetter with a change in the climate regime. In Hungary, there is little chance of this, as the climate scenarios predict further relative drying of the area in the coming decades. This suggests that the climate of drier oak forests and the accompanying slower decomposition also favor the accumulation of more recalcitrant SOC. The principal conclusion of our study is that the increase in SOC suggests that carbon storage in the soils of these ecosystems serves as a counterweight to the declining carbon storage of biomass in drier forest areas.

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