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Effect of Detritus Manipulation on Different Organic Matter Decompositions in Temperate Deciduous Forest Soils

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Abstract: Soil organic matter supply is mainly derived from plant litter. The early stages of litter degradation is a very dynamic process. Thus, its study is important for understanding litter degradation and the control factors of different biomes and ecosystems. In the frame of the Síkfőkút DIRT (Detritus Input and Removal Treatments) Project, the effect of organic matter treatment was studied on the rate of decomposition of organic matter by applying different kinds of organic materials (leaf and wood litter, green and rooibos tea material, and cellulose cotton wool). During long-term experiments, we intended to investigate how the different organic matter manipulations changed by the soil microbial community and how it affects the degradation of different quality organic matter in the soil. The important main purpose of the research was to investigate litter degradation and its main regulators, contributing to both current and future climate scenarios. According to our results, in the case of litter-doubling treatments, we experienced a greater loss of organic matter compared to the weight of the litter bags placed in the soil of organic matter-withdrawal treatments. Furthermore, based on our results, we found that the decomposition rate is influenced by litter quality (leaf and cellulose wool) that is to be decomposed and by the applied litter treatments depending on the time allowed for decomposition. A drier climate by slowing down the degradation processes and by increasing the proportion of recalcitrant molecules in the detritus may increase the turnover time, which may lead to an increase in soil organic carbon (SOC).

Keywords: soil organic matter; soil biology; climate change; tea litter; leaf litter; carbon dynamics; litter bags; litter manipulation

1. Introduction

Numerous literatures and research report that our soils are degraded due to inappropriate land use in recent decades [1–3]. Climate change also has a significant impact and contributes significantly to the current state of soils. A serious problem is the drastic reduction of soil organic matter for the reasons mentioned above. Soil's variable organic matter supply, quality and quantity determine not only the physical and chemical properties of soils but also their biological properties [4,5]. Organic matter

(SOM) forms the C and N reserves of soil; is involved in pH regulation, cation exchange and structure formation; and is a key substrate for soil microorganisms [6,7].

Organic materials in the soil undergo constant transformation. These changes in soils are all effects that have an impact not only on the short-term but also on the long-term future of agriculture [8,9]. Vegetation, including forest cover, plays an important role in the global C cycle and in stabilizing and mitigating the earth's climate [10]. Some of the carbon sequestered in the forests accumulates in the dendromass, and the rest sequesters in the soils. In the forest, only estimates of the carbon content of humus topsoil and mineral soil organic matter are available in the absence of a sufficient number of measurements [11]. At the same time, considerable amounts of carbon are also stored in the forest and soil as well as in dead wood.

More than 50% of net primary production is recycled to the soil through litter decomposition [12] and releases about 60 Pg of carbon per year to the atmosphere [13]. Depending on the type of ecosystem, soil organic carbon (SOC) is up to 30 t/ha at a depth of 1 meter in drier climates and 800 t/ha in wet climates [14]. The amount of SOC is determined by the loss of carbon dioxide from the decomposition of organic material from primary production [15]. Ecosystems and their soils are characterized by a high degree of variability in their steady state [16].

The decomposition of plant litter can be divided into at least two phases [17]. The early stages of degradation (about 0%–40% weight loss) are characterized by the dissolution of easily soluble compounds and the degradation of non-lignin-containing compounds, mainly cellulose and hemicellulose [18,19]. In the late stage (about 40%–100% weight loss), the decomposition of residues containing mainly lignin occurs [20,21].

Microbial degradation of organic substrates is generally regulated by both biotic and abiotic factors [22]. Many authors agree that, both regionally and globally, the degradation of the litter is influenced by the climate, the quality of the litter, and the composition of the soil meso- and microfauna community [23–27].

To understand the different degrees of decomposition and to more accurately estimate current and future global carbon flows, we have little known, often inefficient, or inaccurately measured or hard-to-compare databases. Numerous projects, programs or research networks for studying degradation processes have been established over the past decades and have many important databases over the years that are essential for aerobic (and organic) transformation [28–30]. Previous studies of the DIRT (Detrital Input and Removal Treatment) [31] research network have provided important information and data on the decomposition of litter and organic matter [32,33].

In the 1980s, significant wood loss occurred throughout Europe, resulting in the decay of a large proportion of the tree species that make up the oak forests and the composition of the species [34]. As a result of the decay of trees, the shrub layer of the forests and the soil received more light and moisture, which led to more intensive shrub growth and greater changes in the forest stand [27,35]. Changes in the forest stand also affected the forest soil dynamics. This was partly due to the fact that the soil received more light, that the soil surface was more irradiated and that the amount and quality of the litter covering the forest soil also changed.

For the long-term modeling of all these factors, in 2000, we established experimental plots in the field of research within the framework of the DIRT project. The aim of the project was to investigate how long-term changes in the qualitative and quantitative composition of plant litter input in different climatic conditions affected the soil organic matter accumulation and dynamics. These plots, which have been treated by organic matter manipulation for nearly 20 years, have provided a good opportunity to connect with the "TeaComposition Initiative" [26] and to complement these studies with specific organic matter studies. At the experimental plots, we investigated the degradation ability of microbial communities adapted to the organic matter input quality over the long term. In our investigation, we sought the answer to what was the effect of artificially changing the amount of organic matter on the rate of decomposition of different qualities of organic matter in the soil.

2. Materials and Methods

The experimental forest site (Síkfőkút Project) of 27 ha is located in the southern part of the Bükk Mountains in North Eastern Hungary at 325 m altitude, GPS coordinates N 47°55′, E 20°46′. This forest belongs to the Bükk National Park (Hungary), and the area has been protected since 1976. In the forest (*Quercetum petraeae-cerris* community), there is no active management since 1976, but there has been a legacy of intensive forest management that occurred before that time. The annual average precipitation amounts to 590 mm. The type of the soil according to the FAO (Food and Agriculture Organization of the United Nations) Soil Classification is Cambisol [36]. We examined the annual mean precipitation at the research site in Síkfőkút, based on the data from CARPATCLIM (high-resolution database of the Carpathian Region) and the FORESEE database (Open Database for Climate Change-Related Impact Studies in Central Europe).

The experimental aboveground and belowground litter manipulation plots were established in November 2000. We established one control and five litter manipulation treatments, each with three randomly located 7 × 7 m (49 m^2) replicate plots established under complete canopy cover [31,37]. There were two types of detritus addition treatments (Double Wood (DW) and Double Litter (DL)). In three treatments, detritus inputs were removed: No Litter (NL), No Roots plots (NR) and No Inputs (NI). Short description of the applied treatments are shown in Table 1.

Table 1. The applied DIRT (Detritus Input and Removal Treatments) at Síkfőkút Project site (Hungary) as part of the ILTER (International Long-Term Experimental Research).

Treatments	Description
Double Litter (DL)	Aboveground leaf inputs are doubled by adding leaf litter removed from NL plots.
Double Wood (DW)	Aboveground wood debris inputs are doubled by adding wood to each plot. Annual wood litter amount was measured by boxes placed at the site, and its double amount was applied in the case of every DW plots.
Control (C)	Normal litter inputs: Average litter amounts were typical to the given forest site.
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was totally removed by a rake. This process was replayed continuously during the year.
No Roots (NR)	The plots were trenched around 40 cm wide and 100 cm deep. The soil dug out was placed outside the plot. Root-proof Delta MS 500 PE foil, which was 0.6 mm thick and 1 m wide and of high density, was put in the trenches. Then, the trenches were filled with soil. So as to eliminate root production, plants were cleared (bushes had been cut out at the establishment).
No Inputs (NI)	Aboveground inputs are excluded from plots; the belowground inputs are provided as in NR plots. This treatment is the combination of NR+NL plots.

2.1. Placing Litter Bags in the Soil

Litter decomposition activity was modelled by using litter-test bags. The decomposed organic matter was studied by the litter bag method of Gosz et al. [26,38]. They were placed in the soil in two stages. The first part of the study was carried out 5 years after the establishment of the plots [39], and the second part was carried out after 16–19 years [40]. In the first phase, the bags containing leaf litter, wood and cotton wool were placed in 2004. In the second phase, green tea and rooibos tea were placed into the soil in 2016. The bags were placed at the A_h (humic) level (0–5 cm) for each treatment in both cases. Test bags were filled with known amounts of air-dried leaf litter, branch litter, green tea leaf (*Camellia sinensis*), rooibos tea (*Aspalanthus linearis*) and cellulose cotton wool. In every test plot, 15 of each bag was placed into the soil 5 cm deep. Three of each bag were taken out 3, 6, 12 and 24 months after being placed in the ground for further examination. The bags filled with organic material as the substrate were dried at 70 °C for 48 h. Laboratory processing of the bags was performed within one week from the date of sampling. These were cleaned by hand, taking care to preserve the substrate and to remove any foreign matter (soil and plant residues). Then, the bags were weighed and opened and its contents were poured into a paper bag. We noted separately if the bag was damaged. The weight of the incubated bags was determined, and then, the original weight of the bag was subtracted.

2.2. Laboratory Methods

The soil humus fractions were measured by the methods of Tyurin and Kononova [41] and of Hargitai [42]. The soil respiration was measured monthly by Soda Lime method simultaneously in all 18 plots on each collection date from 2010 to 2012 [43]. The soil moisture was measured monthly by conventional oven dry method at 105 °C [44].

2.3. Statistical Analyzes

For the evaluation of the results, a one-way ANOVA test was applicable to compare means among treatments. Normality assumption was proven by the Kolmogorov–Smirnov test ($p > 0.05$; $p = 0.200$) or by the Shapiro–Wilk test ($p > 0.05$; $p = 0.244$), and the homogeneity of variances was checked by Levene's test. Estimation was investigated by Scheffe post hoc test. SPSS statistics was used to all statistical analyses, and significant differences were set at a 95% confidence level [45].

3. Results

In the organic decomposition experiments (leaf litter, wood and cotton wool measurements) measured in 2004–2005, no significant differences were found in the decomposition of both litter and wood after 3 months of incubation is the soil. However, cotton wool already showed significant differences between the control and no-root treatments (Figure 1).

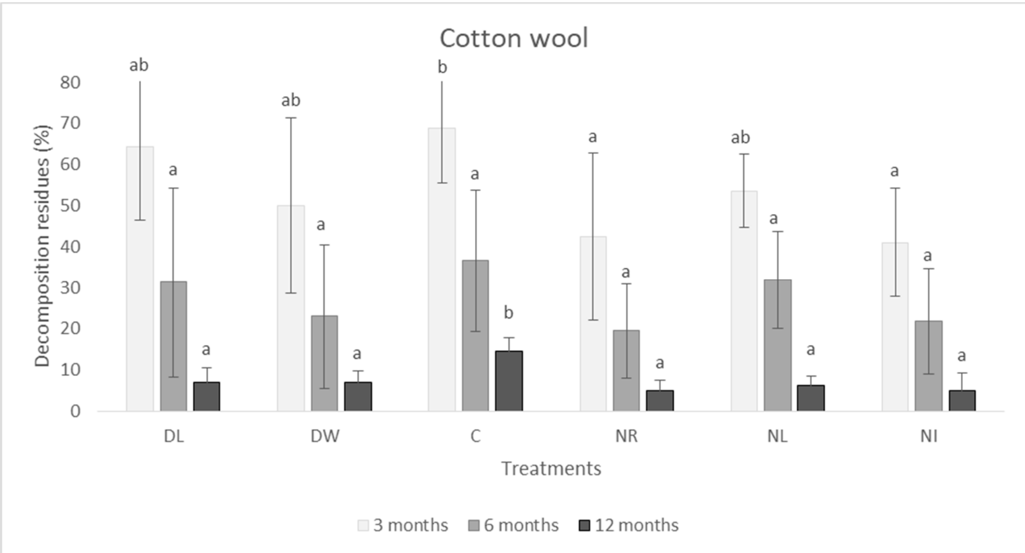

Figure 1. Effect of organic matter manipulation experiments with different DIRT treatments on cotton wool degradation rates after 3, 6 and 12 months: The letters represent significant differences between treatments during the given period ($n = 4$).

One of the wettest years of the last hundred years was 2010. At the Síkfőkút research site, the annual precipitation values exceeded 1000 mm, which means that almost twice as much precipitation fell as the many-year average and that precipitation fundamentally affects soil moisture values (Table 2).

Based on our studies in the summer of 2010, the CO_2 emission of the DL treatment was significantly higher than that of the NL treatment ($p < 0.05$), as was that in the spring for NL and NI and that in the autumn for all withdrawal treatments (NL, NR and NI) (Figure 2). A similar tendency was observed for both types of tea and for the relatively easily decomposing, homogeneously structured 100% cellulose cotton wool.

Table 2. Moisture content (%) of Síkfőkút soils in the studied periods.

Treatments	2004 Spring		2010 Summer		2011 Summer		2012 Summer		2016 Summer	
	Soil Moisture (%)	SE	Soil Moisture (%)	SE	Soil Moisture (%)	SE	Soil Moisture (%)	SE	Soil Moisture (%)	SE
DL	23.13	±1.73	29.55	±1.60	19.91	±3.09	15.52	±3.11	28.53	±2.21
DW	21.31	±1.16	27.18	±2.27	15.27	±1.74	14.07	±2.65	27.91	±3.28
C	23.13	±0.69	27.59	±1.53	16.68	±2.69	12.40	±2.72	27.91	±1.15
NL	21.74	±0.60	24.18	±1.01	13.92	±2.49	9.52	±2.65	23.68	±1.07
NR	25.30	v0.53	26.97	±0.60	17.67	±3.23	12.04	±3.19	25.43	±0.82
NI	24.15	±0.62	25.78	±0.90	14.15	±3.37	9.65	±3.18	24.72	±0.99

Abbreviations: DL = Double Litter; DW = Double Wood; C = Control; NL = No Litter; NR = No Roots; NI = No Inputs.

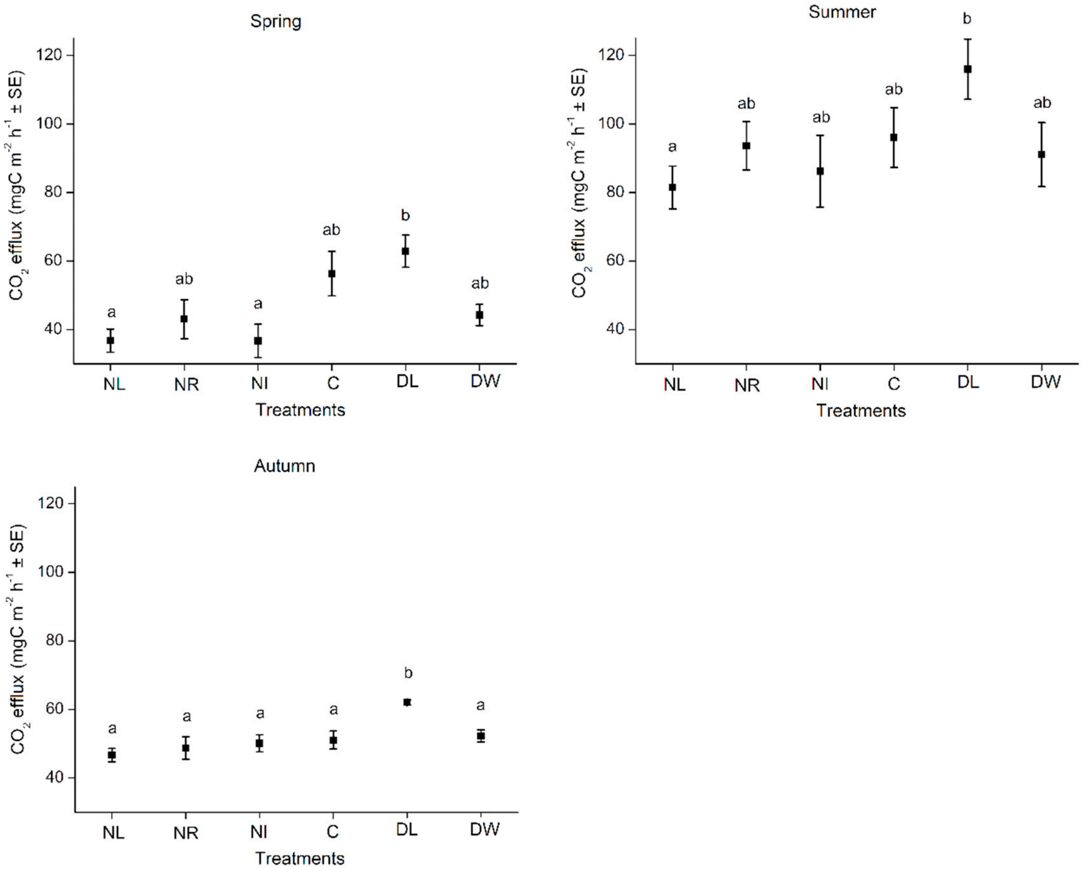

Figure 2. Effect of litter treatments with different DIRT treatments on soil CO_2 emissions by season.

The years 2011 and 2012 were also drier than average, with annual precipitation values of 400 and 450 mm, respectively, which meant that, in these years, one-third and a quarter less precipitation fell, respectively, than the many-year average. In these years, we did not find a significant difference in the values of soil respiration of the treatments. Compared to the summer 2010 values, soil respiration of C, DW and DL treatments showed a 30%–40% decline while that of litter withdrawal treatments decreased by 15%–20%.

The rate of litter degradation was the first to be observed after 6 months of incubation, as the no-root treatment was significantly different from the no-litter and no-input treatments (Figure 3).

No differences in the rate of decomposition were found after 6 months for cotton wool and wood. No significant difference was found in wood degradation after 12 months of incubation between treatments (Figure 4).

After 12 months, all treatments showed a significant difference in the decay residue of cotton wool compared to the control, whereas for leaf litter, the no-root and double-wood treatments were significantly different from the no-input treatment. The degradation experiments were repeated 15 years after the start of the DIRT treatments. While earlier experiments were done with local litter, in this case, we tried to select uniform organic materials.

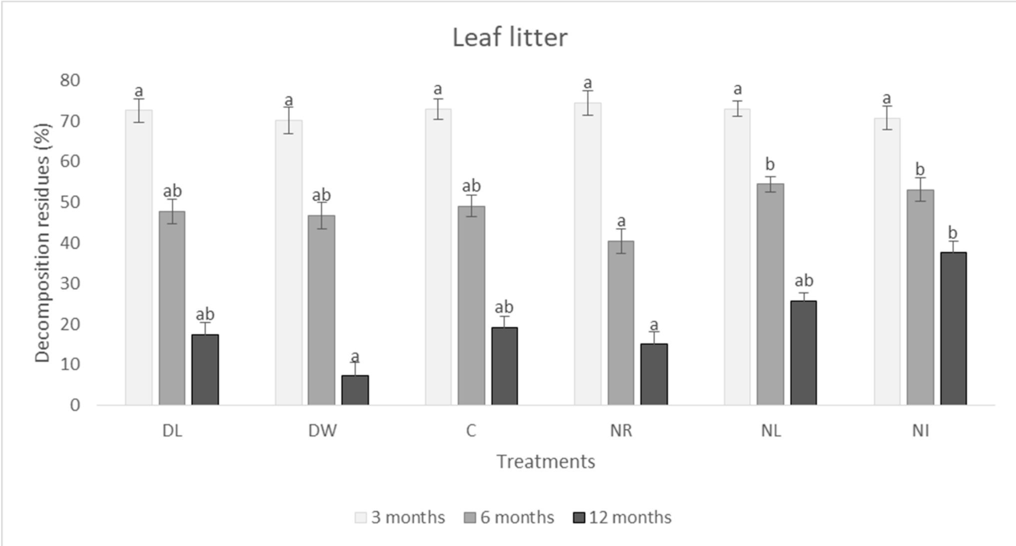

Figure 3. Effect of organic matter manipulation experiments with different DIRT treatments on leaf litter degradation rate after 3, 6 and 12 months: The letters represent significant differences between treatments during the given period ($n = 4$).

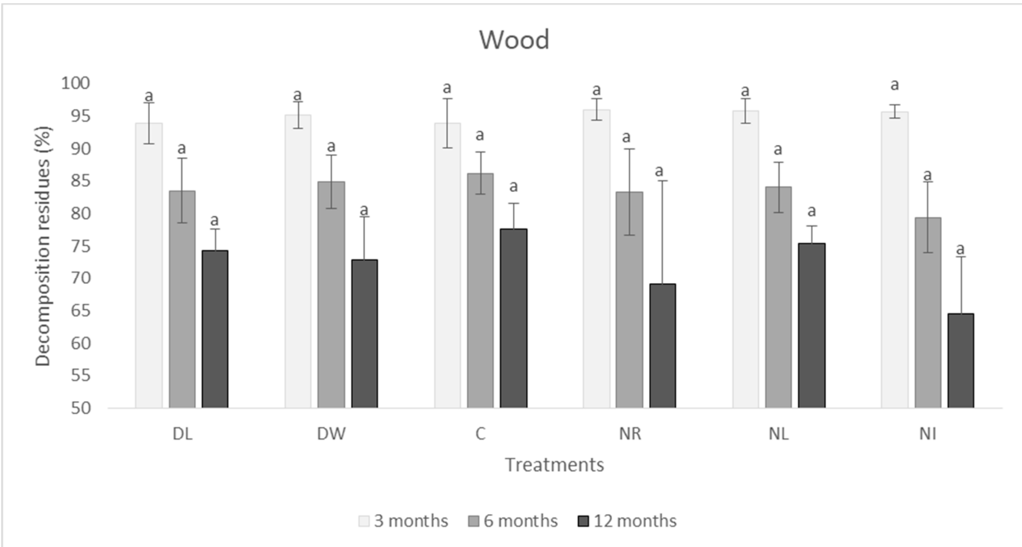

Figure 4. Effect of organic matter manipulation experiments with different DIRT treatments on wood degradation rate after 3, 6 and 12 months: The letters represent significant differences between treatments during the given period ($n = 4$).

In this experiment, leaves similar to green tea leaves and wood similar to rooibos tea were used throughout the experiment to compare the decomposition intensity between treatments. Green tea decomposed faster than rooibos tea according to initial expectations. After the 3-month incubation period, the double-litter and no-root treatments showed significantly higher degradation rates for green tea (Figure 5) than the no-litter treatment. After 12 months, there was no difference in the rate of decomposition of green tea between treatments, whereas after 24 months, there was a significant difference between the control and no-input treatments.

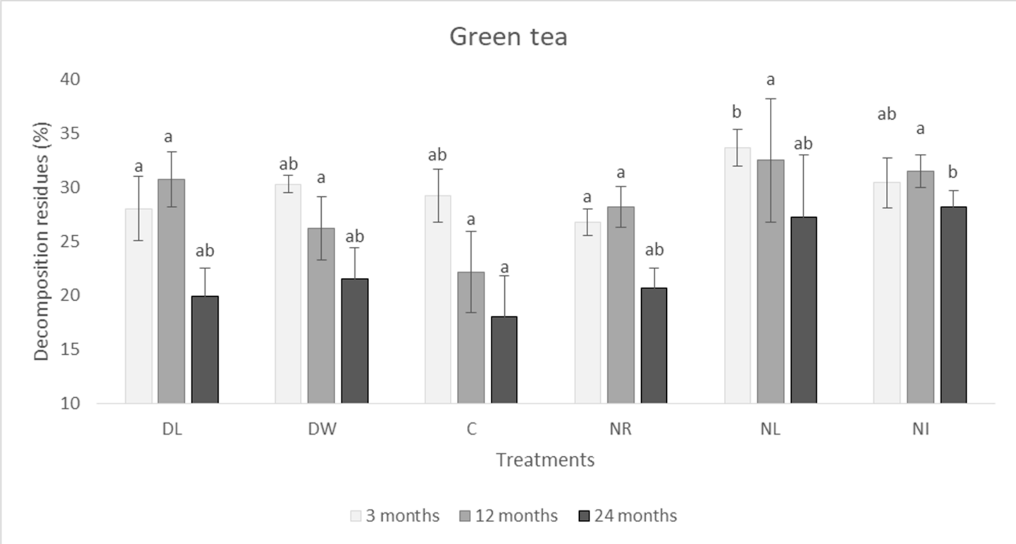

Figure 5. Effect of organic matter manipulation experiments with different DIRT treatments on green tea degradation rate after 3, 6 and 12 months: The letters represent significant differences between treatments during the given period (n = 4).

There was a difference in the decomposition intensity of rooibos tea after 3 months between the double-litter and no-litter treatments, whereas over time, there was no difference between the plots in the degradation rate after either 12 or 24 months (Figure 6).

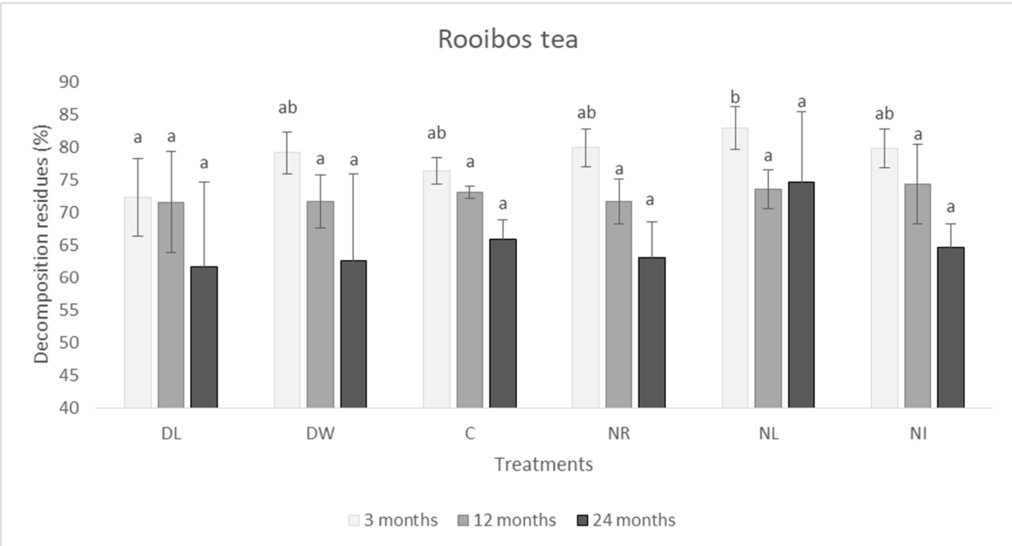

Figure 6. Effect of organic matter manipulation experiments with different DIRT treatments on rooibos tea degradation rate after 3, 6 and 12 months: The letters represent significant differences between treatments during the given period (n = 4).

According to our results, in the case of litter-doubling treatments, we experienced a greater loss of organic matter compared to the weight of the bags placed in the soil of the organic matter-withdrawal treatments (Figure 7).

Similarly, the no-root, no-input and no-litter treatments had similarly slower decomposition rates of the more easily decomposing organic materials compared to the double-litter and double-wood treatments (Figure 8).

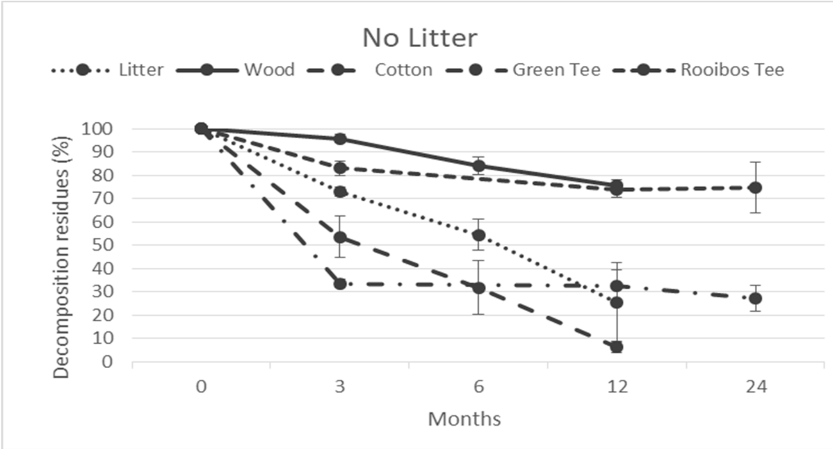

Figure 7. Decomposition rate of different quality organic matter after 3, 6, 12 and 24 months in the soils of No Litter (NL) plots of the DIRT experiment (average of 9 replicates with standard deviation).

Figure 8. Decomposition rate of different quality organic matter after 3, 6, 12 and 24 months in the soils of Double Litter (DL) plots of the DIRT experiment (average of 9 replicates with standard deviation).

At the same time, organic material with higher lignin content (rooibos tea and wood) decomposition was much slower than that of the leaf litter. For the easily soluble humic acid-C (ESHa) and fulvic acid-C (ESFa) fractions, the highest amounts were measured in the soil of DL treatments plots while the lowest was measured in NL plots, but the plots treated with NR and NI showed similarly low values (Table 3).

Table 3. C content of each humus fraction (mg g^{-1}).

Treatments	C-Content (mg g^{-1})	
	ESHa	**ESFa**
NL	19.30	9.21
NR	22.20	10.70
NI	21.50	9.47
C	31.23	15.96
DL	34.30	17.32
DW	30.30	11.73

Abbreviations: ESHa: easily soluble humic acid fraction; ESFa: easily soluble fulvic acid fraction.

4. Discussion

Based on the results, we assume that the faster decomposition in soils with balanced moisture and temperature is due to the higher activity of the persistently formed microbial community in contrast to the withdrawal treatments, where the soil surface is more exposed and the moisture and temperature conditions change under more extreme conditions. Furthermore, due to the persistent lack of organic matter in these treatments, sufficient amounts of microbial mass cannot be sustained, activity is lower and the rate of decomposition is thus slower.

Cellulose-based cotton wool bags were dug in the spring and lifted in the middle of summer after 3 months. In the second half of the 3-month period, the soils of the NR treatment were the wettest during the hot drought period, where surface litter cover prevented soil evaporation and the absence of live roots prevented plant water uptake, so the rate of degradation was highest in this treatment.

The difference in the rate of litter degradation was the first to be observed after 6 months of incubation. Differences in summer moisture value may have played a role in this, as the NI and NL plots had the driest surfaces. Also, these treatments lack the litter surface; thus, the communities of microorganisms capable of degrading them are less diverse on these plots [44,46,47]. Thus, these conditions together may explain the lower level of microbial activity that caused the differences between the three treatments.

For testing the degradation of wood thinner, dry twig and branch pieces were used, although homogeneity was sought, but under the given conditions, it was not fully achieved, so the heterogeneity of the wood in the bags resulted in some heterogeneity in the results obtained, which may have masked any slight differences. The decomposition of high lignin-containing plant tissues in wood is difficult to degrade. Especially if the tiny mesh of the bags seals the members of the macro- and mesofauna away from the substances, they can be degraded even more slowly by microorganisms. Because we were primarily interested in the activity of microbial degradation activity, the hole size of the mesh of the bags excluded the macrofauna as well as the participants of the mesofauna larger than 0.5 mm. More resistant plant tissues as well as the decomposing microorganisms that make up them belong to the "k" strategists, for which the number is probably less influenced by litter treatments. These conditions together may explain why this type of litter found no significant difference between treatments.

One-hundred-percent cellulose cotton wool bags provide a homogeneous food source for the decomposing microbes, as opposed to the highly heterogeneous molecular composition of leaves, wood and tea. This explains that the degradation of cotton wool was the most intense for all treatments.

Once colonized by the cellulose-degrading microorganisms, only local microclimatic conditions influenced the rate of degradation primarily which were the most favorable in the NR plots due to the higher moisture content of the treatment soil. Our previous studies [48] have confirmed this hypothesis. The soil temperature was also higher in this treatment in late spring, summer and early autumn because there were no live plants on this treatment, so slightly more heat (direct irradiation) was applied to the plots, which was the warmer soil temperature combined with higher soil moisture, significantly increasing the activity of microorganisms, including the rate of degradation.

In the case of the decomposition of green tea, the higher moisture content of the plots and the high content of litter and root (the higher content of the more easily decomposable compounds) may increase the number of "r" strategic microorganisms, which decompose readily degradable compounds of the plant materials that enter the soil [27,49].

Both types of tea were characterized by an initial (first 3 months) faster decomposition period, which subsequently decelerated significantly. This could be explained by the fact that both teas contained more readily decomposable substances, after which the residual compounds decomposed much more slowly [26]. In rooibos tea, the amount of readily decomposing substances was lower, so we experienced a much smaller weight loss than the 3-month measurement. Later, the decomposition of the two types of tea followed a similar pattern and their further weight loss was similar in control soils. In the NL and NI treatments, green tea showed a slower decomposition from the beginning than the other treatments, but significant differences were found in only a few cases, probably due to the lower sample number.

However, the trends were clear: there was a real difference between the treatments in the rate of degradation of readily degradable compounds. In the case of rooibos tea (which contains only a small amount of the more easily degradable compound and is also largely degraded in the first 3 months), only the first (3 months) measurement showed significant difference tendencies. No significant difference was observed in the decomposition of the wood pieces during the 1-year study period. This shows that litter treatments have a much lesser effect on the degradation processes of more resistant compounds than on more readily degradable compounds. There was a significant difference between the leaf litter treatments and green tea, which contains several more easily degradable molecules. There was no difference in dry leaf litter (containing whole oak leaves) in the first 3 months, while in tea leaf containing smaller fragments, there was a difference between treatments in the first 3 months. This may also be due to the fact that the tea bags were put into the soil 15 years after the treatments while the leaf litter bags were put into the soil 5 years after the treatments.

The lowest CO_2 emissions were measured on the withdrawal treatments. Due to the cessation of litter additions, the available amount of organic matter in the soil was not sufficient to maintain the degradation processes [50]. Sulzman et al. [51] in the H. J. Andrews DIRT field found that the activity of rhizosphere microorganisms also decreased as a result of reduced nutrient sources. The lowest soil respiration values were measured in the soil of NL and NI plots.

The effect of dredging treatments on degradation processes is also significantly influenced by soil moisture values. It is known that soil respiration is also significantly affected by temperature and soil moisture content [52]. The effect of the treatments on soil respiration and the rate of decomposition of organic matter in the soils are most pronounced at the optimal ambient moisture content. This may explain why we did not find a significant difference between litter treatments in the extremely dry summers in 2011 and 2012 compared to the rainy summers in 2010. In the period of 2016, when the tea filters were placed in the soil, the summer was also wetter than average in the studied area, which was also reflected in the measured soil moisture values and partly due to the fact that we measured a significant difference in the 3-month degradation values between the treatments for both types of tea. The other reason for the significant difference is that the materials in the tea filters were much more fragmented than the previously digested leaf litter and the wood material consisting of smaller twigs and branches.

For the more fragmented tea materials, significant changes were already observed in the first 3 months, as was for the cotton wool, which consists of homogeneous cellulose free of lignin materials. Differences in the rate of degradation, with or without occurrence of different treatments, suggest that the number of microorganisms [53] and microbial activity (enzyme activity and soil respiration [27,47,52]) differing from treatment to treatment can only significantly affect the rate of degradation if the compact structure of the soil decomposes, the molecules are exposed and this facilitates the work of the degrading microorganisms.

There is less available easily degradable C source in the soil of withdrawal treatments, as shown by the study of light humus fractions, and thus, the value of soil respiration is lower. The easily degradable organic C content of the soil is the most active part of the soil organic C fraction, and this easily degradable fraction varies greatly upon disturbance and treatment [54,55]. Crow and Lajtha [56] mention that the rate of degradation may slow down and thus the rate of C-stock that is difficult to degrade increases in enclosed plots.

CO_2 emissions of the double-litter treatment were higher compared to the control, although the difference was significant only in autumn 2010, but higher soil respiration values were measured in spring and summer as well, similar to Crow et al. [57,58], who studied the H. J. Andrews DIRT site in Oregon, USA. In this site, the highest emissions were reported by Sulzman et al. [51] on double-litter-treated plots.

The first 3 months of the decomposition of tea materials fell into the summer, from July to September, which was wetter than average in 2016; thus, in the hot and humid summer months, the readily degradable substances in the tea material decomposed. In the period after the first three months, the weight loss of slowly decomposing substances did not show a significant difference in any of the tea varieties. This can be explained by the fact that "k" strategic microorganisms that degrade more difficult-to-degrade molecules need longer to adapt [54]. In the 2nd year, there were already significant differences between treatments for more easily degradable green tea.

5. Conclusions

As a conclusion of our research, we can say that the fragmentation of the studied organic substances and the resistance of the compounds that make them up fundamentally influence the rate of degradation. Litter varieties, tea materials and cotton wool placed in plastic tulle bags were accessible only to the smaller representatives of the soil fauna members. Thus, the decomposition of various litter groups, especially more compact materials, has slowed sharply. This can be clearly seen in the wood liter where, during the 12 months of the studies, we did not find a significant difference in the decomposition of the smaller twigs and branch pieces in the bags. For dry and even completely intact leaf litter, there was no difference between treatments in the rate of degradation in the first 3 months. After breaking down the matrix of leaf material in smaller soil animals and microorganisms, there was then a difference between the individual treatments, which was evident in the semiannual decomposition data. In addition to the processes mentioned above, soil microclimatic variables are also important. Temperature parameters show a seasonal pattern. The Síkfőkút site is located in the relatively drier forest area of the Carpathian Basin. Thus, one of the main regulators of the decomposition processes here is soil moisture. During the drought period, the effect of detritus treatments on microbial activity may not be adequate. This may also result in the fact that, if some areas become drier due to climate change and as a result the detritus input increases due to dying vegetation, the amount of organic matter entering the soil due to decreasing microbial activity may temporarily accumulate, increasing the SOC stock of soils.

Author Contributions: All authors contributed equally to the development of the ideas and authoring of the paper. Z.K.: manuscript preparation, field work and laboratory analyses; K.J.: field work and identification of soil type; B.B.: data interpretation and analysis; T.K.: statistical and data analysis; S.A.P.: field work and data analysis; C.V.: field trials; I.F.: statistical analyses and manuscript preparation. All authors have read and agreed to the published version of the manuscript.

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

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