



# Carbon sequestration of forest soils is reflected by changes in physicochemical soil indicators – A comprehensive discussion of a long-term experiment on a detritus manipulation

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## ABSTRACT

The interactions of climatic and geochemical factors control soil organic carbon storage capacity and turnover. The comprehensive evaluation of the effect of long-term detritus manipulation on the soil organic carbon, soil-forming processes and the soil physical and chemical properties will help us better understand the carbon sequestration of forest soils. The long-term (19 years) effect of detrital input and removal treatments (DIRT) on physicochemical soil properties were investigated at a Central-European forest site (Sikfőkút, Hungary). In contrast to the results of similar experiments in other parts of the world, the detritus input treatments affected the soil organic carbon and almost all of the soil physicochemical indicators for the upper 15 cm layer. Soil pH, potential acidity and base saturation decreased in the litter removal plots and increased in the detritus doubling treatments. A decrease in organic matter content in the litter removal plots explained the changes in bulk density, as the stability of aggregates also decreased with the decrease of exchangeable bases and organic colloids. In this respect, compared to the other DIRT sites and other similar experiments in the world, our experimental site is considered unique, as it has the highest clay content and the driest climate. We conclude that potential cation exchange capacity and base saturation (exch. Ca) play a fundamental role in predicting the occurrence of the carbon sequestration mechanisms. We suggest to include these parameters into current SOC models.

## 1. Introduction

Soils are the largest reservoirs of organic carbon on Earth (Schmidt et al., 2011). Soil organic carbon (SOC) storage depends on the inputs of plant material and losses of SOC through decomposition (Fekete et al., 2008; Sulman et al., 2014; Chenu et al., 2019). Most models consider net primary productivity, soil temperature and moisture as the main drivers of SOC storage (Todd-Brown et al., 2013; Fekete et al., 2017). How climate change affects forest ecosystems and thus the carbon sequestration capacity of forest soils is an important issue. Climate warming is expected to induce SOC losses in forest soils that result, in turn, in reduced soil fertility, reduced water storage capacity and positive feedback on climate change (Feng et al., 2008; Prietzel et al., 2016).

The latest studies indicate that soil organic matter turnover is governed by accessibility, not recalcitrance (Baldock and Skjemstad, 2000; Dungait et al., 2012; Lavalley et al., 2020). Recalcitrance and accessibility/aggregation seem to determine the turnover dynamics in fast and intermediate cycling organic matter pools, but for long-term organic carbon preservation, the interactions with mineral surfaces are a major control in all soils (Kögel-Knabner et al., 2008). Consequently, the stabilization of SOC is governed not only by microbial activity but also by physical protection within soil aggregates (Denef et al., 2001; Kaiser and Asefaw Berhe, 2014) and chemical protection via associations with soil minerals (Bruun et al., 2010; Chenu et al., 2019; Cotrufo et al., 2015; Rasmussen et al., 2018; Sayer et al., 2019). The share of carbon between mineral-associated and particulate organic matter and the ratio between

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carbon and nitrogen affect soil carbon stocks and mediate the effects of other variables on soil carbon stocks (Cotrufo et al., 2019). Six et al. (2002) suggest that physicochemical characteristics inherent to soils define the maximum protective capacity of these pools, which limits increases in soil organic matter (i.e. C sequestration) with increased organic residue inputs.

It is a complex issue of how environmental factors affect carbon sequestration. Doetterl et al. (2015) conclude that the interactions of climatic and geochemical factors control SOC storage and turnover, and must be considered for robust prediction of current and future soil carbon storage. Precipitation and temperature were only secondary predictors for carbon storage, respiration, residence time and stabilization mechanisms. Using observations from a 3,000-kyr-old soil chronosequence preserved in alluvial terrace deposits of the Merced River, California, Doetterl et al. (2018) demonstrate that biogeochemical alteration of the soil matrix (and not short-term warming) controls the composition of microbial communities and strategies to metabolize nutrients. Indeed, soils change dynamically due to the interaction of environmental factors, and changes in individual soil properties can only be investigated in a complex and interrelated way. Therefore, ecological research networks functioning across climatic and edaphic gradients are critical for the understanding of biogeochemical carbon cycle and related soil-forming processes.

The Detrital Input and Removal Treatment (DIRT) network was established to assess how rates and sources of plant litter inputs influence accumulation or loss of organic matter in forest soils (Crow et al., 2009). DIRT employs chronic additions and exclusions of aboveground litter inputs and exclusion of root ingrowth to permanent plots at forested sites in the USA, Germany, Hungary and China. Many of the results from the DIRT network have been synthesized by Lajtha et al. (2018). Besides, we find a similar experiment in a lowland tropical forest in Panama (Sayer et al., 2011, 2020). Bruun et al. (2010) conducted a comprehensive study about the lability of SOC in tropical soils with different clay minerals. As a result of these studies, the microbial background of decomposition processes, their dependence on soil moisture and temperature conditions (Veres et al., 2013; Kotroczo et al., 2014), the quality and origin of soil organic matter (Pisani et al., 2016; Wang et al., 2017; VandenEnden et al., 2018; Kotroczo et al., 2020) are already well known for forest soils.

In the DIRT research network, the soil diversity and changes in soil formation processes and resulting soil chemical and physical properties have not been studied comprehensively. We hypothesize that due to carbon sequestration or carbon loss, soil physicochemical properties can change significantly over 19 years, which is of great importance for ecosystems. The evaluation of the effect of long-term detritus manipulation on SOC and the soil physical and chemical properties will help us better understand the carbon sequestration of forest soils. Given the differences between the soil-forming factors of other DIRT sites, the Central European forest site (Síkfőkút) is probably unique because it has the driest climate of all DIRT sites and distinct mineralogy. In our study, we investigated the long-term effect of detrital input and removal treatments on SOC, cation exchange capacity, bulk density, water holding capacity, exchangeable bases, potential acidity and soil structure at the Síkfőkút DIRT site. Our second hypothesis is that the carbon sequestration capacity of soils resulting from the complex interaction of climatic and mineralogical factors could be most simply summarized and characterized by the potential cation exchange capacity and base saturation (or potential acidity) of the soil. These parameters are also able to predict other physicochemical changes due to organic matter input, such as soil porosity, structure, pH, nutrient turnover, microbiological activity, and so on.

## 2. Material and methods

### 2.1. Site description and treatments

The study area is situated in the hill-country of North Hungary in Central Europe (47°55'34" N and 20°26' 29" E) (Fig. 1) at an elevation of 320–340 m a.s.l. The site has a temperate continental climate with hot summers with low overall humidity levels. Long-term meteorological data indicate that the climate at the site has become drier and warmer over the past decades (between 1953 and 2014) (Berki et al., 2014). The mean annual temperature is 9.9 °C, and the mean annual precipitation is 590 mm. July is the hottest month with an average temperature of 20.4 °C, and January is the coldest with −2.7 °C. The deciduous forest at the study site is a semi-natural stand (*Quercetum petraeae-cerris community*) with no active management since 1972. In this previously coppiced forest, the Sessile oak (*Quercus petraea* L.) and Turkey oak (*Quercus cerris* L.) species that make up the overstory are approx. 110 years old. According to the soil survey of Switonik et al. (2014), the soils are Chromic Protovertic Luvisols (Clayic, Cutanic) and Protovertic Endostagnic Abruptic Luvisols (Clayic, Cutanic) (FAO, 2014). Inside the Síkfőkút forest stand, 7 × 7 m permanent experimental plots were set up in 2000 following the protocol used in the DIRT plots established in the USA (<https://dirtnet.wordpress.com/>). During the experiment, two addition treatments (*Double Litter*, *Double Wood*) and three removal treatments (*No Litter*, *No Input*, *No Roots*) were applied (Table 1). Each treatment and the *Control* were conducted in three replicates.

### 2.2. Soil analyses

For physical and chemical analyses, soil sampling was carried out in 2017 and 2019, almost two decades after the start of treatments. Samples were taken from a depth of 0–15 (A horizon) and 15–25 cm depths (AB horizon) in two replicates on each plot. Samples were taken in 2017 to determine SOC. Soil samples were dried, ground and sieved (2 mm). To determine the SOC, soil samples were pretreated with 10% hydrochloric acid to eliminate inorganic carbonate content before organic carbon analysis by dry combustion (Matejovic, 1997) using an CHNS elemental analyzer (Elementar Vario EL). The particle size distribution was determined after chemical treatment using laser diffraction. After treating the samples with HCl and H<sub>2</sub>O<sub>2</sub> to remove the carbonate and organic material (Buurman et al., 1996), 0.5 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> was added to the samples to disperse the particles. A Fritsch Analysette 22 Microtech device was used in the range of 0.2–2000 µm. Grain sizes were calculated applying the Mie theory with 1.45 refractive index (Centeri et al., 2015; Makó et al., 2017). Soil pH was determined potentiometrically in 0.01 M CaCl<sub>2</sub> at a soil/solution ratio of 1:2.5 (Hungarian Standard, MSZ-08-0206-2, 1978). Exchangeable bases (Ca, Mg, Na and K) and base saturation percentage were determined using buffered 1 M ammonium



Fig. 1. The geographical location of the experimental site (Síkfőkút Forest, Hungary).

**Table 1**

The detritus input and removal treatments in the Sífökút oak forest (Hungary) performed every year since 2000.

Treatments	Description
Control (C)	Normal litter inputs. Average litter amount typical of the forest site.
Double Litter (DL)	Above-ground leaf inputs are doubled by adding litter removed from No Litter plots.
Double Wood (DW)	Above-ground wood debris inputs are doubled by adding wood to each plot. Annual input of wood litter was measured in boxes placed at the site, and a double amount of that value was applied in the case of every DW plot.
No Litter (NL)	Above-ground inputs are excluded from plots. Leaf litter was removed by a rake. This process was repeated continuously every year.
No Root (NR)	Roots are excluded by inserting impenetrable barriers in backfilled trenches to the top of C horizon of soil. Root resistant plastic foil was placed around the plot to the depth of 1 m, hindering the roots developing outside the plot to get into the NR plot. Trees and shrubs were eradicated when the plot was established, and plant roots decayed with time.
No Input (NI)	Above-ground inputs are excluded from plots and the below-ground inputs are also eliminated as in NO ROOTS plots. This treatment is the combination of NR + NL plots.

acetate (pH 7.0) extraction. As we did not have the opportunity to directly measure Al and Fe ions, the full potential cation exchange capacity (CEC) was determined by the Na-acetate/ethanol/NH<sub>4</sub>-acetate replacement method (Chapman, 1965). As the sodium acetate results in artificially high CECs in acid forest soils due to increases in pH-dependent charges (Ciesielski et al., 1997), we also considered it necessary to determine hydrolytic acidity using soil extraction by 0.5 M Ca-acetate (pH 8.2) in the ratio of 1:2.5 (Kappen, 1929). The extractants were titrated with 0.1 M NaOH and the hydrolytic acidity was calculated from the amount of alkali consumed. The bulk density (BD) and water holding capacity (WHC) were measured using undisturbed soil cores prepared with the excavated method (McKenzie et al., 2002). Undisturbed flat horizontal surfaces were prepared with a spade at a depth of 15 cm and on the surface. Steel rings of 150 cm<sup>3</sup> volume were gently hammered into the soil. The rings were excavated without disturbing or loosening the samples and carefully removed. BD calculated to dry mass per unit volume (g cm<sup>-3</sup>). After saturating soil samples with tap water for 24 h, soil water content at maximum water holding capacity was measured and calculated to volume percent. Microaggregate stability (MiAS) was calculated according to Vagelar's structure factor (Horel et al., 2019) from the rate of < 0.02 mm fractions determined with dispersion using laser diffraction (a) and without any dispersion using pipette method (b):  $MiAS = \frac{a-b}{a} \cdot 100$ .

Using the bulk density, the SOC concentrations were converted to the kg m<sup>-3</sup> dimension, which refers to the upper 15 cm soil layer. This was necessary because SOC values expressed as wt% are not comparable due to different bulk density of plots.

### 2.3. Statistical analyses

To determine the effect of the detritus input and removal treatments on the individual soil physical and chemical variables for each sample, one-way ANOVA was conducted using the Tukey post-hoc test to separate the groups. Normality of data was analysed by the Saphiro-Wilk test. The paired relation between the variables was examined by the Pearson correlation coefficient (r). To determine intercorrelation among the indicators, we also performed a Principal Component Analysis (PCA). The suitability of the sampling (selected variables) was determined with Kaiser-Meyer-Olkin (KMO) and Bartlett tests. Only principal components with eigenvalue greater than 1.0 were analysed as a complex soil quality index (Juhos et al., 2019). Variable means per plot values were used in the PCA and Pearson correlation analyses. All data were statistically processed using IBM SPSS Statistics 22 and MS Excel.

## 3. Results

### 3.1. Changes in SOC

After 17 years since the start of the experiment, the averages of SOC for the 0–15 cm layer per treatment reflect the volume of detritus input (Table 2). Lower SOC was observed everywhere in case of the removal treatments compared to the Control and input treatments. SOC was significantly higher in the Double Litter and Double Wood treatments compared to the No Litter and No Input plots but the differences were not significant compared to the Control and No Root plots.

### 3.2. Changes in physical soil properties

There were significant changes in soil bulk density and porosity in the upper 0–15 cm: the BD of the No Root and No Input treatments were significantly higher compared to the Control, Double Wood and Double Litter plots (Table 2). The removal of litter alone caused an increase in BD and a decrease in soil porosity on the No Litter plots as well, but the difference was not significant compared to the Control. The Control did not show a significantly lower BD than the Double Litter and Double Wood, but No Input and No Root plots had significant higher BD than the Control.

The input (Double Litter and Double Wood) treatments did not show significantly higher WHC than the Control (Table 2). Among the removal treatments, the No Input and No Litter sites had significantly lower WHC than the Control. There was a significant moderate linear relationship between the SOC and WHC as well as between BD and SOC (Table 3). In contrast with doubling the detritus inputs and higher SOC, there were no significant changes in soil physical properties at the Double Wood and Double Litter plots compared to the Control.

The minor differences in the particle size distribution of the plots were not significant, so no effect of the treatments was observed (Table 2). The soils were generally characterized by clay loam texture. The clay content was between 30.7% and 46.2% and the silt content was typically between 50.9 and 60.4%. Clay content was moderately correlated with WHC (Table 3).

Due to the natural vegetation and the undisturbed state, the MiAS was high in all plots (Table 2) and significant differences were observed between treatments. The No Input plots had the significantly lowest MiAS, but the MiAS of the No Root and No Litter treatments was also significantly lower than that of the Control, Double Litter and Double Wood treatments. We did not find a significant difference between Control, Double Litter and Double Wood. There was a significant moderate correlation between the SOC and MiAS and a strong correlation between the BD and MiAS (Table 3). The physical properties of the 15–25 cm soil layer were also investigated, but no significant differences were observed.

### 3.3. Changes in chemical soil properties

Chemical changes were limited to A horizons, so only the changes in soil properties for the upper 0–15 cm are presented (Table 2).

The pH values ranged from 4.82 to 6.20 over the entire experimental area. The pH of the No Input and No Litter treatments were significantly lower compared to the Control, Double Litter and Double Wood plots (Table 2). The highest pH values were observed with Double Litter treatment, but the increase in pH was not significant compared to the Control and Double Wood treatments.

CEC showed a strong linear correlation with SOC and pH (Table 3). However, no significant linear correlation was found between clay content and CEC. The Double Litter treatment showed significantly higher CEC compared to all of detritus exclusion treatments, but the difference was not significant compared to the Control and Double Wood treatment (Table 2). Changes in CEC and pH separated detritus removal treatments from the plots that received more organic matter inputs

**Table 2**

Effect of detritus input and removal treatments on physicochemical soil properties at 0–15 cm layer after 19 years of treatment (C: Control; DL: Double Litter; DW: Double Wood; NL: No Litter; NR: No Root; NI: No Input) in Síkfőkút forest in Hungary. The result of the ANOVA test for the means in the six treatments. Different letters represent significant differences within each group ( $p < 0.05$ , ANOVA and Tukey's test). SOC: Soil organic carbon; WHC: water holding capacity; BD: bulk density; MiAS: microaggregate stability; clay:  $< 2 \mu\text{m}$ ; silt:  $2\text{--}20 \mu\text{m}$ ; sand:  $20\text{--}2000 \mu\text{m}$ ; HA: hydrolytic acidity; K, Na, Ca, Mg: exchangeable cations; BS: base saturation; CEC: cation exchange capacity; pH: reaction measured in 0.01 M  $\text{CaCl}_2$ ; PC1: Principal Component (see Table 4).

Indicators	Treatments										
	C		DL		DW		NL		NR		NI
SOC ( $\text{kg m}^{-3}$ )	43.32	ab	47.14	b	47.32	b	33.75	a	35.69	ab	33.99
SOC ( $\text{m/m}\%$ )	6.93	abc	7.27	bc	7.43	c	5.16	abc	4.64	ab	4.33
WHC ( $\text{V/V}\%$ )	53.45	c	50.22	bc	52.87	c	45.89	ab	48.90	abc	44.22
BD ( $\text{g cm}^{-3}$ )	0.87	a	0.87	a	0.87	a	0.99	ab	1.04	b	1.02
MiAS ( $\text{m/m}\%$ )	91.89	bc	92.34	bc	94.98	c	87.32	b	87.34	b	81.33
clay ( $\text{m/m}\%$ )	32.58	a	40.01	a	35.51	a	41.32	a	38.14	a	38.96
silt ( $\text{m/m}\%$ )	57.95	a	54.07	a	56.07	a	53.61	a	56.42	a	55.03
sand ( $\text{m/m}\%$ )	9.47	a	5.92	a	8.42	a	5.07	a	5.44	a	6.01
HA ( $\text{cmol}(+) \text{kg}^{-1}$ )	49.10	ab	37.03	a	44.50	ab	77.33	b	59.97	ab	64.33
K ( $\text{cmol}(+) \text{kg}^{-1}$ )	0.69	a	0.69	a	0.69	a	0.44	a	0.39	a	0.42
Na ( $\text{cmol}(+) \text{kg}^{-1}$ )	0.17	a	0.17	a	0.18	a	0.18	a	0.15	a	0.17
Ca ( $\text{cmol}(+) \text{kg}^{-1}$ )	20.25	ab	26.82	b	22.41	ab	12.07	a	16.37	ab	13.29
Mg ( $\text{cmol}(+) \text{kg}^{-1}$ )	5.58	c	6.18	c	5.03	bc	3.33	ab	3.39	ab	2.83
BS ( $\text{cmol}(+) \text{kg}^{-1}$ )	68.54	a	72.95	a	65.64	a	49.69	a	56.92	a	49.46
CEC ( $\text{cmol}(+) \text{kg}^{-1}$ )	39.43	abc	46.26	c	43.53	bc	32.56	a	35.68	ab	32.75
pH	5.88	bc	6.10	c	5.85	bc	4.97	a	5.38	ab	5.15
PC1	0.70	b	1.06	b	0.94	b	-0.97	a	-0.53	a	-1.20

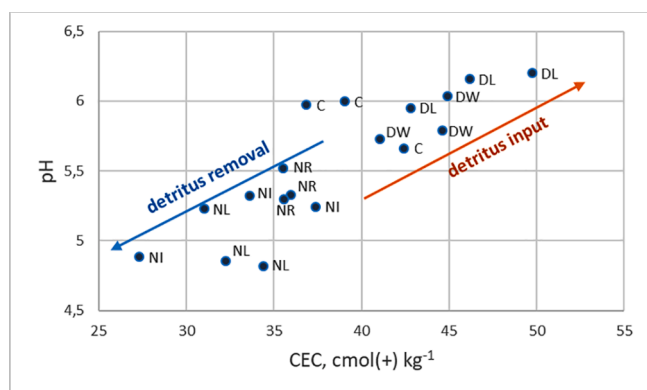
**Table 3**

The Pearson correlation coefficients ( $r$ ) of the measured soil indicators at 0–15 cm layer after 19 years of the detritus input and removal treatment in Síkfőkút forest (Hungary), where WHC: water holding capacity; BD: bulk density; clay:  $< 2 \mu\text{m}$ ; HA: hydrolytic acidity; CEC: cation exchange capacity; BS: base saturation; pH: reaction measured in 0.02 M  $\text{CaCl}_2$ ; SOC: soil organic carbon; MiAS: microaggregate stability.

	WHC	BD	clay	HA	CEC	BS	pH	SOC	MiAS
WHC	1								
BD	-0.756**	1							
clay	-0.693**	0.584*	1						
HA	-0.506*	0.635**	0.465	1					
CEC	0.623**	-0.658**	-0.250	-0.716**	1				
BS	0.572*	-0.653**	-0.493*	-0.858**	0.511*	1			
pH	0.736**	-0.761**	-0.415	-0.795**	0.843**	0.659**	1		
SOC	0.563*	-0.566*	-0.170	-0.439	0.734**	0.325	0.709**	1	
MiAS	0.775**	-0.709**	-0.430	-0.606**	0.675**	0.598**	0.844**	0.659**	1

\*\*. Correlation is significant at the 0.01 level (2-tailed).

\*. Correlation is significant at the 0.05 level (2-tailed).



**Fig. 2.** The relationship between soil pH and cation exchange capacity (CEC) at 0–15 cm depth after 19 years of detritus input and removal treatments (where C: Control; DL: Double Litter; DW: Double Wood; NL: No Litter; NR: No Root; NI: No Input) in the Síkfőkút deciduous forest in Hungary. Arrows show the direction of change in soil pH and CEC in response to detritus removal (blue) or addition (red) treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

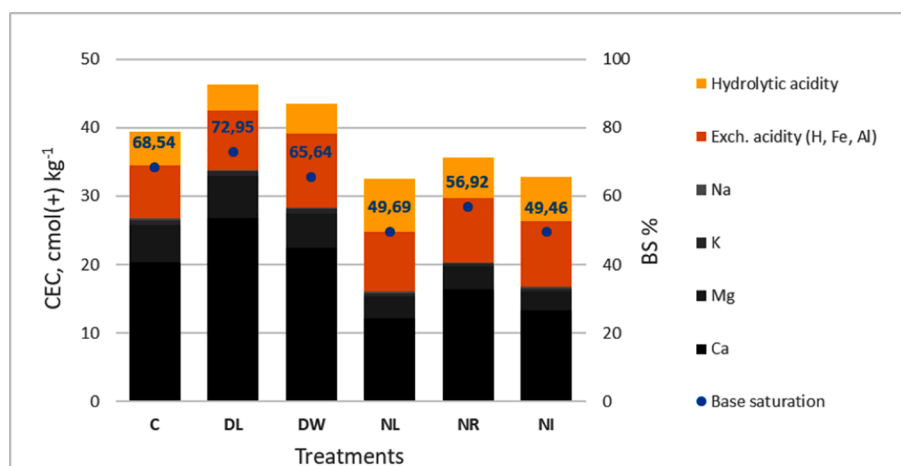
(Fig. 2).

Base saturation showed no significant difference between the treatments, but significant differences in the case of the exchangeable Ca and Mg content was observed (Table 2). Exchangeable Ca was significantly higher in the *Double Litter* plots compared to the *No Input* and *No Litter* treatments. Exchangeable Mg was significantly higher in the *Control*, *Double Wood*, and *Double Litter* treatments compared to the detritus removal treatments. No significant linear correlation was found between base saturation and SOC (Table 3). The hydrolytic acidity of soils in the *Double Litter* treatment was significantly lower than that of the *No Litter* treatment (Table 2). The differences between the other treatments were not significant. The changes in the adsorption complex and cation composition of the soil are summarized in Fig. 3. The correlation between base saturation and pH as well as between hydrolytic acidity and pH was significant (Table 3). But no significant linear correlation was found between hydrolytic acidity and SOC.

#### 3.4. Multivariate statistical analyses (PCA)

According to our results, the detritus input and removal treatments primarily affected SOC, pH, CEC, base saturation, hydrolytic acidity, MiAS, BD, and WHC indicators for the upper 15 cm layer. Therefore, these were included in the PCA. According to the eigenvalues greater than 1, the PCA yielded one principal component (PC1) explaining a total of 70.62% of the variance for the entire set of variables (Table 4).



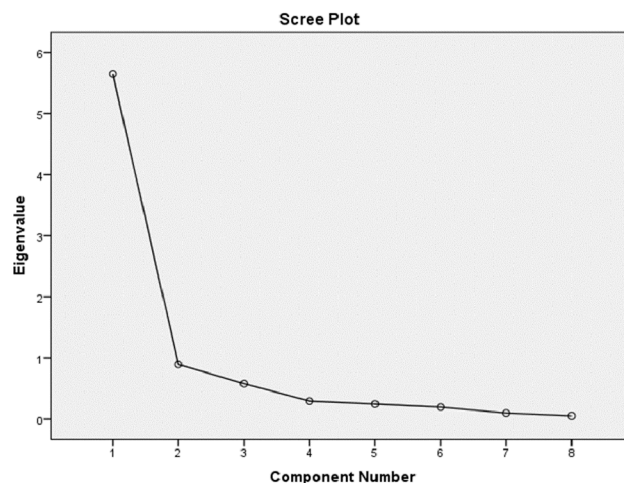


**Fig. 3.** The changes in the adsorption complex and cation composition (CEC: cation exchange capacity) of the soil by treatments (where C: Control; DL: Double Litter; DW: Double Wood; NL: No Litter; NR: No Root; NI: No Input) at 0–15 cm depth after 19 years in the Síkfőkút deciduous forest (Hungary).

**Table 4**

Results of the principal component analysis of soil indicators at 0–15 cm layer after 19 years of the detritus input and removal treatment in Síkfőkút forest (Hungary), where pH: reaction measured in 0.02 M CaCl<sub>2</sub>; MiAS: microaggregate stability; CEC: cation exchange capacity; WHC: water holding capacity; BS: base saturation; SOC: soil organic carbon; HA: hydrolytic acidity; BD: bulk density; clay: < 2 µm; PC1: the first of the principal components; Commonalities: the rate of preserved heterogeneity of the given parameter (the square of their correlation with the PC1); Factor loadings: the Pearson correlation of the variable with the PC1.

Principal component	PC1	Scree Plot	
Eigenvalue	5.65		
% of variance	70.62		
Indicators	Factor loadings	Commonalities	
pH	0.949	0.901	
MiAS	0.877	0.769	
CEC	0.860	0.740	
WHC	0.825	0.681	
BS	0.768	0.590	
SOC	0.741	0.549	
HA	−0.828	0.685	
BD	−0.856	0.733	



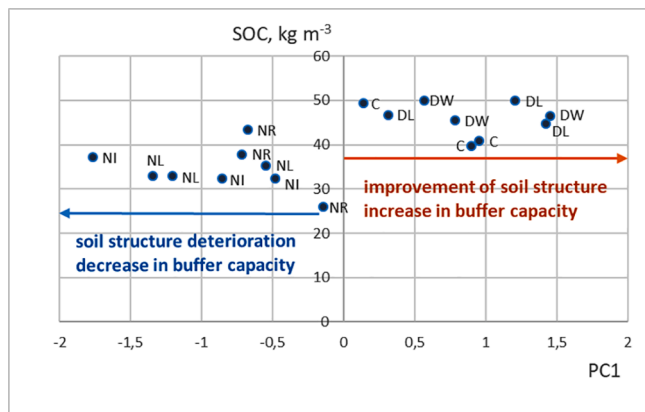
The eigenvalues of the other principal components explained a very small proportion of the total variance. The high communality values also show that there is a high intercorrelation between the variables, i.e. the soil properties change together as a result of the detritus manipulation. The direction of the changes is indicated by the sign of the factor loadings. Detritus input and removal treatments were separated along the first PC axis (Fig. 4). PC1 can be considered as a specific physico-chemical index which expresses in a complex way the changes in the soil caused by carbon turnover and detritus inputs. PC1 was negative for removal treatments and significantly lower than PC1 values for input and Control treatments (Table 2). The PC1 values of the Double Litter and Double Wood plots were the highest, but the difference was not significant compared to the Control.

## 4. Discussion

### 4.1. C-sequestration in the light of the climate and mineralogy of Síkfőkút site

Similar to the other DIRT sites, SOC pools decreased in response to the chronic exclusion of detritus at the Síkfőkút site, but there was no significant difference between the effect of root removal and above-ground litter exclusion (Table 5). At the US DIRT sites, the exclusion of aboveground litter had a more significant effect on SOC than did excluding roots.

In contrast to our Síkfőkút site, at the more humid DIRT sites in USA (Bousson, Andrews, Harvard, Michigan, Wisconsin) and Germany (Steinkreuz), soil organic matter (SOM) pools responded only slightly, or not at all, to the chronic doubling of aboveground litter inputs after



**Fig. 4.** The principal component (PC1) significantly separates the experimental plots depending on their soil organic carbon (SOC) and detritus input or removal treatment (where C: Control; DL: Double Litter; DW: Double Wood; NL: No Litter; NR: No Root; NI: No Input) at 0–15 cm depth after 19 years in the Sífökút deciduous forest (Hungary). Arrows show the direction of change in physicochemical properties in response to detritus removal (blue) or addition (red) treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

10–20 years of treatment (Table 5). The only exception is the Wisconsin Arboretum, where significantly higher SOC<sub>s</sub> were measured on detritus input treatments after 50 years. Explanations for the even negative response of SOM to litter additions include increased decomposition of new inputs and old SOM (so-called priming effect) (Lajtha et al., 2018). Since our Central European Sífökút DIRT site has the driest climate in DIRT network and among similar detritus manipulation experiments, the fact that double-litter treatments resulted in higher SOC suggests that C sequestration of soils is greatly influenced by rainfall conditions (Doetterl et al., 2015).

The significance of precipitation in a temperate climate is confirmed by Fekete et al. (2020), who investigated 17 deciduous forests along a precipitation gradient in the Carpathian Basin including also the Sífökút site. Although net primary production decreases with decreasing rainfall, the carbon sequestration capacity of their soils increases due to reduced leaching and slower degradation processes. Along with this gradient, the pH and Ca content of the soil showed significant differences. Due to the higher degree of leaching in the humid areas, the Ca content and pH of the soils significantly decrease, and the leaching may have affected, directly or indirectly, the SOM (Rowley et al., 2018). The higher amount of litter as a substrate in humid forest areas and higher soil moisture values lead to the formation of higher fungal biomass and this may be the reason for the faster decomposition of organic materials (Fekete et al., 2020). So, the potential effects of less precipitation similar to our experimental site in Sífökút (590 mm per year) may be the formation of higher base saturation and higher pH, resulting in higher carbon sequestration capacity depending on the amount of exchangeable Ca and clay minerals.

Reviewing the soil texture and mineralogy of the DIRT network (Table 5), it can be seen that the smectites are the predominant clay minerals at almost all sites (Madarász et al., 2013) (except for the Andrews site where allophanes are dominant) and Sífökút site has the highest clay content. This suggests that the soil texture and more clay content may play a major role in the Sífökút site's highest carbon sequestration capacity and lower mineralization rate among DIRT sites. This is also supported by Zacháry et al. (2018). Their results confirm the fact that texture primarily affected the size of the fast carbon pool, which was increased by the clay content and decreased by the sand content. Texture has a significant role in soil organic matter mineralization, however, other parameters also play a crucial role in the decomposition. Under given climate conditions, their role in the carbon sequestration capacity of soils depends primarily on their amount and the associated

Ca and Mg (base saturation) as confirmed by Rowley et al. (2018).

#### 4.2. Relationship between carbon sequestration and changes in soil properties

According to our results at the Sífökút DIRT site, soil pH and base saturation decreased in the litter removal plots. The reason for this is, on the one hand, that litter removal reduced Ca and Mg inputs into the soil. Besides, the precipitation reached the surface directly on the removal plots, without litter interception, so the leaching depth may have been greater than on the plots covered with litter (Jiang et al., 2018). Double detrital inputs increased SOC, Ca and Mg input, which resulted in a higher potential CEC and lower acidity. So the increasing SOC and bases increased soil buffering capacity against the leaching and acidic substances from decomposition. Stabilized as Ca humates, more stable mineral-associated organic compounds form than in more humid forest soils (Fekete et al., 2020). The results of the Ca and pH tests carried out 8 years after the establishment of the Sífökút DIRT have already indicated this tendency in the chemical changes (Tóth et al., 2011) since then the differences have become more significant. As the sodium acetate resulted in artificially high CEC in acid forest soils high in organic matter, due to increases in pH-dependent charges, it is not surprising that CEC shows a strong linear correlation with SOC and pH (Skinner et al., 2001; Solly et al., 2019). No significant linear correlation was found between hydrolytic acidity and SOC which shows that with the accumulation of organic matter, the amount and saturation of the variable charges also increase (Vorob'eva and Avdon'kin, 2006). Together with a decrease in organic matter content, explains well the changes in soil structure, as the stability of aggregates also decreases with the decrease of bases and organic colloids (Rhoton et al., 2002; Bai et al., 2020). This is supported by the strong linear correlation between SOC and MiAS at the Sífökút DIRT site. Increase in SOC also increases the potential CEC of soils, which increases water holding capacity as well (Rhoton et al., 2002). So, changes in SOC content play an important role in the formation of soil structure and porosity.

Contrary to our results, most DIRT experiments in temperate climates did not show a significant difference in pH between treatments (Table 5). Moreover, at the Harvard and Bavaria sites, a small decrease in pH was observed for *Double Litter* treatments compared to the *Control* (Huang and Spohn, 2015; Rousk and Frey, 2015). In Wisconsin, where SOC increased as a result of detritus input, a higher pH was measured compared to the control, similar to our results (Lajtha et al., 2014b). A comprehensive study of cation exchange capacity and exchangeable cations in these experimental areas has not been performed or published. We hypothesize that where there is no significant change in pH and SOC, there is no significant change in CEC compared to control. This is because the increase in the CEC of soils is caused by the increase in the amount of organic matter and the decrease in acidity (Parfitt et al., 1995; Emamgolizadeh et al., 2015). Tanner et al. (2016) measured soil carbon after 6 years of continuous litter removal and litter addition in a semi-evergreen rain forest in Panama. Soils in litter addition plots, compared to litter removal plots, had higher pH and contained greater concentrations of exchangeable Ca and Mg and lower BD. Over 15 years at this research site, Sayer et al. (2019, 2020) showed that these changes were also significant compared to the controls, although BD did not differ between controls and litter addition treatments, it has increased in litter removal plots.

Until recently, research into SOC stabilization has predominantly focused on acidic soil environments and the interactions between SOC and aluminium (Al) or iron (Fe) (Rowley et al., 2018). Although it has widely been established that exchangeable Ca positively correlates with SOC concentration and its oxidation resistance. Calcium carbonate can influence occluded SOC stability through its role in the stabilisation of aggregates. Although the addition of Ca<sup>2+</sup> generally improves microbial conditions for decomposition by increasing pH and reducing stress from H<sup>+</sup>, it can counterintuitively reduce respiration rates through the

**Table 5**  
An overview of the effects of worldwide detritus manipulation treatments (DL: Double Litter; C: Control; NL: No Litter) on soil organic carbon (SOC), pH and bulk density (BD) (means or ranges) in the light of different climatic and soil conditions. Different letters represent significant differences within each group ( $p < 0.05$ ).

Site	Climate; mean annual temp. (T); annual precip. (P)	Soil texture; parent material	Duration of treatment; sampling depths	References	SOC (m/m%)		pH		BD	
					DL	C	DL	C	DL	NL
Sikfökt Forest (Hungary)	Temperate; T = 9.9 °C; P = 590 mm	Clay loamy; marine sediments (clay)	17–19-years-old; 0–15 cm		7.27 a	6.93 a	6.1b	5.9 ab	0.87 a	0.87 a
Bousson Experimental Research Reserve (Pennsylvania)	Temperate; T = 8.4 °C; P = 1125 mm	Coarse loamy; glacial till that overlies shale and sandstone	20-years-old; 0–10 cm	<a href="#">Bowden et al., 2014</a>	5.68	6.04	nd	3.12–3.25	0.7	0.9
H.J. Andrews Experimental Forest (Oregon)	Temperate climate; T = 8.8 °C; P = 2200 mm	Coarse loamy; volcanic (strong andic properties)	10-years-old; 0–10 cm	<a href="#">Lajtha et al., 2018</a>	4.9	6.2	5.2–5.4		nd	0.82
Harvard Forest (Massachusetts)	Temperate climate; T = 78 °C; P = 1100 mm	Coarse-loamy; eolian deposits over granite and mica schist	20-years-old; 0–10 cm	<a href="#">Lajtha et al., 2014a</a>	6.85	7.24	4.2	4.3	0.59	0.71
University of Michigan Biological Station (Michigan)	Temperate climate; T = 5.5 °C; P = 817 mm	Sandy; derived from glacial sediments	10-years-old; 0–10 cm	<a href="#">VandenEnden et al., 2018</a>	7.38	6.47	nd	nd	nd	nd
University of Wisconsin Arboretum (Wisconsin)	Temperate climate; T = 7.5 °C; P = 928 mm	Silt-loam; glacial deposits overlaid by a loess cap	50-years-old; 0–10 cm	<a href="#">Lajtha et al., 2014b</a>	4.743.89	3.432.84	5.90	4.90	0.901.01	1.051.04
Steinkreuz, Bavaria (Germany)	Temperate climate; T = 7.5 °C; P = 750 mm;	Sandy to loamy; developed from sandstone	14-year-old; 0–4/8 and 4/8–10 cm	<a href="#">Huang and Spohn, 2015</a>	1.73*	1.43*	3.9	4.0	nd	nd
Heshan National Field Observation and Research Station of Forest Ecosystem (China)	Subtropical monsoon climate; T = 22.3 °C, P = 1700 mm	Sandy loam; sandstone	10-years-old; 0–10 cm	<a href="#">Cao et al., 2020</a>	0.77*	0.86*	4.1	4.2	nd	nd
Barro Colorado Nature Monument (Panama)	Tropical climate; T = 27 °C; P = 2600 mm;	Oxisols with kaolinite as the dominant clay mineral; developed on basalt	10-years old; 0–5 cm (dry and wet season)	<a href="#">Cusack et al., 2018</a>	nd	1.6–2.1	0.9–1.5	4.13	nd	nd

\* The dimension of SOC is  $\text{Mg ha}^{-1}$ .

stabilization of SOC (Gaiffe et al., 1984).

According to our results, the BD values of *No Root* and *No Input* are higher than in the plots *Double Litter*, *Double Wood* and *Control*. The possible reason for this is the lack of roots and fewer surface detritus. As a result, there is less food for soil-dwelling organisms e.g. also for earthworms. Besides, the soil-dwelling organisms that make up the structure have a harder time getting into these soils because of the foils (*No Roots*), so presumably, there may be fewer of them. Kotroczó et al. (2020) and Fekete et al. (2014) found that the *Double Litter*, *Double Wood* and *Control* plots at the Sífölkút DIRT site have a higher average moisture content and a more balanced microclimate. These conditions and higher litter input are favorable for soil organisms. All of these ecological and environmental differences between treatments can also be measured in terms of enzyme activity, soil respiration, the number of microorganisms, and fungal biomass values (Beni et al., 2017).

The relationship between carbon sequestration and soil structure is supported by the fact that the changes in soil structure at the US DIRT sites are less significant than at the Sífölkút DIRT sites. However, even though differences occurred in SOC concentrations in removal treatments, no significant differences were found in BD across treatments in Bousson site in Pennsylvania (Bowden et al., 2014) and Harvard Forest sites (Lajtha et al., 2014a) after 20 years from the start of treatment. Examined at the level of soil aggregates, treatments had a significant effect on soil structure at Bousson site (Mayzelle et al., 2014). The degree of macroaggregation and structural stability of the 0–5 cm depth soil generally increased with increasing detritus input in the following order: *No Root* > *Double Litter* > *Control* > *No Litter* > *No Input*. In Wisconsin Arboretum, the BD decreased in *Double Litter* plots and increased significantly in the *No Litter* plots in the 50th anniversary of the experiment (Lajtha et al., 2014b). Similar to our results, most literature reports that changes in the soil physical and chemical properties are limited to the topsoil. A decade or two is considered as a short time in soil development, so we may not expect much faster change. However, these long-term experiments are suitable for identifying trends in carbon sequestration of soils.

## 5. Conclusion

According to our results, the 19-year detritus manipulation treatment on the Sífölkút DIRT site resulted in a significant change in the SOC content and chemical properties of the soils, and consequently also in the soil structure. As a result of carbon sequestration, the physico-chemical properties of the soil change together, which can further increase the carbon sequestration in the soil as positive feedback. In this respect, compared to other similar experiments in the world, our Central European territory can be considered unique.

The accumulation of organic matter in soils and formation of stabilized mineral-associated organic matter is determined both by mineralogy and climate as well as detrital inputs besides that regulated by several physicochemical properties such as clay, CEC and Ca content of soils. Quantifying the protective capacity of soils requires careful consideration of all mechanisms of SOM protection and the implications of experimental procedures. In this regard, the maintenance and establishment of DIRT and similar continental research networks are very important.

Based on a review of the literature data and our results, we conclude that potential CEC, the exchangeable cations and base saturation could also play a fundamental role in predicting the occurrence of these stabilisation mechanisms. We suggest to include these parameters into the current SOC models.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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