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Variability in litter inputs affecting soil fungi and bacteria through moisture and carbon content in forest soil

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Abstract

Soil organic matter content is a main driver of soil functions and ecosystem services. Various quantity of litter inputs was studied in a *Quercetum-petraeae-cerris* forest in northeastern Hungary at the Síkfőkút DIRT (*Detritus Input and Removal Treatment*) experimental site. The goal of the project was to assess how rates and sources of plant litter inputs might control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal time scales. Six treatments were applied at the experimental site. Beside the control (CO) condition, two detritus addition (double litter (DL) and double wood (DW)) and three detritus removal (no litter (NL), no roots (NR) and no input (NI) treatments were applied in which detritus quantities were manipulated above and below ground. Our aim was the study of the relationship between the litter treatments, their carbon (C) content and the number of microorganisms and biomass of fungi. Litter treatments also had a significant effect on soil microorganisms and soil organic carbon (SOC) content. These effects decreased in parallel with soil depth. Fungal biomass values were more than five times higher for DL (2 mg fungi g^{-1} soil) than for the soils of NI (0.4 mg fungi g^{-1} soil) condition in the upper 5 cm layer, while 0.57 (DL) and 0.08 (NI) values were measured in the 15–25 cm layer. The most probable number (MPN) method, which measures the number of certain groups of living and active microorganisms (fungi and bacteria), showed even greater differences between the treatments. Positive direct and indirect effects of greater organic matter inputs is affected the soil functioning through on better moisture and C content in soils. Litter entering the forest floor resulted in a larger amount of organic substrate and inorganic nutrients. In addition, it resulted in more favorable microclimatic conditions (lower temperature and soil moisture fluctuation) in the soils, which increased the number of microorganisms and the biomass of fungi. There is no significant difference in the number of microbes between the control and doubling treatments (DL, DW). Furthermore, in the case of fungal biomass, there is a significant difference only in the upper 5 cm layer of the DL. These results explain the significantly higher SOC content of the DL treatment compared to the other treatments, suggesting a weaker priming effect. In summary, the results of our research suggest that litter removal had a much greater effect on soil microbial number and fungal biomass as well as SOC content than the addition of a similar amount of litter.

1. Introduction

Soils are critical components of terrestrial ecosystems. Soil properties can affect many functions of biosphere, such as production of biomass, exchange of materials among pedosphere, atmosphere, hydrosphere and living organisms (Eldor, 2014). Forest soils store two-thirds of all terrestrial SOC (Xia et al., 2015). Temperate deciduous forests at northern mid-latitudes play an important role in the global C cycle (Barr et al., 2002), and forests of temperate zone are actively accumulating C in large enough quantities to affect global C budget (Canadell et al., 2007). The role of forest soils in global C balance is also critical. Soils are important components of global C storage, containing about two and a half times as much C as vegetation (Batjes, 1998; Field and Raupach, 2004). Globally CO_2-C release from soil is estimated to be 8×10^{16} g $year^{-1}$ (Raich et al., 2002), more than ten times the

amount of C derived from fossil fuel combustion (Schlesinger and Andrews, 2000).

Forest ecosystems have a high C content stored on their soil surface and C could accumulate in soil as a result of both the deposition of photosynthesis-derived C by roots and detritus decomposition (Baldrian et al., 2013). Soil microorganisms play a very important role in soil nutrient and soil C cycling, thereby in forest productivity, because they drive biogeochemical processes (Kotroczó et al., 2009; Blagodatskaya and Kuzyakov, 2013; Błońska et al., 2018). Decomposer organisms, and especially fungi are particularly important parts of these ecosystems, and their biomass is high in acidic soils of forests (Wallander et al., 2001; Osono, 2007). Fungi are represented by both the saprotrophs, which decompose detritus and soil organic matter, and by mycorrhizal fungi. Soil microbial biomass responds rapidly to changes of the external environment, so it can be a good indicator of environmental change (Classen et al., 2015). Soil fungal biomass and diversity was found to be strongly associated with plant biomass and diversity in forest soils (Peay et al., 2013). Detritus production and climate both influence the number, biomass and activity of decomposing microorganisms (Fekete et al., 2014).

Our studies were carried out in Síkfőkút International Long-Term Research Site. This site is suitable to exemplify climatological and forest compositional changes that are likely to affect leaf and root litter inputs, as well as soil organic matter (SOM) content (Fekete et al., 2014). Long-term meteorological data show that the site has become drier and warmer in the last 40 years, typical to many other areas of Hungary. Frequency of summer drought events increased in the last few decades; summer weather in the Carpathian Basin is increasingly similar to that in the Mediterranean areas (Domonkos, 2003). The structure of Síkfőkút forest has changed significantly for last half century: the number of sessile oak trees (*Quercus petraea*) has decreased by 71%, and the number of Turkey oak trees (*Quercus cerris*) by 15.8%, while number of Hedge maple trees (*Acer campestre*) has increased from 0% to 28%, and some other tree species (e.g. *Acer tataricum*, *Cerasus avium*) have also appeared (Kotroczó et al., 2012; Fekete et al., 2017). Leaf-litter production as an average has decreased by 13% (Kotroczó et al., 2012). By contrast, the amount of wood detritus (branches, twigs, bark and trunk) has been increasing for the last 40 years. The proportion of *Q. cerris* and *A. campestre* litter has been growing as these species had increased their dominance, following the mortality of *Q. petraea* (Kotroczó et al., 2012).

Present research with Síkfőkút DIRT is part of the international DIRT effort to explore how changes in quality and quantity of detritus inputs might affect biological activity, chemical composition as well as numerous physical variables, especially soil organic matter composition and content (Nadelhoffer et al., 2004). In this study, the effects of changes in these variables were measured by microbial (bacteria and fungi) number and fungal biomass of soils of litter treatments. Microbial communities in detritus and in soils consist of a very broad range of creatures in different physiological states (e.g. active, passive, dying and dead) (Blagodatskaya and Kuzyakov, 2013). Methods used can measure the different physiological groups within them.

Differences in mass of detritus, chemistry of leaves and of root litter can influence the abundance, distribution and composition of any decomposer microorganisms (Gholz et al., 2000). Furthermore, alterations in vegetation biomass and composition can influence microbial biomass, soil organic matter (SOM) content, pH and chemistry.

According to our hypothesis, litter removal has a greater effect on the number of microbes and the amount of fungal biomass than the addition of litter to the same extent. According to our other hypothesis, the microbial counts and fungal biomass values of the litter doubling treatments in the soils of the Síkfőkút DIRT site do not show a significant difference compared to the control, as the priming effect here is not as significant as for wetter US DIRT sites. As a result, in Síkfőkút DIRT site more SOC can accumulate in soil of DL treatment than in the soil of control. Effect of such various litter treatments might be dependent also on the measured soil moisture, which can be significantly influenced by depth of soil layers, as well. Furthermore, it is expected that more favorable soil conditions (temperature, soil moisture) will result in a greater increase in abundance of microbes in soils of treatments receiving extra litter than in the treatments of litter removal and the missing soil organic matter, as driving force in any soil functioning. Model experiment of DIRT site is serving as appropriate tool to study the role of SOM in any of soil functioning and ecosystem services.

2. Materials and methods

2.1. Site description and experimental design

Our investigation has been carried out in Síkfőkút Experimental Forest in northeastern Hungary (Fig. 1). Study area (27 ha) is located on the southern slopes of Bükk Mountains at an average altitude of 325 m a.s.l (47°55' N; 20°26' E). The area has been protected since 1976. Currently it is part of the Bükk National Park. The climatic data of the site was compiled from the CARPATCLIM (high-resolution database of the Carpathian Region—www.carpatclim-eu.org). The timeframe was between 1961-2010. Mean annual temperature is 10.4°C and mean annual precipitation is 586 mm (Fekete et al. 2021). According to the soil surveys the soils are Chromic Protovertic Luvisols (Clayic, Cutanic) and Protovertic Endostagnic Abruptic Luvisols (Clayic, Cutanic) (Switoniak et al., 2014; Juhos et al., 2021). The forest covering the area (*Quercetum-petraeae-cerris* community) has had no active management since 1976 (Jakucs, 1987), but it has a legacy of intensive forest management that occurred before hand.

Experimental aboveground and belowground litter manipulation (DIRT) plots were established in November 2000 (Table 1). We established one control and five litter manipulation treatments each with three randomly located 7 × 7 m replicate plots established under complete canopy cover (Nadelhoffer et al., 2004). The first DIRT site was established in 1956 by Francis Hole in the University of Wisconsin Arboretum in two forest and two prairie sites, where the manipulations included doubling and removal of aboveground litter inputs annually (Lajtha et al.

Fig. 1. Location of the Sikfőkút DIRT (Detritus Inputs and Removal Treatment) Project, as part of International Long-term Ecological Research (ILTER) network.

Table 1
The DIRT (Detritus Input and Removal Treatments) treatments at the Síkfőkút ILTER (International Long-Term Experimental Research) oak forest (Hungary).

Treatments	Description
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from NL plots annually after defoliation.
Double Wood (DW)	Aboveground wood debris inputs are doubled by adding wood to each plot annually after defoliation. Annual wood litter amount was measured by boxes placed to the site and its double amount of that was applied in the case of every DW plots.
Control (CO)	Normal litter inputs. No manipulation
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was totally removed in autumn after defoliation by raking.
No Roots (NR)	Roots within the plots were severed from surrounding trees by excavation of trenches to the C horizon (1 m); (the trenches were 0.4 m wide), impervious barriers (0.6 mm thick high density Rootproof Delta MS 500 PE foil) were inserted into the trenches which were then backfilled. Trees and shrubs in the plot were removed when the plot was established. Chemical weed control was also applied used by Medalon (agent: 480 g l^{-1} glyphosate-ammonium) and dry plant residues were removed. Spraying was applied once or twice a year. The leaf litter and wood debris providing the trees in the surrounding plots was not removed. The amount of surface litter was similar to the control plots.
No Inputs (NI)	Both above and belowground inputs are excluded as in NL and NR plots.

2018). The International DIRT Project assesses the role of plant litter input quantity and quality (leaf, wood and roots litter) on the accumulation and dynamics of organic matter in forest soils. DIRT uses an experimental approach of chronically adding aboveground litter, excluding litter, and preventing root ingrowth to long-term experimental plots to assess the importance of plant litter sources and loading rates on SOM formation and accumulation or loss (Lajtha et al. 2018).

2.2. Soil sampling and measurements

Soil samples (0 to 25 cm, separating three layers: 0–5, 5–15 and 15–25 cm) were collected for biological analysis from 5 randomly chosen locations of every plot.. Soil samples for determining fungal biomass and microbial number were stored at 4°C and the measurements were performed within 1 week after sampling. Dates of soil sampling: 03.07.2015; 02. 10. 2014; 07.12.2015; 07.11.2016, 01.12.2017; 13.04.2018; 03.07.2018; 22.02.2019. Soil samples were sieved (2 mm). Determination of soil fungal biomass was based on alkali saponification followed by solid-phase extraction for separation, purification and enrichment. After the sample preparation, the ergosterol content of the soil sample was measured by HPLC and the soil fungal biomass was calculated from the amount of the ergosterol (Beni et al., 2014). Montgomery et al. (2000), Wallander et al. (2013) and Béni et al. (2021) suggested that fungal biomass could be calculated from a fungal biomass: ergosterol ratio of 250:1. We also counted on this ratio.

The soil microbiological status of aerobic bacteria and microscopic fungi were determined by Most Probable Number (MPN) method, described by Reichart (1991) and Libisch et al. (2010). The commonly used Nutrient liquid broth was used to determine the number of bacteria. The number of microscopic fungi that could be cultured was determined using Sabouraud liquid broth. In the assay, 9 mL of sterile physiological saline was added to 1 g of fresh soil, and after one minute of vortexing, the dilution series was performed in 10-fold dilutions on a microplate using the above mentioned liquid broths. During the

method microorganisms are grown in a liquid medium on microplate. In triplicate, prepare a series of dilutions in the appropriate (selective) broth to read the number of positive tubes at the end of the incubation period. Using the 3-digit code number obtained from this, the most probable number of living cells is determined on a statistical basis (Cochran, 1950).

Soil samples for SOC analysis were passed through a 2 mm sieve and were dried and pre-treated with 10% hydrochloric acid to eliminate inorganic carbonate content before organic C analysis by dry combustion using an elemental analyzer (Elementar Vario EL CHNS, Elementar Analysen systeme GmbH, Germany) (Matejovic, 1997). Soil moisture contents were examined by determining the mass of wet and dry samples after drying in oven at 105°C for 24 hours.

2.3. Statistical analyses

The comparison of the physicochemical soil properties among detritus input and removal treatments was made with ANOVA and Tukey's post-hoc test, representing significant differences within each group at $p < 0.05$. The distribution of fungi and bacteria numbers and fungal biomass among treatments were compared with 'ggstatsplot' package, pairwise Dunn's test calculated significant differences. The relationships between certain measured variables were explored and modelled using linear regression analyses (Patil, 2021).

3. Results

SOC was significantly lower for the litter withdrawal treatments than for the litter doubling and control treatments, as well as for the soil-dwelling organisms as well.

Parallel to the depth of the soil, we experienced a significant decrease in the fungal biomass in the deeper soil layers (Fig. 2).

Considering the average values of fungal biomass in the six treatment conditions, it was 238% higher in the upper 5 cm layer than in the 5–15 cm layer and 3200% higher than in the 15–25 cm layer. The highest differences of fungal biomass between soil layers were detected for DL and NR treatment conditions, while the smallest ones for NL and Control treatment conditions. There was no significant difference between the 0–5 cm and 5–15 cm layers for the NL treatment, and a significant difference was found between the 3 layers for all other treatments. The litter treatments also generated significant fungal biomass differences in the soils of the treatments at all 3 soil depths. Litter growth (in the soils of DL and DW) increased and litter removal (in the soils of NL, NR, NI) decreased the fungal biomass of the soils. In all three layers, the DL treatments showed about 5 times higher fungal biomass than the lowest NI treatment (but showed almost similar differences between DW and NI). The absence of live roots also results in a significant reduction in the biomass of fungi, which is well demonstrated by the finding that the fungal biomass of the control was 127% bigger than that of the NR. Doubling of wood material input (branches, twigs, bark) has increased the fungal biomass to a lesser extent (by 36% compared to control), and that rise is not sufficient to make it significant. However, doubling of the leaf litter has resulted in a significant increase of fungal biomass (by 83%) compared to the control.

In deeper layers of soils the influence of the surface litter seems to decrease, which is well demonstrated by the fact that in these layers no significant difference was found between the 2 doubled treatments and the control. The lack of both leaf litter and live roots has found to reduce the amount of soil microorganisms often significantly, as measured by the MPN method. However, doubling of leaf or wood litter did not cause a significant increase in the amount of bacteria and fungi, as compared to the control condition (Fig. 3 and 4).

Number of fungi in the group with litter doubling and Control treatments was 452% higher in the upper 5 cm layer than in the 5–15 cm layer (for litter withdrawal treatments, the difference was only 287%). In the case of bacterial counts, the difference between the two soil layers was even greater: 1014% for the doubling and Control treatments, and 633% for the litter withdrawal treatments.

In the upper 5 cm layer, the bacterial count of doubled litter (DL, DW) and control (CO) treatments showed a 16.5-fold

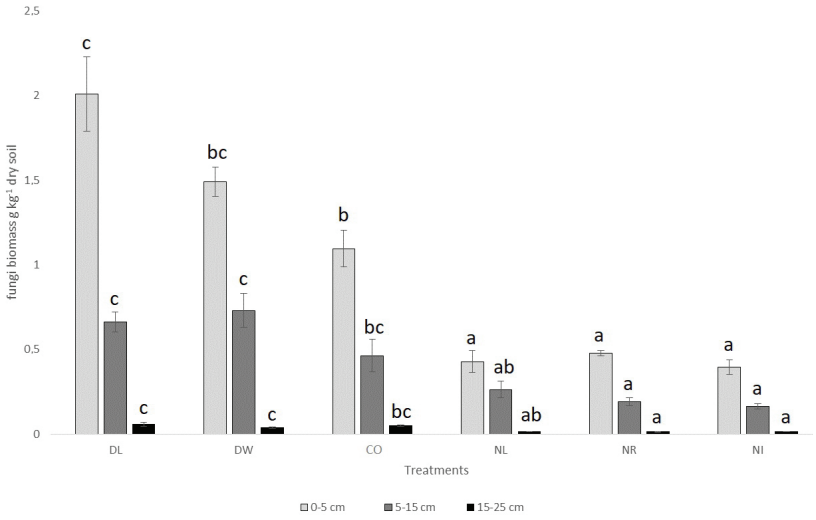

Fig. 2. Fungal biomass in the upper 25 cm (0–5, 5–15, 15–25 cm of soil layers) in Síkfőkút DIRT Project. Different letters indicate significant differences among treatments in each of the soil layers (0–5, 5–15, 15–25 cm).

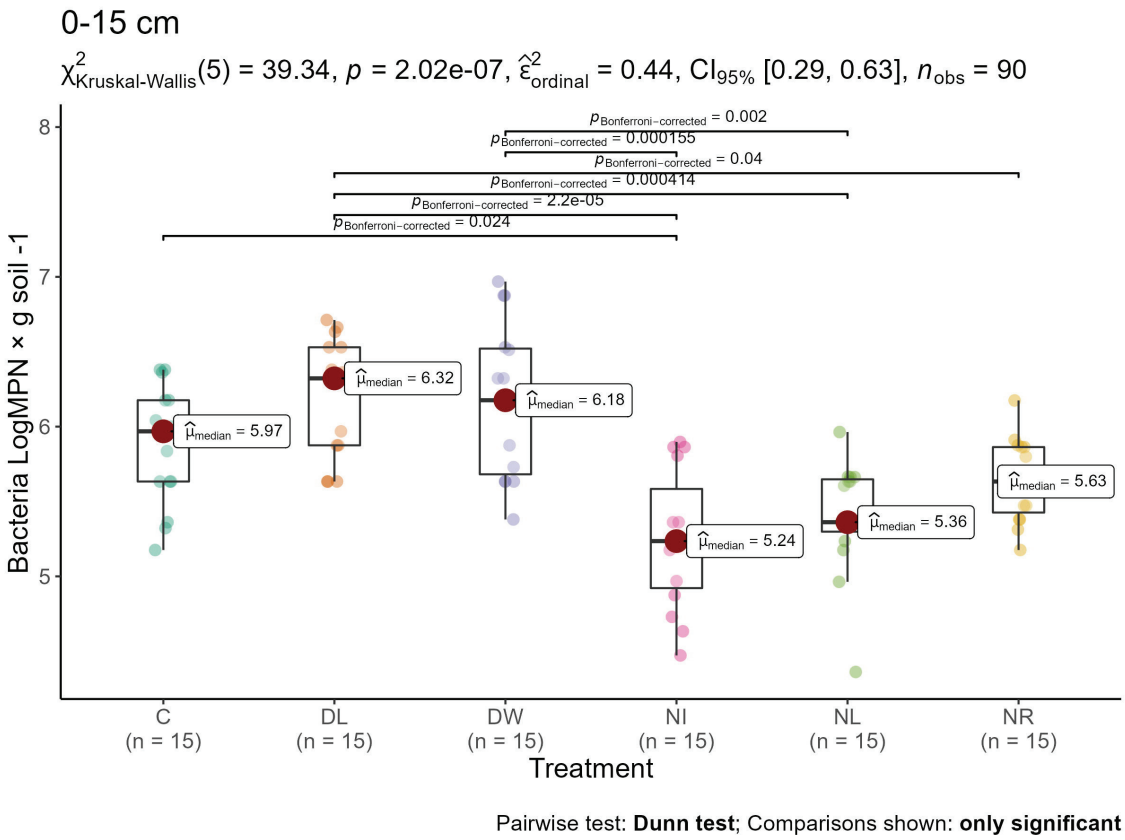

Fig. 3. Abundance of cultivable bacteria in treatments of Síkfőkút DIRT site in the upper 15 cm of soil layer by MPN test.

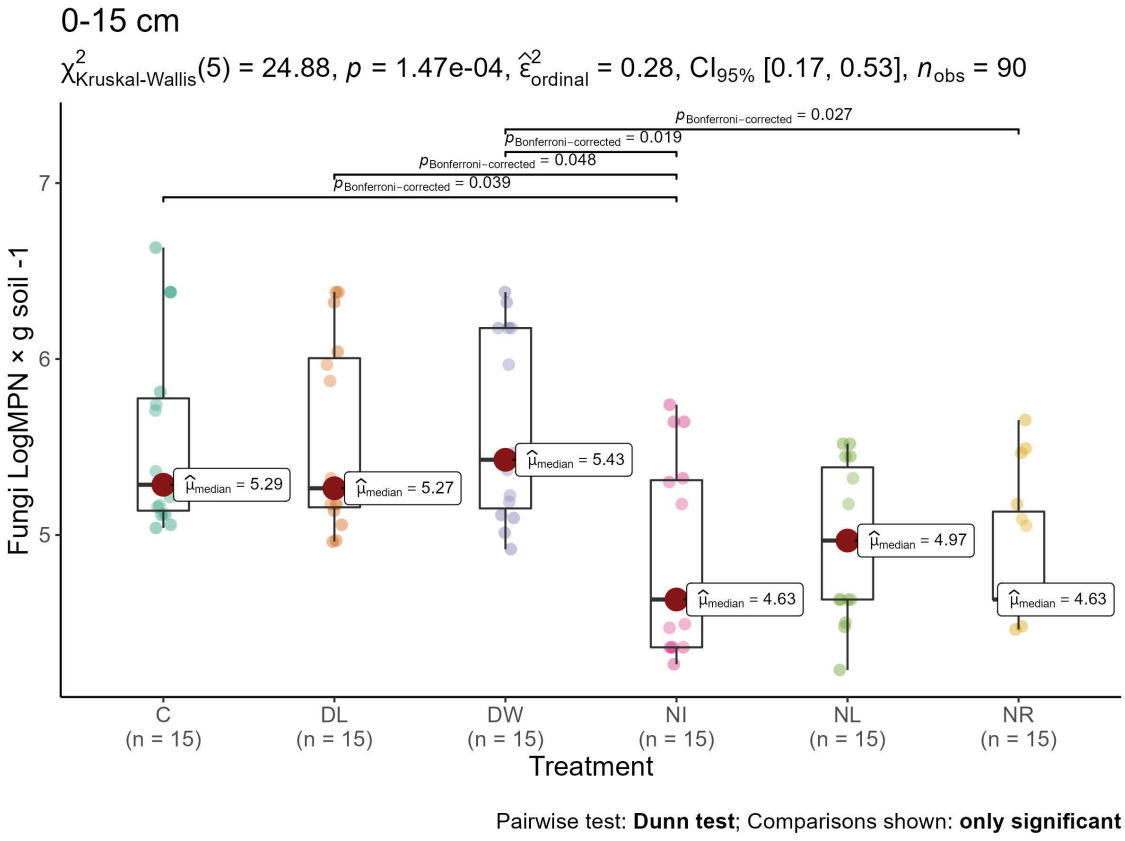

Fig. 4. Abundance of cultivable fungi in treatments of Síkfőkút DIRT site in the upper 15 cm of soil layer by MPN test.

difference between lowest and highest values at times of measurement. The same value was 6.5-fold for the litter withdrawal (NL) treatments. For the number of fungi, these values differed 62-fold and 30-fold, respectively. The greatest difference was found in the soils of DL treatment with a 26-fold difference in the number of bacteria (it was only 6-fold in the case of NL) and an 83-fold difference in the number of fungi with DL (17-fold in the case of NR).

Both bacterial (Fig. 5) and fungal MPN counts (Fig. 6) as well as fungal biomass (Fig. 7) showed a strong correlation with C content of examined soils in the upper layer (0–5 cm). However, the correlation between SOC content and fungal biomass showed a rapid decline with soil depth (Fig. 7). In the top soil layer (0–5 cm) R^2 value was 0.76, which indicates a strong correlation between these two variables. In the deeper layer (5–15 cm) this value was lower (R^2=0.59); while in the deepest examined layer (15–25 cm) there was no correlation between these two variables.

3.1. Effect of soil moisture on microbial counts

The correlation between soil moisture and fungal biomass also shows a downward trend as soil depth increases (Fig. 8). In the upper layer R^2 was 0.77; in the middle layer it was lower (R^2 = 0.42), while in the lowest layer there was no significant relationship between the two variables. MPN bacterial cell counts showed that litter withdrawal treatments (combined 3 treatments) did not show a significant relationship with soil moisture, while litter doubling treatments and Control combined showed a positive correlation with soil moisture (Fig. 9). For microscopic fungi, both groups showed a positive correlation with soil moisture (Fig. 10).

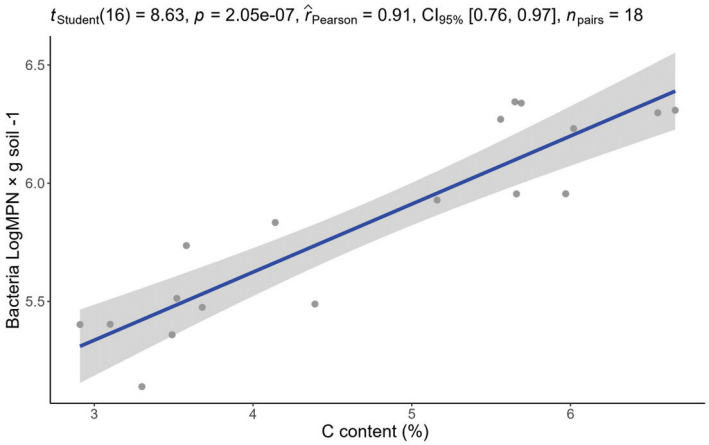

Fig. 5. Correlation between SOC content and bacterial MPN counts in the top soil layer (0–5 cm) in Síkfőkút DIRT site. Mean values of MPN were calculated for the 18 plots.

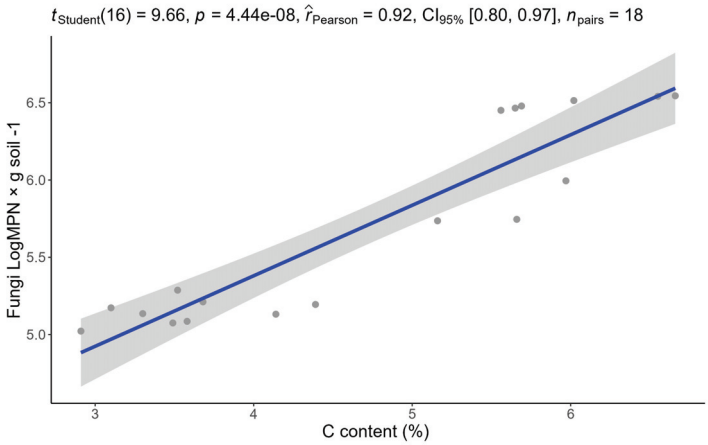

Fig. 6. Correlation between SOC content and fungal MPN counts in the top soil layer (0–5 cm) in Síkfőkút DIRT site. Mean values of MPN were calculated for the 18 plots.

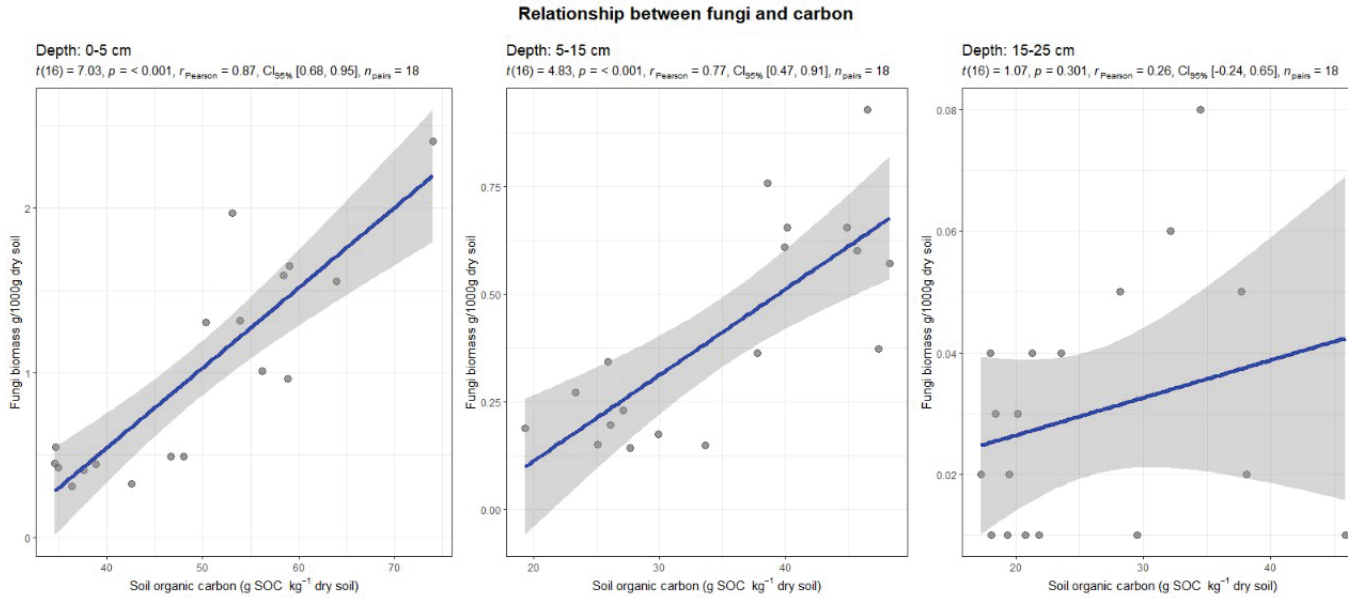

Fig. 7. Correlation between SOC content and fungal biomass in the three soil layers (0–5, 5–15, 15–25 cm) in Síkfőkút DIRT site.

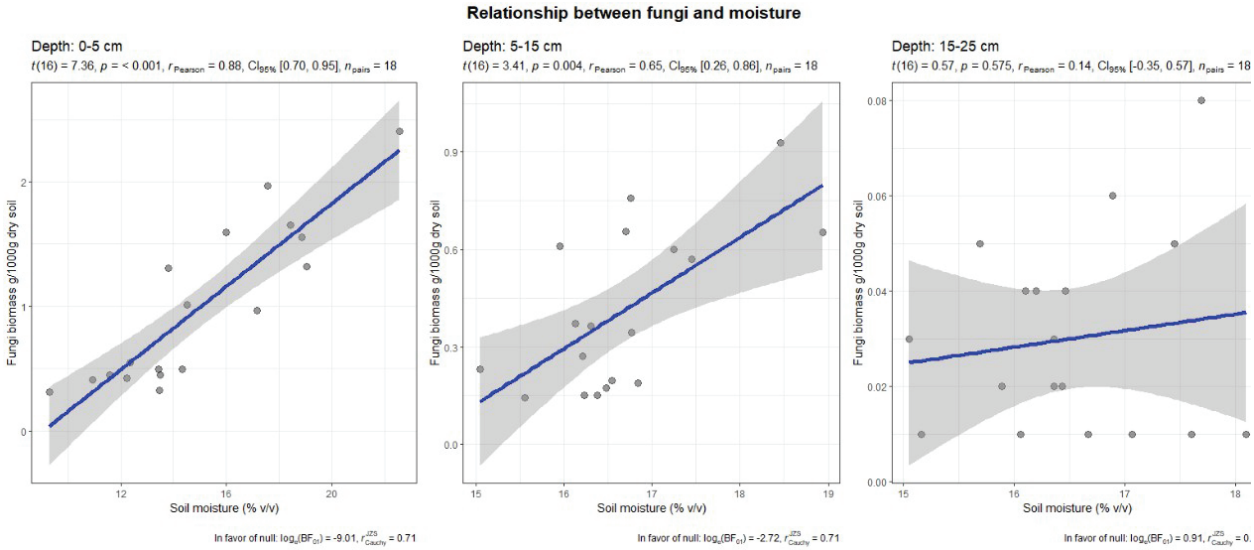

Fig. 8. Correlation between soil moisture content and fungal biomass in the three soil layers (0–5, 5–15, 15–25 cm) in Síkfőkút DIRT site.

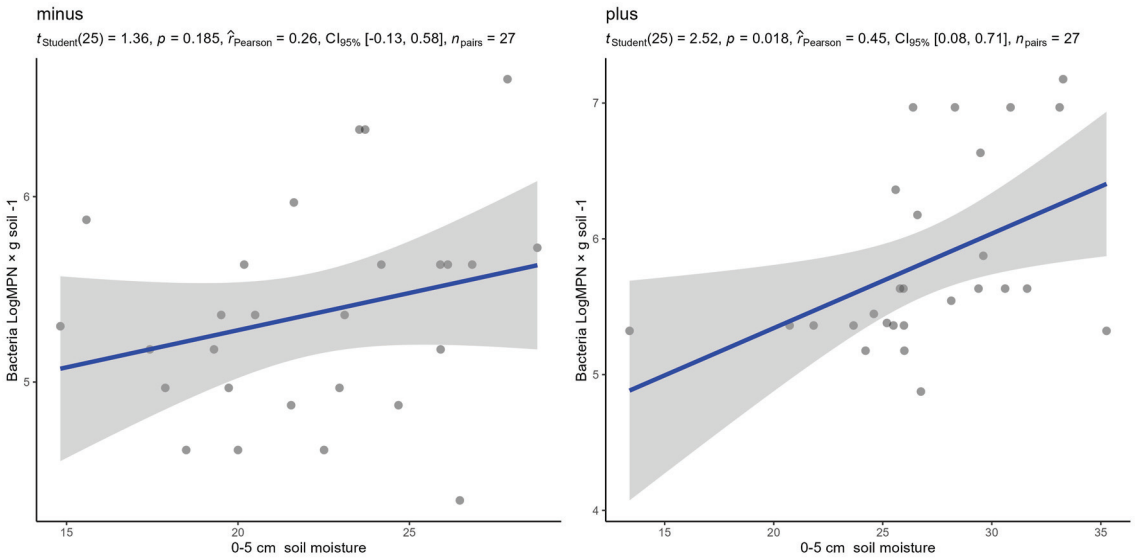

Fig. 9. Correlation between soil moisture content and bacterial MPN counts in the top soil layer (0–5 cm) in Síkfőkút DIRT site. „minus" means litter withdrawal treatments (combined 3 treatments), while "plus" means litter doubling treatments (DL, DW) and Control.

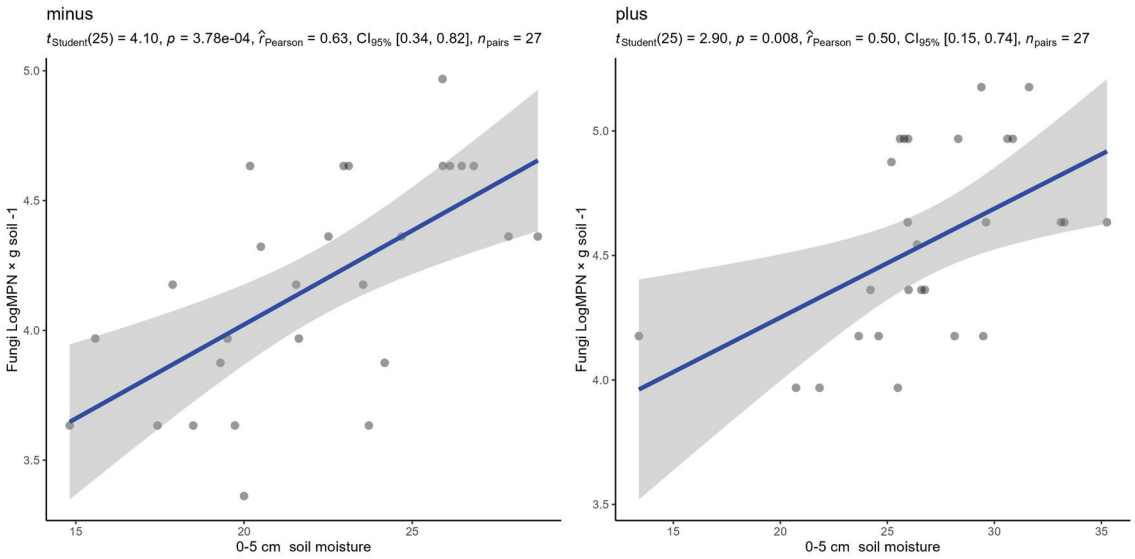

Fig. 10. Correlation between soil moisture content and fungal MPN counts in the top soil lay-er (0–5 cm) in Síkfőkút DIRT site. „minus" means litter withdrawal treatments (combined 3 treatments), while "plus" means litter doubling treatments (DL, DW) and Control.

4. Discussion

4.1. Effects of various litter treatments on soil microorganisms

The soils of litter withdrawal treatments (NL, NR, NI) are characterized by lower values of litter production, drier conditions, higher temperature extremes (and higher temperature fluctuations) and lower pH values (Fekete et al., 2016; Juhos et al., 2021). These parameters also affected the SOC content of the soils. Higher amount of leaf and wood litter entering the soil of DL and DW, and in connection with this the higher organic matter content of the soils, provides more nutrients and organic substrates for soil microorganisms. So if the background parameters (e.g. soil moisture and soil temperature) becomes favorable for microbes of soils with doubling and control treatments showed a more significant increase than those with litter withdrawal soils. Litter treatments also have a significant effect on bulk density, which can affect water holding capacity of soil and the aeration in the deeper layers (Minasny and McBratney, 2018; Schütte et al., 2021), which in turn has a significant effect on the fungal biomass and microbial content of the soils. Lack of live, continuously growing roots enriching the soil with nutrients and organic residues in the root withdrawal treatments (NR, NI) and lack of surface litter for 2 decades in NL and NI treatments significantly reduces the SOC (Fekete et al., 2014; Juhos et al., 2021); thus, it affects not only the microorganisms involved in decomposition processes, but also the soil-dwelling animal organisms that moving the soil particles and thus continuously loosen the soil. This lower activity and the significant decrease in SOM can be explained by the higher bulk density values of soils of litter withdrawal treatments; these effects also reduce the water storage capacity of soils, which also has an indirect negative effect on microorganisms (Li et al., 2002; Juhos et al., 2021).

The number of bacteria and fungi in soils is affected by a number of parameters that often interact at the same time. For example, the fungal growth / bacterial growth ratio is strongly influenced by soil pH (Rousk et al., 2009). According to this, the fungal / bacterial ratio should be higher in lower pH (Table 2) litter removal soils. Our studies did not support this statement in this case. This is probably also due to the high levels of lignin-rich organic matter in the soils of the litter addition and control plots, which promotes the growth of fungi, as lignin is typically considered to be highly resistant to degradation (Kögel-Knabner, 2002) and only specialized biota (predominantly white-rot fungi) are able to synthesize the special extracellular enzymes that are necessary to break down these recalcitrant structures (Swift et al., 1979; Yue et al., 2016). Furthermore, fungi also play a dominant role in the degradation of intact cellulose content of forest litter (Sun et al., 2020).

The fungal biomass of the litter doubling treatments also increased compared to the control, but only in the upper 5 cm layer, while no significant difference was found in the number of fungi and bacteria. Enzyme activity studies also showed no significant difference between control and litter addition treatments (Fekete et al., 2014; Veres et al., 2015). Because significant extra litter only slightly increased the organic matter degrading activity of microorganisms, significantly more C could accumulate in the DL soils than in the control treatment soils. This was not observed for the wetter US DIRT sites (HD Andrews (OR), Bousson (PA), Harvard (MA)), and in fact, control treatment at these sites showed higher C content than control (Juhos et al.,

Table 2
Effect of detritus input and removal treatments on physicochemical soil properties at 0–25 cm layer 19 years after the site was founded (C: Control; DL: Double Litter; DW: Double Wood; NL: No Litter; NR: No Root; NI: No Input) in Síkfőkút forest, 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 (m/m%); SM: Soil moisture (m/m%) in the given soil layers on 25.06.2016, CCSL: the C content of surface litter kg/plot (49 m^2); fluct: Maximum yearly fluctuation of the temperature in the soil of litter treatments (in °C) based on (Fekete et al., 2016), DTR: diurnal soil temperature range (daily average over all season, based on (Fekete et al., 2016)), WHC: water holding capacity (based on (Juhos et al., 2021)); pH: reaction measured in 0.01 mol L^{-1} $CaCl_2$(based on (Juhos et al., 2021)). Diferent letters represent significant differences ($p < 0.05$) among the treatments.

	DL	DW	C	NL	NR	NI
SM in 0–5 cm	19.5±1,54c	18±0.98bc	15.2±1.03abc	12.2±0.74a	13.8±0.29ab	11±0.89a
SM in 5–15 cm	17.2±0.21a	17.8±0.92a	16.4±0.17a	16.5±0.16a	16.1±0.54a	16.1±0.29a
SM in 15–25 cm	17.2±0.38a	17.3±0.62a	16.3±0.35a	16.5±0.35a	15.8±0.4a	16±0.41a
SOC 0–5 cm	6.69±0.22d	5.68±0.07c	5.78±0.23c	3.29±0.13a	4.28±0.19b	3.52±0.23a
SOC 5–15 cm	3.7±0.15c	3.15±0.07b	3.01±0.09b	2.27±0.05a	2.48±0.04a	2.29±0.03a
SOC 15–25 cm	2.53±0.24b	2.22±0.12ab	2.15±0.11ab	1.95±0.07a	1.78±0.05a	1.69±0.02a
fluct	20.03	22.12	22.12	25.88	26.61	28.3
DTR	0.63±0.02a	0.74±0.03a	0.85±0.04a	1.46±0.07b	1.48±0.06b	1.87±0.07c
pH*	6.1c	5.85bc	5.88bc	4.97a	5.38ab	5.15a
CCSL*	29.8	23.3	15.5	–	15.5	–
WHC (V/V%)*	50.22bc	52.87c	53.45c	45.49ab	48.9abc	44.22a

2021). The difference between the mentioned American sites and the Síkfőkút DIRT site can be explained by the strength of the priming effect, which is much stronger in the case of American sites with wetter climates and wetter soils than in the case of the often drought Hungarian DIRT site (Fekete et al., 2014; Lajtha et al., 2018).

4.2. Effect of soil depth on soil microorganisms of litter treatments

The largest amount of fungal biomass was found in the topsoil of treatments plots. This is attributable to several factors. First, this layer is in direct contact with the litter layer on the surface (except for NL and NI treatments where it is missing), and second, here the highest organic matter content of soils results in higher numbers and larger biomass of decomposing microorganisms (Šnajdr et al., 2008). At the same time, these soil layers lacked any stagnant water, so the microorganisms present were primarily aerobic decomposers, which favor the upper soil layer with better oxygen supply. Furthermore, only aerobic microorganisms were studied by the used MPN method. Fungal biomass in the topsoil of the NL treatment plot was only 39% of that of the control treatment, which shows the importance of fresh plant litter.

Belowground biomass accounted for between 20–40% of the biomass of trees, depending on species (Jackson et al., 1997), therefore the role of living roots in deeper layers is decisive (Kocsis and Biró, 2015). This also applies to our study. However, difference between root removal treatments (NR, NI) and the Control treatment was found to be larger in deeper layers, showing the in-creasing role of organic material from living roots (e.g. exudation from tree roots) and that of mycorrhizal fungi. This finding can be partly explained by the roots and rhizospheres of trees as well as by ground vegetation associations with fungi to form mycorrhizae. More than 90% of forest trees in temperate and boreal zones use ectomycorrhizal fungi (ECM) to obtain nutritive materials from the soil (Markkola et al., 1996). In fungal biomass study, we measured quite small values in 15–25 cm layer of soils, but even here the difference was significant when comparing DL and DW treatments with the three litter-withdrawal treatments. Compared to Control only live root-free (NR, NI) treatments resulted significant difference, which shows the importance of living root systems in improving fungal biomass production.

In case of litter doubling treatments and Control, number of bacteria and fungi decreases much more with the soil depth than in the case of litter withdrawal treatments. This shows the increasing effect of the litter in microbial counts on the surface layer. The results of number of fungi and bacteria between the soil layers suggest that the surface litter exerts its effect most in the upper 5 cm layer, but even in the 5–15 cm layer it results in significantly higher values compared to the litter withdrawal treatments. Another reason for the difference in litter withdrawal treatments may be the lack of surface protection effect of leaf litter coverings in NL and NI treatments. In these treatments, the surface layer is directly exposed to UV radiation from the Sun, which can reduce the number of microorganisms on and near the surface (Formánek et al., 2014). As it is a forest area, these effects are insignificant due to the shading effect of the canopy. Other effect of the litter cover is that the soils of NL and NI treatments dry out more easily during the dry period, which can significantly limit the number of microorganisms and the amount of living microbial biomass (Borowik and Wyszkowska, 2016; Beni et al., 2017). Microclimate is widely considered as a key constraint regulating rates of litter decomposition (Gholz et al., 2000; Zhang et al., 2008; Both et al., 2017). In our previous studies, we have shown that the degree of temperature fluctuation (both annual and daily fluctuations are significantly higher in the soils of litter removal treatments than in the others) and the number of frosty days are much smaller in litter-covered soils (Table 2) (Fekete et al., 2016). In the soils of the NI, 4 and a half times as many frosty days were measured at a soil depth of 10 cm as in the Control, while in the DL we did not measure any temperature below freezing during 3 years. These effects also can significantly affected the number of microorganisms and the amount of living microbial biomass (Biederbeck and Campbell, 1973; Nottingham et al., 2019).

4.3. Effect of soil moisture on soil microorganisms

It was previously reported that soil moisture content has an effect on and positively correlate with microbial biomass and activity in a certain moisture range (Baldrian et al., 2010). Effect of soil moisture on microorganisms is significantly affected by litter treatments. This effect is strongest near the soil surface. Current findings therefore confirm such a correlation, which decreases rapidly with the depth of the soil.

5. Conclusions

Soil microorganisms react very quickly to changes in environmental variables of the soil. Our current findings have showed higher bacterial and fungal MPN numbers and fungal biomass in the soils of detritus addition treatments and control treatment than in detritus withdrawal treatments. In the soils of doubling treatments (DL, DW) the presence of hot spots of microbial activity was amplified with increasing amount of plant debris, including litter, organic matter from living roots and deadwood; so naturally the microbial biomass of soils also was enlarged. Getting the right amount of organic debris to the surface creates more favorable microclimatic conditions in the soils. This and proper nutrient supply can result in more active soil life, which increases the healthy functioning and stress resistance of soils. It can be also stated that missing and/or improper organic matter content in soils might enhance on soil vulnerability to environmental changes and reduces proper soil functioning, as well. This fact could be considered of developing appropriate soil management practices.

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