Final professional report on research carried out in the framework of application KH_126478 for the period from December 2017 to January 2020

Introduction

Soil organic C and N are critical components of ecosystems. Soil organic matter (SOM) accounts for virtually all of the ecosystem N reserve, it provides cation exchange and water holding capacity, and it acts as a major control on soil pH. SOM also strongly promotes soil aggregation, which in turn accounts for much of a soil's ability to drain freely (and thus resist erosion) yet retain water for use by plants. Soil C accumulation and turnover are important processes globally: soils contain about 1.5×10^{18} g C), which is 2-3 times that in vegetation (Reiners 1968, Schlesinger 1990). The C flux between soils and the atmosphere is large, with soil respiration representing about 10 times the C flux due to fossil fuel combustion (Watson et al. 1990). Thus, any change in rates of soil C turnover will markedly affect the global C cycle. SOM levels are determined by the balance between inputs and losses, the latter arising primarily from respiration, leaching, and erosion. The rate of C loss due to respiration depends on the extent to which OM inputs are stabilized against degradation. Such stabilization can be viewed as involving three overall processes:

1. increased recalcitrance (due to changes in chemical composition of the OM, which are due in turn in large part to microbial degradation and synthesis),
2. chemical interactions (especially with clay surfaces and aluminum),
3. decreased accessibility (due to physical protection within soil aggregates).

The first process operates in both O horizon and in mineral soil. The second two are restricted mainly to mineral soil where clay particles can sorb organics and form aggregates that protect the organics from access by microbes (decreased accessibility). At present, however, none of these processes are well understood.

Inputs from belowground plant sources (roots and exudates) are less easily measured, but also vary widely among forests (Aber et al. 1985,). The wide ranges in rates, sources and chemical compositions of plant inputs to forest soils leads to important questions about how different amounts and forms of plant inputs influence soil processes at different levels of organization. Soil organic matter supply is mainly derived from plant litter. The early stages of the litter degradation is a very dynamic process. The vast majority of soil organisms are heterotrophic microorganisms that derive metabolic energy by oxidizing organic C originating as plant inputs (litter, exudates, sloughed roots, etc.) to soils. Likewise, most nutrients used by soil organisms are cycled through plants, which in turn link soil systems to more complex soil-plant-atmospheric systems. Therefore, study this is important for understanding litter degradation and the control factors of different biomes and ecosystems.

Recognizing the lack of knowledge about the long-term, decades-long effects of plant litter on the qualitative and quantitative composition of SOM, American researchers have initiated a DIRT Project to study this. The goal of the DIRT project is to assess how rates and sources of plant litter inputs control the accumulation and dynamics of organic matter and nutrients in forest soils over decadal time scales. In 2000, the Síkfőkút DIRT Project joined this international network. We have investigated this as part of this concept in our research proposal.

Methods

The Síkfőkút ILTER site was established in 1972 (Jakucs 1973). The long-term research connected to the IBP (*International Biological Programme*) and the MAB (*Man and Biosphere*) international programs and concentrated on the structure, production and function of the ecosystem. The experimental site of 27 ha is located in the south part of the Bükk Mountains in North Hungary at 325 m.a.s.l (47°55' N; 20°26' E). This forest has belonged to the Bükk National Park since 1976, which protects this territory from anthropogenic influence. The site has brown forest soils with clay illuviation (Stefanovits 1985) and the type of the soil according to the FAO Soil Classification is Cambisols (IUSS WRB 2006). The site has *Quercetum petraeae-cerris* community

The present research with Síkfőkút DIRT (*Detritus Input and Removal Treatments*) is a part of the international DIRT effort to explore how changes in the quality and quantity of detritus inputs affect biological activity, chemical composition as well as numerous physical variables, especially soil organic matter composition and content (Nadelhoffer et al. 2004; Kotroczó et al. 2008).

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. 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 treatments in Síkfőkút Project site (Hungary).

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 to the site and its double amount was applied in the case of every DW plots.
Control (C)	Normal litter inputs. Average litter amount typical to the given forest site
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was totally removed by 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.

Renewal of the experimental plots

The experimental plots were renovated in 2019 according to proposal's plans. As a first step, several fallen trees that fell on the parcels were removed with the help of a woodcutter (Fig 1). Then trenched NI and NR treated plots were dug again and the root-resistant plates were checked (Fig 2a and 2b). Since the root-resistant foils were laid 20 years ago, they had to be replaced in many cases. This required a Bobcat trencher, which we rented for the duration of the work. In 2017, data loggers were placed on plots (Fig 3) and in 2018, soil solution collectors (Fig 4a and 4b) were prepared (lysimeters) and were placed into the plots.

Fig 1. Removal of fallen tree trunks on parcels

Fig 3. Datalogger

Fig 2a. Trench digging with BobCat trencher

Fig 2b. Trench digging with BobCat trencher

Fig 4a. Preparation of soil solution collectors for plots

Fig 4b. Preparation of soil solution collectors for plots

Presentation of our main results based on our publications and manuscripts under review

Degradation studies of various organic substances

Based on our 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, 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 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 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 surface. 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 materials dry twig and branch pieces were used. 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. More resistant plant tissues, as well as the decomposing microorganisms that make up them, belong to the “k” strategists, whose number is probably less influenced by litter treatments. These conditions together may explain why this type of litter found no significant difference between treatments.

100% cellulose cotton wool bags (as a control organic matter) 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. 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 increases the activity of microorganisms, including the rate of degradation.

In the case of decomposition of green tea (easy degradable organic matter), the higher moisture content of the plots, 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.

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 (Djukic et al. 2018). In Rooibos tea (difficult to decompose organic material) the amount of readily decomposing substances was lower, so we experienced a much smaller weight loss than the 3-months 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 tee 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 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 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.

Microbiological studies of different 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. 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 MPN method. The 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 biomasses accounted for between 20-40% of the biomass of trees, depending on species, therefore the role of living roots in deeper layers is decisive. This also applies to our study. However, the difference between the root removal treatments (NR, NI) and the Control treatment was found to be larger in deeper layers, showing the increasing role of organic material from living roots (e.g. exudation from tree roots) and that of mycorrhiza 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 mycorrhiza.

Bacterial abundance in fresh wood material is very low. MPN measurements showed a significant difference between C and DW soils of treatments in fungal numbers. At the same time there was no significant difference in the number of bacteria between C and DW condition plots. In our opinion, the reason for this is that wood debris represents a rich and reliable source of organic matter which is hard to transform, mainly due to its impermeability and high lignin content. These characteristics benefit filamentous, macroscopic fungi, which are consequently the dominant decomposers of deadwood. Soil moisture content has an effect on and positively correlate with microbial biomass and activity in a certain moisture range. Current findings therefore confirm such a correlation, which decreases rapidly with the depth of the soil.

Soil microorganisms react very quickly to changes in environmental variables of the soil. Current findings have showed a significantly higher amount of bacteria in case of soils of detritus addition treatments and control treatment than in detritus withdrawal treatments. In soils of DL and DW treatments significantly higher amount of fungi and larger fungal biomass was found than in the detritus withdrawal treatment condition. In the soils of double treatments (DL, DW) the presence of hot spots of microbial activity was amplified with increasing amount of plant debris, including litter and deadwood; so naturally the microbial biomass of soils was enlarged, too.

Utilization of the results obtained

Our research aimed to determine the effect of different amounts and quality of organic matter inputs on soil / in soil on the biological-chemical-physical processes. These processes were examined in particular through changes in biomass and biological activity joining international research teams (‘TeaComposition’ and ‘DIRT’) through the proposal. Our results of organic matter decomposition are of great practical importance qualitatively and quantitatively. Climate change and the accompanying changes are a major challenge not only for farmers but also for decision makers, since many times they have to plan ahead for decades. The knowledge, results or methods that help agriculture or the agricultural sector, answer questions on land use, cultivation methods, or a combination of these, in making decisions and choosing the right technologies, are of great importance. Knowledge and dynamics of the carbon storage capacity of soils (which depends not only on the quantity but also on the quality of organic matter in the soil) is also important because their knowledge will be a crucial economic factor in the near future, rising CO_2 quota prices and tightening CO_2 emission values.

Presentation of publications and study trip from the last two years

Publications in progress

We have completed the publications undertaken in the proposal. As promised in the proposal, a large amount of data has been generated from the numerous studies carried out. Some of the data have been successfully published in collaboration with international research networks (*Djukic et al. 2018*; *Lajtha et al. 2018*). Both manuscripts were published in journal Q1, as planned in the proposal. This is remarkable because the data series measured on the Síkfőkút DIRT site have been compared with the data of international research stations working with similar or identical methods. In the second part of the application further series of data are in the process of being published and will be published in the coming period as ***we have submitted the following manuscripts with the help of this proposal***:

1)

Zsolt Kotroczó, Katalin Juhos, Borbála Biró, Tamás Kocsis, Sándor Attila Pabar, Csaba Varga, István Fekete (2020): *Effect of litter manipulation treatments on degradation rates of different type of organic matter in a deciduous forest*. Journal: ***Soil Research***

2)

István Fekete, Áron Béni, Gábor Várbíró, Péter Jeager, Katalin Juhos, Marianna Makádi, Ibolya Demeter, Zsolt Kotroczó (2020): *Effect of Detritus Input Change on Soil Microorganisms in a Forest Site of Central Europe*. Journal: ***Soil Research***

3)

Katalin Juhos, Zsolt Kotroczó, Áron Béni, Balázs Madarász, István Fekete (2020): Long-term effect of detritus manipulaton on soil chemical and physical properties in a Central Europen decidous forest. Journal: ***Geoderma***

4)

Fekete, István; Imre Berki; Kate Lajtha; Susan Trumbore; Ornella Francioso; Paola Gioacchini; Daniela Montecchio; Gábor Várbiró; Áron Béni; Marianna Makádi; Ibolya Demeter; Balázs Madarász; Katalin Juhos; Zsolt Kotroczó (2020): *Soil biological activity and carbon content change significantly in Central European forests along a climatic gradient*. Journal: ***Nature Geoscience***

The research results of the competition have been presented at numerous prestigious international and domestic conferences. Of these, it is worth highlighting the ***7th International***

Symposium on Soil Organic Matter, October 6-11. 2019. Adelaide and the ***21st World Congress of Soil Science: beyond food and fuel.*** Rio de Janeiro. Among the domestic conferences the following should be highlighted: ***Hungarian Congress of Ecologists, Nyíregyháza*****; "Talajtani Vándorgyűlés" Conference**, Pécs and the *XIV. Carpathian Basin Environmental Science Conference*, Gödöllő.
The results of the research (publications) are also available on the website: (homepage)

Study visits in the application

Our professional study trip to Canada and the United States, where we met not only the leader of the ILTER DIRT research network (of which the Síkfőkúti DIRT Project is a member), but also visits to several DIRT research areas was very significant.
The invitation parties:

1. **University of Toronto** (1265 Military Trail, Scarborough, ON M1C 1A4, Canada);
2. **Natural Resources Canada**, **Canadian Forest Service, Laurentian Forestry Centre** (1055 du P.E.P.S., P.O. Box 10380, Stn. Sainte-Foy, Québec, QC, G1V 4C7, Canada);
3. **Allegheny College** (520 N Main St, Meadville, PA 16335, Egyesült Államok);
4. **University of Michigan, Biological Station** (9133 Biological Rd, Pellston, MI 49769, Egyesült Államok);
5. **Oregon State University** (2750 SW Campus Way, Corvallis, Oregon 97331, Egyesült Államok)

Within the framework of this proposal we visited international research sites where, similarly to the Hungarian Síkfőkút DIRT Project, to which this proposal is linked, there are also DIRT research sites. We also visited the University of Toronto where we had a successful discussion with Prof. Dr. Myrna Simpson for further joint research. The purpose of our trip was specifically to discuss further cooperation besides visiting international DIRT research stations and exchanging practical experiences. Our visit was particularly successful in this respect, because we agreed on microbiological (PLFA), organic matter (NMR), humus fractionation (Density fraction) tests that could not be carried out in Hungary or only at extremely high costs. In addition, we aimed to visit the new DIRT research area in Canada, established two years ago, where the new plots were developed using a similar method to the Síkfőkút DIRT research area.

6. **University of Bologna**, Department of Agricultural and Food Sciences, Bologna, Italy: Preliminary thermogravimetric studies were carried out on the organic matter content of the soils of the Síkfőkút DIRT project. In addition, we presented our research to colleagues and students at the University in a one-and-a-half-hour lecture.
7. **University of Naples Federico II**, Naples, Italy (UNINA): Preliminary THM-GCMS studies were carried out on the molecular structures of soil organic matter. These results were presented at the above-mentioned *Adelaide Conference*.
8. **Università degli Studi di Palermo**, Palermo Italy (UNIPA): the organic matter content of the soils was analyzed by refractometric NMR.

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