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How Does Long-Term Organic Matter Treatment Affect the Biological Activity of a Centre European Forest Soil?

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Abstract: A significant portion of the increase in atmospheric CO_2 enters the environment through a decrease in the level of organic matter (SOM) in soils. One of the reasons for this is the cutting of forests and the conversion of growing areas into arable land, thus changing land use. As a result, SOM today only has approx. 70–80% of the period before the spread of intensive farming. For the long-term study of the effect of varying amounts of SOM, we set up experimental plots for litter manipulation in 2000. In the course of our investigations, we studied how changing the amount of organic matter input the soil affects the CO_2 emissions of the soil and its closely related biological activity after five or ten years, in addition to the continuous maintenance of the treatments. According to our assumption, after 10 years, the biological activity of the soil will decrease as a result of the removal treatment of organic matter, and the biological activity will increase as a result of the doubling. The pH value of the soil shifted in the acidic direction over 10 years as a result of the removal of organic matter, while it did not change as a result of the increase. In the first year, we could not detect any significant differences in the enzyme activity values. From our later results, we found that a drastic reduction in the amount of leaf litter has a greater effect on soil enzyme activity and soil respiration to a greater extent than an increase in litter production above natural levels. The pH of the soil was as expected, with litter withdrawal shifting the pH towards acidic over the years.

Keywords: soil biology; organic matter; DIRT; luvisol; ß-glucosidase; soil respiration

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1. Introduction

The carbon storage and accumulation capacity of soils are very significant in the global carbon cycle [1] as SOM forms the N reserve of ecosystems, participate in the formation of soil pH conditions, cation exchange capacity, and the formation of soil structure [2]. Another major feature of organic matter is that it forms the most important substrate for hetero-organotrophic soil microorganisms [3,4]. Raich [5] estimated that decomposing litter (including root materials) for about 70% of the total carbon outflow from soils, the amount of which was estimated at 68 Gt per year. Chemical and biological processes in soils have an influence on global climate change through the production and consumption of greenhouse gases. The quality and quantity of litter input to the soil varies greatly in different ecosystems [6,7].

Climatic factors (temperature, water) and soil organisms are of key importance in the input–output processes, which together affect the decomposition of organic matter and the abiotic release of nutrients from the soil [8–10]. A significant part of the carbon dioxide entering the carbon cycle comes from the respiration of living organisms, the weathering of rocks and volcanic activity, while anthropogenic industrial activity is responsible for 5–15% of the CO_2 released into the atmosphere [11]. The latter value is only apparently small since, compared to the gases formed through natural processes, this appears as an additional amount in the atmosphere [12]. Although, according to the majority of researchers, the increase in CO_2 entered the atmosphere primarily due to the burning of fossil fuels, a considerable proportion of it was caused by the reduction of the level of organic matter in the soils, which was caused by the cutting down of forests and the incorporation of virgin lands into cultivation [13]. Several studies [14,15] report that the organic matter content of soils today is only approx. 70–80% of the period before the spread of farming.

Quantitative and qualitative changes in litter production significantly influence the decomposition processes taking place in the soil, but their extent and sometimes even their direction are not exactly known. The thickness of the litter clearly reduces the effect of extremes of soil temperature and minimum and maximum temperature values, creating a more balanced microclimate for soil organisms. Furthermore, the quantitative change in litter production (depending on the climatic conditions) has a different effect on the changes in the SOC content of the soils [3]. Fekete et al. [16] found that the average annual litter quantity of 0.354 kg m^2 of dry leaves moderates the cooling of the soil by 1.1 °C in winter and 0.6 °C in summer. In warmer and drier climates, litter production decreases, and a thinner litter cover may develop, which may increase daily and seasonal temperature extremes. The effects of the thickness of the litter layer on soil processes are of particular importance, as it is the most important influencing factor of soil carbon reserves, biological activity, and soil respiration [17].

According to [18], global warming affects the decomposition of organic matter in the soil and, through this, the global carbon cycle of the biosphere. Several researchers assume that an increase in temperature induces the decomposing processes more strongly than the building processes [19,20]. Therefore, an increased outflow of CO_2 from soils can begin, which—as positive feedback—can cause a further increase in the atmospheric CO_2 level [21–23]. The basis of the studies in an old Douglas-fir reported that increasing litter input (if it has a high C/N ratio) accelerates the decomposition of organic matter in the soil so that the increase in litter input increases the amount of CO_2 released to the atmosphere rather than the amount of carbon stored in the soil [24]. An increase in soil respiration, as well as a decrease in litter production, can result in a decrease in soil organic matter, which leads to land degradation.

Macro- and microclimatic as well as seasonal changes strongly influence the values of soil temperature, soil respiration, and soil moisture, which have been proven to affect microbial processes, including enzyme activity [25–27]. According to several studies, there is a close relationship between the enzyme activity of soils and the mineralization of nutrients, which is also shown by the fact that the accumulation of inorganic forms of the given nutrients can reduce the activity of the enzymes that help in their production [28–30]. This suggests that the amount of accumulating products exceeding the requirement can lead to competitive inhibition of enzyme activity. According to studies, the background of the process is a repression mechanism, which blocks the synthesis of the affected enzymes by the accumulation of simple inorganic compounds (serving as nutrients), while in their absence, the accumulation of the organic molecules to be degraded stimulates the synthesis of the enzymes responsible for their degradation [31].

During our study, we primarily sought answers to how important biological parameters such as soil respiration and ß-glucosidase enzyme activity change during a long-term litter treatment, where we artificially reduced or increased the amount of organic matter reaching the soil in the form of leaf litter.

2. Materials and Methods

2.1. Research Area

The model area is located in the Bükk Mountains belonging to the Northern Central Mountains, 6 km from Eger (Hungary) [32]. GPS coordinates of the Síkfőkút Project: 47°55′34″ N and 20°26′29″ E, altitude 330 m (Figure 1). 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 cutting forest, the Sessile oak (*Quercus petraea* L.)

and Turkey oak (*Quercus cerris* L.) species that make up the overstory are approximately 110–115 years old. The soils of the study area are Chromic Protovertic Luvisols (Clayic, Cutanic) and Protovertic Endostagnic Abruptic Luvisols (Clayic, Cutanic) [33,34]. The detrital input and removal treatments (DIRT) research has been ongoing in the area since 2000, which is part of the USA ILTER (International Long Term Ecological Research) research network [35].

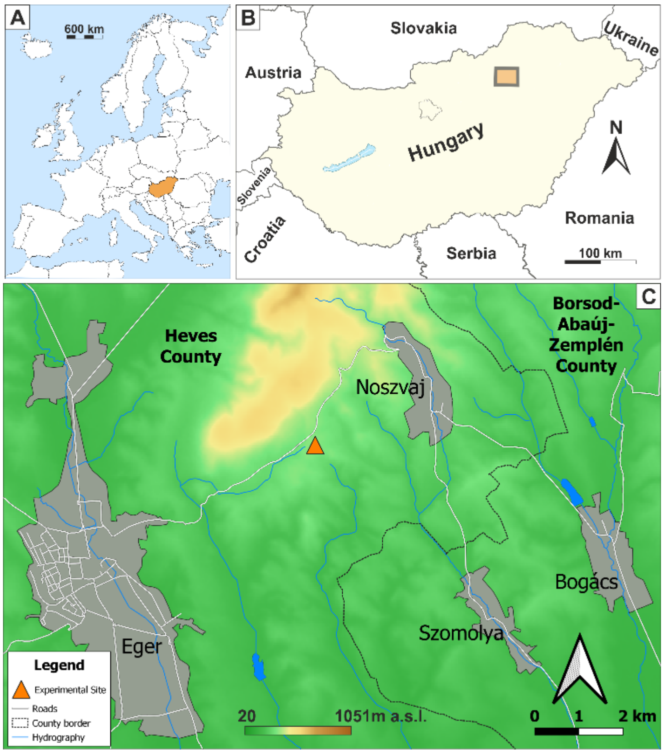

Figure 1. The Síkfőkút DIRT Project research area (Hungary). (**A**) Hungary in Europe; (**B**) Síkfőkút Proejct in Hungary; (**C**) Location of Síkfőkút Project.

We set up 9 experimental plots of 7 × 7 m (Table 1). The plots were established in November 2000. In the litter manipulation field experiment, 3 types of treatments were used: Control (Co), No Litter (NL), Double Litter (DL) in three replicates [36]. The plots are maintained regularly. About 160 kg of leaf litter is transferred from the NL plots to the DL plots every year.

Table 1. Treatments of DIRT plots.

Treatments	Description
Control (Co)	Normal litter inputs. Average litter amount typical of the forest site.
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was removed by a rake. This process was repeated continuously every year.
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from No Litter plots.

2.2. Soil Sampling and Test Methods

Sampling took place during the establishment of the treatments and after 5 and 10 years in the spring time (when biological activity starts after the winter rest period). Soil cores were collected from the 0–15 cm layers in mineral soil with a 20 mm diameter Pürckhauer 1175/1000 mm soil corer (Bürkle GmbH). In the process, we made an average sample from 5 point samples for each plot (9 plots). These average samples were well homogenized and sieved through a 2 mm soil sieve. These average samples were used for the tests.

The method of β-glucosidase enzyme activity is based on the determination of p-nitrophenol released during the enzymatic hydrolysis of a synthetic substrate, p-nitrophenyl-β-glucopyranoside (pNP-β-G). P-nitrophenyl-β-glucopyranoside is converted to colorless p-nitrophenol by β-glucosidase in soil. Upon addition of Tris, the reaction stops, the pH becomes alkaline, and the formed colorless pNP transforms into a yellow phenolate. Its color intensity is proportional to the β-glucosidase activity of the soil [37]. Enzyme activity was measured in six replicates. The soil moisture measurements were performed with a TDR 300 (Time Domain Reflectometer) instrument. The instrument measures moisture in percent by volume. We performed two measurements per plot, the results of which were averaged and then used for further calculations. The soda lime (SL) method [38] was used to measure soil respiration. The tests were performed monthly 3 times, 2 measurements per plot, so a total of 6 measurements per treatment. Soil pH was measured from an aqueous suspension. We used a Testo 206 type digital pH meter. The soil moisture and pH were measured in three replicates. Soil respiration measurements were carried out in the field with six replicates per treatment.

2.3. Statistical Methods

One-way ANOVA was applied to the test result. The Kolmogorov–Smirnov test ($p > 0.05$, $p = 0.200$) or the Shapiro–Wilk test ($p > 0.05$) were used to prove the assumption of normality, while Levene's test ($p > 0.05$) was used to prove the homogeneity of variances. to determine the significant differences, Tukey's honestly (HSD) post hoc test was used. Pearson correlation analysis (2-tailed) was carried out to identify relationships between the measured soil variables. Values of R for significant correlations (*, $p < 0.05$ or **, $p < 0.01$) and correlation trends ($p < 0.1$) were reported. The statistical software used was IBM SPSS 22 (IBM, Armonk, NY, USA).

3. Results and Discussion

At the beginning ($p = 0.642$) and 5 years after the start of the treatments ($p = 0.552$) there was no significant difference in the pH values of the examined treatments. After 10 years, compared to Co (pH = 6.32), the pH of the NL (pH = 5.41) treatment shifted to a more acidic direction (Figure 2). The decreasing litter input lowered the pH of the soil. This can be explained by the fact that the acid intermediates and humic substances produced during litter decomposition cannot be sufficiently buffered by the decreasing basic cation content of the decreasing litter input [39,40]. Similar results were experienced by [41] when, after forest clearcutting, they found that after the reduced litter input, the organic matter content of the soil began to decompose rapidly and the pH also changed in an acidic direction due to the change of cation/anion conditions in the soil. Due to the higher basic cation content dissolution and larger buffer capacity associated with a larger liter intake, we would have expected that the pH in the DL plot would be more basic compared to Co. On the other hand, in the DL treatment (pH = 6.41), the pH of the soil developed similarly to Co (Figure 2).

Regardless of the treatments, almost the same soil moisture values were measured every year. The soil moisture content of the DL and NL plots did not differ significantly from Co (Figure 3).

The similar soil moisture values between the treatments are typically the result of snowmelt and heavy rainfall at the beginning of the vegetation period. The literature and our previous studies confirm that soil moisture affects soil respiration only in extreme cases. Based on this, due to the exceptionally high precipitation, the higher soil moisture content showed a stronger correlation with soil respiration in the case of the Co and DL treatments [16,42]. In our case, the annual precipitation in the forest was in accordance with the long-term average, so it had no significant effect on the examined parameters. Sulzman et al. [24] also found no significant relationship between soil respiration and soil moisture in their similar litter manipulation experiment, except when the water content is extreme (biological activity or physical diffusion is limited). Bowden et al. [43] also found, in the case of laboratory-incubated forest soils, that CO_2 emissions are lower when the moisture content is too high or too low.

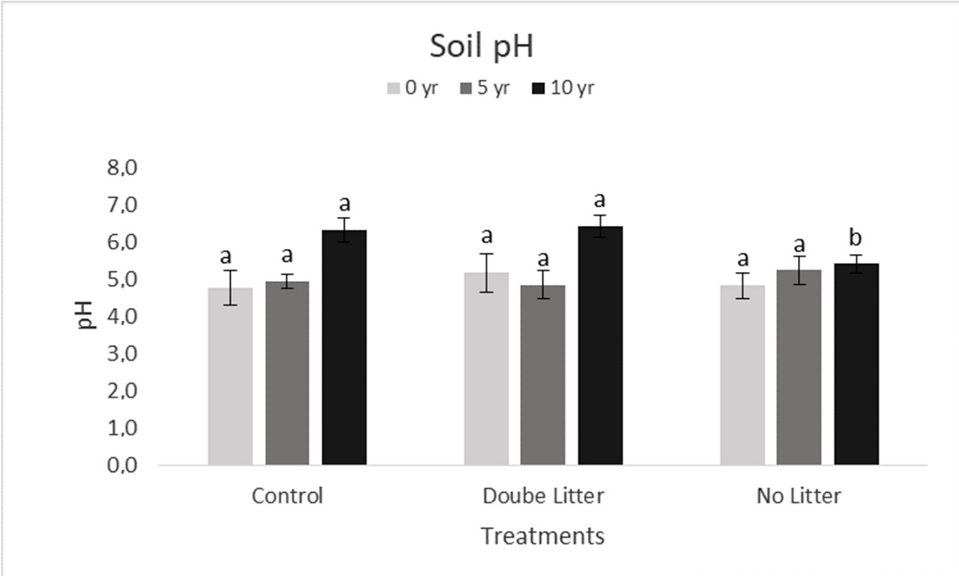

Figure 2. Soil pH at the start of the treatments and after 5 and 10 years, based on spring measurements (average of 6 samples per treatment; letters indicate the significant difference between treatments within the same sampling time).

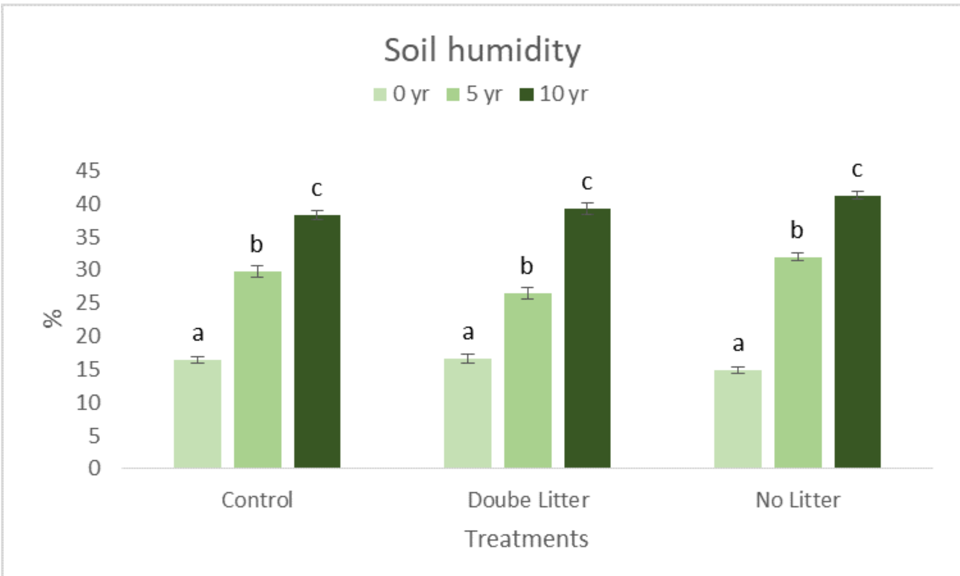

Figure 3. Soil moisture at the start of the treatments and after 5 and 10 years, based on spring measurements (Average of 6 samples per treatment; Letters indicate the significant difference between treatments within the same sampling time.).

In the period after the establishment of the plots, no significant differences were observed in the CO_2 emissions of the different treatments (p = 0.886). This can be explained by the fact that the effect of the treatments was not yet detectable when the plots were established. After 5 years, there was significant difference between the treatments (p = 0.012). In the NL plots (22.348 mgC/m^2/h), soil CO_2 emissions were significantly lower compared to Co (29.832 mgC/m^2/h) plots. However, the effect of the increased amount of litter did not yet show the expected results in the DL (28.963 mgC/m^2/h) treatments. After 10 years, the CO_2 emissions of the NL plot continuously decreased compared to the control site. This is attributed to the fact that the amount of organic matter in the soil has been reduced by litter withdrawal due to the lack of continuous replenishment. As a result of the decreasing organic matter intake, the soil microorganism communities adapted to the limited resources, their metabolic activity became less intense, and thus the CO_2 emission of the soil decreased. In the case of the DL treatment, contrary to our expectations, the microbial activity of the soil, i.e., the CO_2 emission, did not increase significantly (p = 0.075).

A certain time must elapse between litter manipulation and the appearance of changes in the stock and dynamics of organic matter, which depends on the characteristics of the area (speed of decomposition, ability to bind soil particles) [44]. In the beginning, there was a higher CO_2 emission in the litter removal plots after their establishment because the remains of plant roots left in the soil during their decomposition served as an easily available and easily usable nutrient source for the microorganisms, similar to the studies of [45]. However, Sulzman et al. [24] reported that shortly after treatment, microorganisms in the root zone quickly go to the dormant stages if they do not have a sufficient source of nutrients. As a result of the treatment, the litter accumulates in the upper of the soil, the activity of the microorganisms does not immediately follow the rate of this accumulation, therefore, in contrast to the litter withdrawal, the effect of the excess organic matter did not prevail during the spring investigation (Figure 4).

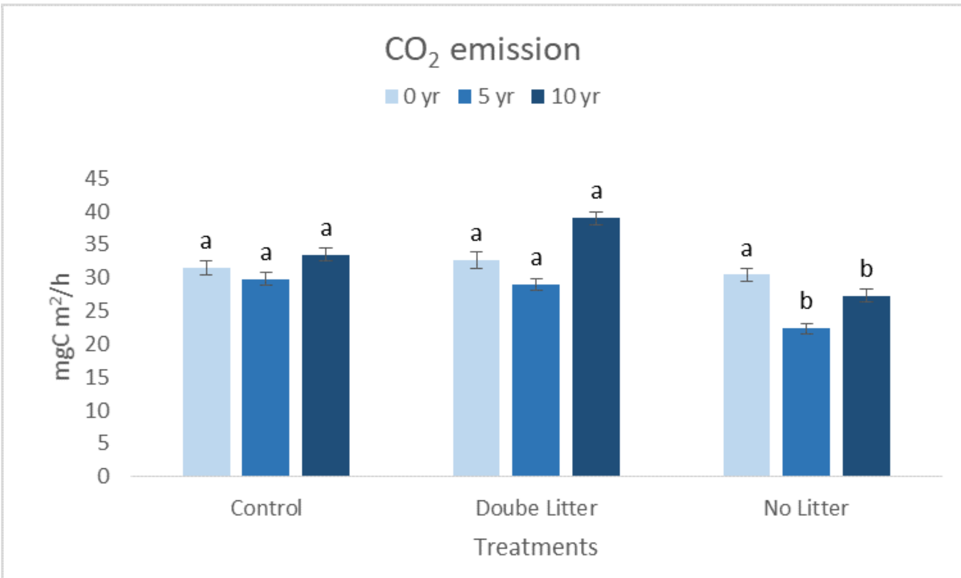

Figure 4. Carbon dioxide emissions from the soil at the start of the treatments and after 5 and 10 years, based on spring measurements (Average of 6 samples per treatment; Letters indicate the significant difference between treatments within the same sampling time.).

In the case of the β-glucosidase enzyme (Figure 5), spring activities increase because this is when the degradation of the previous year's litter becomes more intense as a result of the rising temperature. Under the experimental conditions we used, the temperature does not play the main role in the development of enzyme activity (similar results were obtained by [46] for arylsulfatase and sucrose), but we could not demonstrate a significant relationship with average soil moisture in the initial period either. The activity of the ß-glucosidase enzyme (Figure 5) developed similarly to the trend observed in soil respiration 5 and 10 years after the establishment of the plots. As a result of the correlation analysis, no significant relationship can be detected between the two measured variables (ß-glucosidase enzyme and soil respiration) (p > 0.050, R = 0.213).

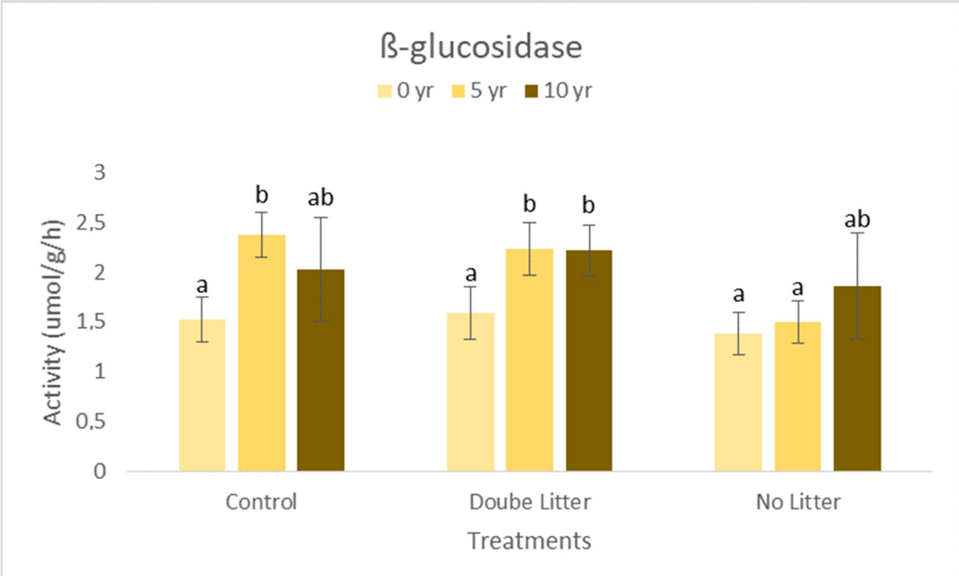

Figure 5. Soil ß-glucosidase enzyme activity at the start of the treatments and after 5 and 10 years, based on spring measurements (average of 6 samples per treatment; letters indicate the significant difference between treatments within the same sampling time.).

However, there is a significant relationship ($p = 0.0433$, R = 0.392) between extremely high spring precipitation (high soil moisture) and ß-glucosidase enzyme activity in the 10th year after the treatments were set up. These results agree with [16,39], who also found that higher soil moisture has a positive effect on the activity of certain soil enzymes. SOM supports more physical, chemical, and biological processes sustaining vital ecosystem functions in addition to C sequestration and as a source of nutrients and energy for biota.

Contrary to our expectations, the increased aboveground organic matter production in the area and soil we examined did not result in an increase in CO_2 emission. In contrast, the removal of organic matter (decreasing litter input) caused a decrease in total soil respiration. Based on literature data, the increase in CO_2 emissions from heterotrophic respiration can indeed cause positive feedback on climate change due to the rather long residence time of the carbon in the soil, however, if the abiotic factors (mainly soil moisture) do not change between extreme limits, then they do not have a significant influence on soil respiration intensity. Abs et al. [47] reported similar results that the responses of microbes to climate change will determine the amplitude of the feedbacks between the carbon cycle and climate. McElliot et al. [42] also reported in their work that, among the abiotic factors, soil respiration was more intense on the forest floor in case of increased soil moisture content. Abiotic factors (temperature, humidity) were also mentioned as main influencing factors by [48,49].

4. Conclusions

We conclude that changes in soil enzyme activity induced by plants and soil microorganisms are primarily influenced by climatic factors (mainly soil moisture) and secondarily by the input of aboveground organic matter. We found that ß-glucosidase was sensitive to soil pH changes and extremely high soil moisture, so it should be a good biological indicator for measuring ecological changes resulting from soil acidification. We found that the effect of the withdrawal treatments was stronger in the long-term period than the effect of the double litter treatments. In our study area, the increased aboveground organic matter production did not result in an increase in CO_2 efflux, contrary to our expectations. In contrast, organic matter withdrawal (reduced litter input) caused a decrease in total soil respiration. We found that, contrary to our expectations, soil pH was unaffected by increased organic matter input, but with decreasing organic matter input, soil pH showed a significant decrease after 10 years. A decrease in the SOM content of soils can cause a number of unfavorable changes in the biological activity of soils, the intensity of the decomposition processes, and thus the emission of carbon dioxide from the soil. These processes significantly contribute to climate change, which, by impacting the soil, can further modify these variables. In conclusion, soil organic matter analysis contributes to a better understanding of the impact of climate change on soil. This will also help to better adapt to future changes, which will contribute to the practical implementation of appropriate and sustainable land use.

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