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EFFECTS OF DIRT TREATMENTS ON THE ENZYME ACTIVITIES OF THE SOIL IN SIKFOKUT SITE

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ABSTRACT. The soil organic matter is affected among others by the quality of litter input i.e. the kind of litter material that is deposited on soil surface. Leaf, root and wood litter addition were applied and the effect of these treatments on soil enzymes activities (polyphenoloxidase, saccharase and phosphatase). The rapidly decomposing leaf and root litter had a great influence on enzyme activities. First the decomposition of fine roots affected in the case of polyphenoloxidase and phosphatase activity. The investigations of phenoloxidase and phosphatase which were carried out right after the establishment of parcels, showed absolutely higher values in soil samples of NI and NR treatments in the first years. Then the leaf litter decomposition was the most important influencing factor. Later these values decreased and was exceeded by the DL (Double Litter), C (Control), DW (Double Wood) treatments, in which surface leaf-litter was added to the soil. The wood litter decomposition did not affect the polyphenoloxidase activity substantially during the experimental period. The phosphatase activity showed the same tendency as polyphenoloxidase, but wood litter played a greater role in phosphatase activity, and the activity declined rapidly in the case of root treatments. The examination of the saccharase activity was launched three and a half years after the establishment of the plots; thus having enough time for the development of the different ecological conditions at our disposal. Significant differences were detectable between the treatments in the case of saccharase and phosphatase.
Keywords: polyphenoloxidase, phosphatase, saccharase, soil enzyme activity, litter decomposition, DIRT treatments

INTRODUCTION

The much talked-of global climatic change is also indicated by the long term meteorological measurements of the Sikfokut Project. The climate of the forest has become warmer and dryer for the last three decades (Antal et al., 1997; Tóth et al, 2007). The functional and regulation processes of the forest ecosystem are influenced variously by the climate change, as well as it has important effects on the structure and species composition of the forest, the quality and quantity of organic materials and the biological activity in the soil, both directly and indirectly. Since the early 70's the species composition and the quantity and structure of the litter production in the Síkfőkut *Quercetum petrea-cerris* have greatly changed, and several researchers consider the climate change to be the reason (Tóth et al, 2007). The experimental plot, the Sikfokut DIRT Project, is member of the DIRT intercontinental project organized by the USA ILTER (International Long-Term Ecological Research). The general aim of the project is to explore the changes in litter production due to changes of climate or land-use. Furthermore, it aims to examine the processes concerning the different chemical, biochemical and biological parameters in the given soils caused by the changes of litter production. An important part of the research involved soil enzymes (Fekete et al., 2007).

The soil enzyme activity is an indicator of stress meeting ecosystems (Sowerby et al., 2005). Extracellular enzymes play an outstanding role in litter decomposition and nutrient cycling whose processes are directly controlled by factors belonging to the given site such as temperature, moisture, nutrient availability and chemical properties of the litter (Sinsabaugh, 1994). Organic matter accumulation is the effect of balance between plant inputs (litter, roots, and root exudates) and outputs (exports of gases, dissolved carbon and other elements). Outputs result from complex interplays among plant inputs, processing of inputs by soil and forest floor biota and climatic factors (temperature, water balance) that affect rates of biotic processing of organic matter and abiotic leaching of material from soil profiles (Qualls et al., 1991; Kotroczó et al., 2008). An increase of the amount of decomposed litter generally implies the increasing enzyme activity (Larson et al., 2002). Harrison and Pearce (1979) found two to 29 fold higher phosphatase activity in the leaf litter than in the soil. Anderson et al., (2004) came to similar conclusion in case of other enzymes. Tarafdar and Jungk (1987) found that root proximity stimulated the soil phosphatase activity.

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Polyphenols (a major class of aromatic carbon) are substrates for polyphenoloxidases and peroxidases that can cause both the oxidative breakdown (e.g. lignolytic activity) and coupling of aromatic carbon compounds (synthesis of humic substances, (Freeman et al., 2001; Larson et al., 2002). Phenoloxidase and peroxidase take part in oxidative decomposition of lignin and tannin or in polymerization of polyphenols (humus forming). Mass loss of litter shows a strong connection with the amount of polyphenoloxidase enzyme (Sinsabaugh et al., 2002). A remarkable part of the soil phosphate content could be found in organic bound, and mineralization of these organic fractions is an important factor in the nutrient supply of plants. Phosphate release from organic phosphate sources is performed by phosphatase enzyme (Sinsabaugh et al., 1993).

The objectives of this study were to examine the connection between the litter amounts and soil enzyme activity.

MATERIALS AND METHODS

The DIRT treatments are derived from a project started in 1957 in forest and grassland ecosystems at the University of Wisconsin (Nielson and Hole, 1963). Our researches constitute an important part of a long term international project which includes five more experimental sites (Nadelhoffer et al., 2004) in USA (Harvard Forest, H. J. Andrews, Bousson, University of Michigan Biological Station) and Germany (Universität Bayreuth BITÖK) apart from Síkfőkút Project. The Síkfőkút DIRT project (located in Hungary) joined with the American ILTER DIRT network in November 2000.

The experimental site of 64 ha is located in the south part of the Bükk Mountains in North Eastern Hungary at 325 m altitude. GPS coordinates N 47°55' E 20°46'. This forest has protected since 1976 and it is part of the Bükk National Park at present. The annual precipitation amounts to 550 mm. The type of the soil according to the FAO Soil Classification is Cambisols. This forest is a semi-natural stand (*Quercetum petraeae-cerris* community) without forest management. Six treatments were established in the experimental site (Table 1). Each plot is 7m wide and 7m long (49 m^2), and every treatment was set up in three replications.

Table 1 The applied DIRT treatments in open-field experiment (Síkfőkút, Hungary).

Treatments	Description
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.
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from NO LITTER 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 doubbled amount of that was applied in case of every DW plots.
No Roots (NR)	Roots are excluded by inserting impenetrable barriers in backfilled trenches to the top of the horizon C. Root resistant plastic foil was placed into the plot in the depth of 1 m hindering the roots developing outside of the plot to get into the NR plot. Trees and shrubs were eradicated when the plot was established, and plant roots decayed in time
No Inputs (NI)	Aboveground inputs are excluded from plots, the belowground inputs are provided as in NO ROOTS plots. This treatment is the combination of NR+NL plots.

The soil samples were taken randomly from 5 places of each plot from the top 15 cm layer, using an Oakfield soil sampler (Oakfield Apparatus Company, USA). Samples were homogenized and transported to the laboratory. The polyphenoloxidase (PPO) activity was measured 18 times under laboratory conditions from July 2002 to June 2005 during the vegetation growing periods. The measurement was carried out according to the method of Sinsabaugh et al. (1999). One hour incubation period and 30 °C incubation temperature was applied. Measurement of phosphatase (P) activity was also carried out according to Sinsabaugh et al. (1999), the activity was measured 15 times under laboratory conditions from April 2001 to September 2005 by Zsolt Krakomperger.

The absorbance was measured by spectrophotometer (Zeiss Spekol 07). Between June 2004 and Oktober 2006, saccharase activity of the collected soil samples was measured 13 times according to Frankenberger and Johanson, (1983),

For detecting the soil temperature, an ONSET, StowAway TidbiT-type data-logger (Onset Computer

Corporation, USA) was put into the centre of each parcel, at 10 cm depth. Data-loggers were programmed to measure the soil temperature in every hour. Data were downloaded at stated intervals, generally once a year. The soil moisture content was determined in a drying oven at 105°C.

The experimental data were statistically evaluated by one-way ANOVA, (Statistica 5.5 version). Calculations including Student's t-test were carried out using Microsoft® Office 2003 Excel®. At the beginning of the experiment, we ensured the random sampling and the independence of each sampling elements. Kolmogorov – Smirnov test helped to determine the possible normal distribution of actual data, while variance homogeneity was examined with Fmax-probe. Correlation analysis, paired and two sampled t-probe and variance analysis were also carried out. When groups were significantly different, variance analysis were completed with Tukey's test. Non-parametric probe was needed in only one case (for the examination of phenoloxidase enzyme), when we used Kruskal – Wallis test and Mann-Whitney U test. When 'p' values were equal or less than 0.05, the examined values were considered to be significantly different.

RESULTS AND DISCUSSION

The enzyme examinations (phenoloxidase, phosphatase), which were carried out right after the construction of parcels, absolutely showed higher values in soil samples of NI and NR treatments in the first years (Fig.1. and Fig. 2.).

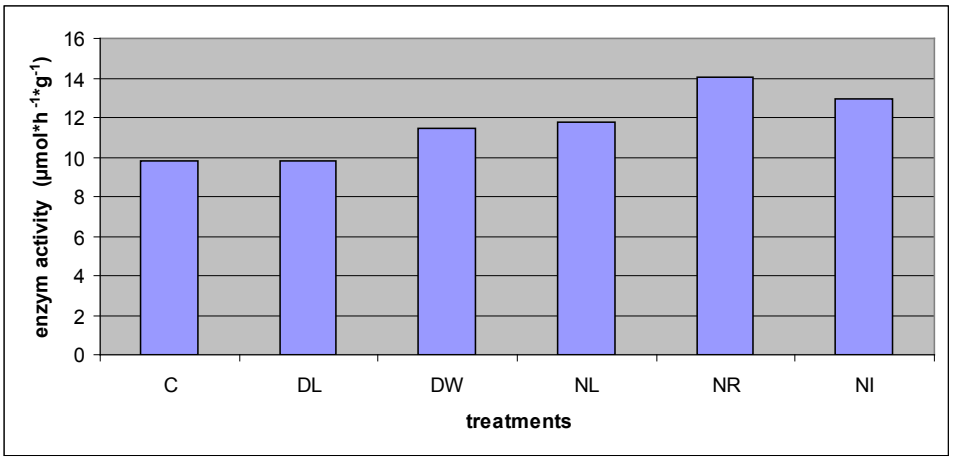

Fig. 1 Comparsion of the phospatase activities according to treatment types based on the total results measured between April 2001 and December 2001 (C: Control, DL: Double Litter, DW: Double Wood, NL: No Litter, NR: No Root, NI: No Input)

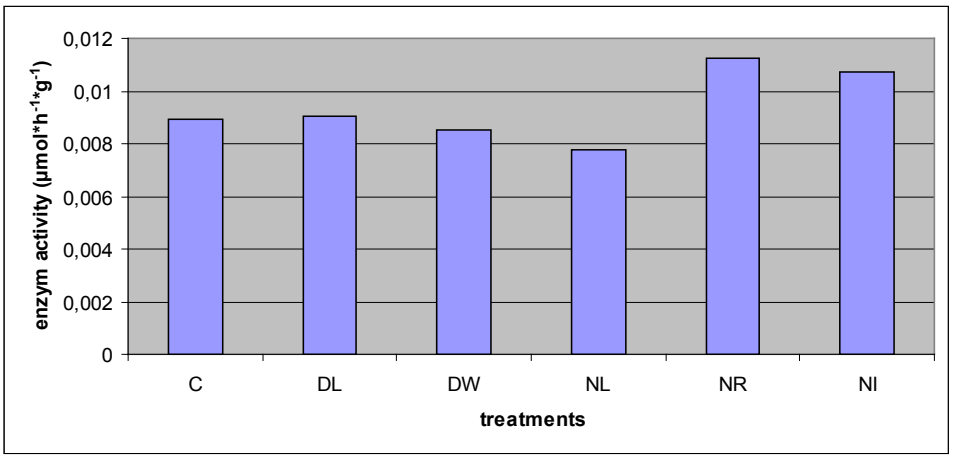

Fig. 2 Comparsion of the phenoloxidase activities according to treatment types based on the total results measured between July 2002 and July 2003 (C: Control, DL: Double Litter, DW: Double Wood, NL: No Litter, NR: No Root, NI: No Input)

Later these values decreased and was exceeded by the DL, C, DW treatments, in which surface litter was added to the soil (Fig. 4.).

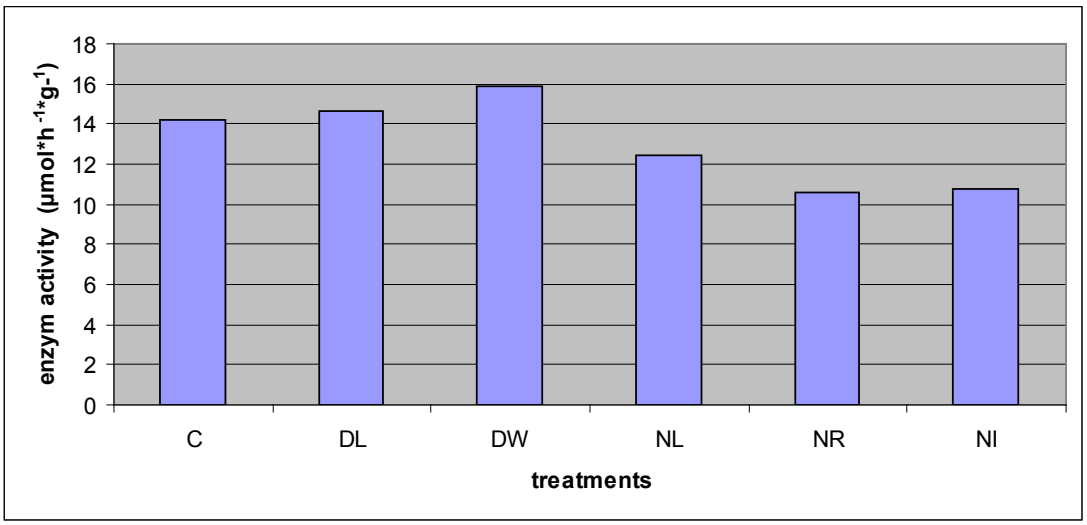

Fig. 3 Comparsion of the phospatase activities according to treatment types based on the total results measured between April 2003 and September 2005 (C: Control, DL: Double Litter, DW: Double Wood, NL: No Litter, NR: No Root, NI: No Input)

In our opinion, the previous results can be explained by the following facts: as a consequence of plant removal, some enzymes may have got into the soil from the lysis of root cells, after the construction of NI and NL parcels (Burns, 1982); and additionally, lytic roots served as substrates for microorganisms. Otherwise, humidity of soil is higher in these parcels (because of the lack of evapotranspiration), which leads to quick decomposition of soil nutrients, since high humidity increases the microbial activity. Evidently, these effects cease to exist when all substrates are used up and root enzymes are degraded. In case of phosphatase, these processes passed in 2001 and 2002. ANOVA statistical analysis resulted in significant differences between treatments ($F_{(5;54)}$=8,32; P<0,001), from April, 2003 to the end of the measurements (September 2005). According to Tukey-probe, DL, C and DW showed significant differences in case of NR and NI, while DW showed significant difference in case of NL. The more slowly regression of phenoloxidase became apparent for 2004-2005 (Fig. 4.). Even at that time, only DL surpassed the average enzyme activity of root-treated parcels.

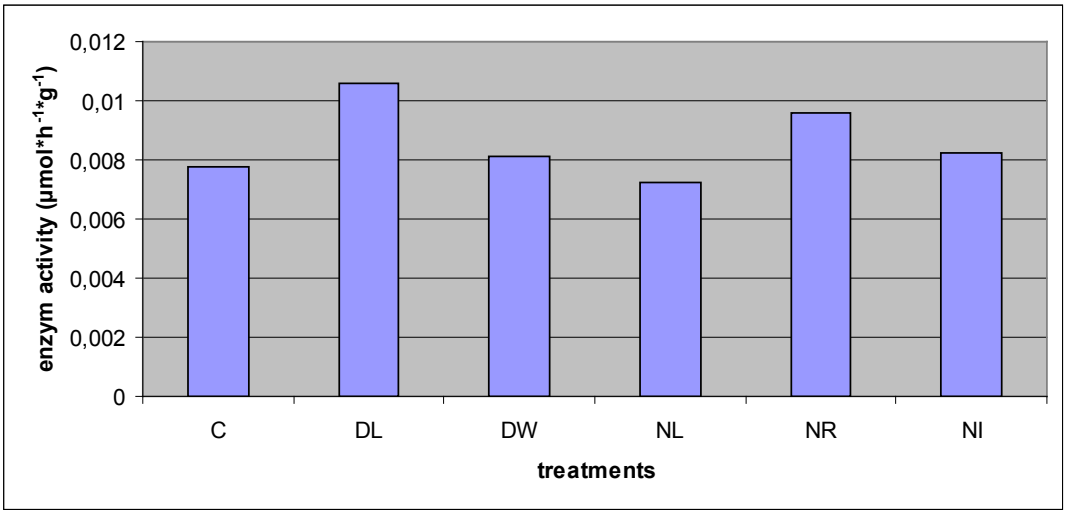

Fig. 4 Comparsion of the phenoloxidase activities according to treatment types based on the total results measured between September 2003 and June 2005 (C: Control, DL: Double Litter, DW: Double Wood, NL: No Litter, NR: No Root, NI: No Input)

In our opinion, this can be explained by the fact that lignin is a resistant compound, the decomposition of which is very slow in the soil. The slow decomposition of root-lignin maintains the necessary amount of substrates for a long time. As lignin is a substrate of phenoloxidase, this leads to higher phenoloxidase activity. Examination of saccharase began three and a half year after the construction of parcels (Fig. 5.). ANOVA showed significant differences between treatments in case of saccharase ($F_{(5;72)}$=3,25; p=0,011).

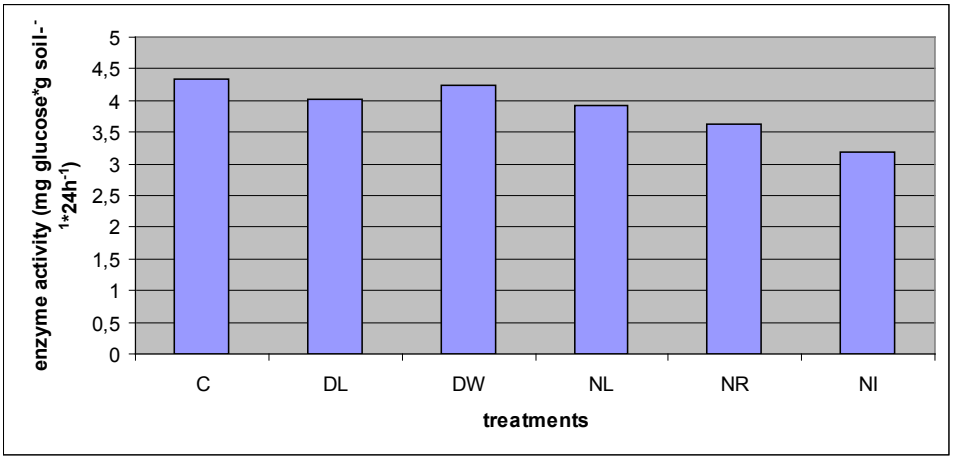

Fig. 5 Comparsion of the saccharase activities according to treatment types based on the total results measured between June 2004 and October 2006 (C: Control, DL: Double Litter, DW: Double Wood, NL: No Litter, NR: No Root, NI: No Input)

Treatments with litter addition (DL, DW) and control samples (C) showed the highest activities, while treatments with litter withdrawal (NL, NR, NI) could be described lower (often significantly lower) activities (such as phosphatase and glucosidase in the first two years (Krakomperger, 2008)). Remarkably, dominancy of DL (which treated with the largest amount of biomass) against C and DW could not be detected by none of the examined enzymes (with the exception of phenoloxidase). In case of saccharase and glucosidase (Krakomperger, 2008), its activity can be ranked to the third place, while in case of phosphatase its activity can be ranked to the second place (after DW). Tukey-probe did not result in significant differences between the three treatments.

REFERENCES

Anderson M., Kjøller A., Struwe S., Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry, 36: 1527-1537, 2004.

Antal E., Berki I., Justyák J., Kiss Gy., Tarr K., Vig P., A síkfőkúti erdőtársulás hő- és vízháztartási viszonyainak vizsgálata az erdőpusztulás és az éghajlatváltozás tükrében, Debrecen, 83 p, 1997.

Burns R. G., Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem. 14:423-427, 1982.

Fekete, I., Varga, Cs., Kotroczó, Zs., Krakomperger, Zs., Tóth, J. A., The effect of temperature and moisture on enzyme activity in Síkfőkút Site. Cereal Research Communications Volume 35, Issue 2, 381-385, 2007.

Frankenberger W. T., Johanson J. B., Method of measuring invertase activity in soils. Plant and Soil 74: 313-323, 1983.

Freeman C., Ostle N., Kang H., An enzymic 'latch' on a global carbon store – A shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature, 409: 149, 2001.

Harrison A. F., Pearce T., Seasonal variation of phosphatase activity in woodland soils. Soil Biol. Biochem. 11: 405-410, 1979.

Kotroczó, Zs., Fekete, I., Tóth J. A., Tóthmérész B., Balázsy S., Effect of leaf and root-litter manipulation for carbon-dioxide efflux in forest soil. Cer. Res. Comm. Volume Suppl. 36: 663-666 pp., 2008.

Krakomperger Zs, Tóth J. A., Varga Cs., Tóthmérész B., The effect of litter input on soil enzyme activity in an oak forest. Cereal Research Communications Vol. 36. 323-326, 2008.

Larson J.L., Zak D.R., Sinsabaugh R.L. Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Science Society of America Journal, 66: 1848-1856., 2002.

Nadelhoffer K., Boone, R., Bowden, R., Canary, J., Kaye, J., Micks, P., Ricca, A., McDowell, W., Aitkenhead, J. The DIRT experiment. In: Foster, D. R., Aber, D.

J. (eds) Forests in Time. Yale Univ. Press, Michigan, 2004.

Nielson G.A., Hole F.D., A study of the natural processes of incorporation of organic matter into soil in the University of Wisconsin Arboretum. Wisconsin Academic Review, 52:231-227, 1963.

Qualls R.G., Haines B.L., Swank W 332 .T., Fluxes of dissolved organic nutrients and humic substances in a deciduous fores. Ecology, 72: 254-266, 1991.

Sinsabaugh R. L. – Klug M. J. – Collins H.P. – Yeager P.E. – Petersen S.O., Characterizing Soil Microbioal Communities. In: Robertson G.P. – Coleman D.C. – Bledsoe C.S. – Sollins P. (ed): Standard Soil Methods for Long-term ecological research. Oxford University Press 318-348, 1999.

Sinsabaugh R.L., Enzymic analysis of microbial pattern and process - Biol. Fertil. Soils, 17: 69-74, 1994.

Sinsabaugh R.L., Antibus R.K., Linkins A.E., McClaugherty C.A., Rayburn L., Repert D., Weiland T., Wood decomposition: Nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology, 74: 1586-1693, 1993.

Sinsabaugh R.L., Carrerio M.M., Repert, D.A., Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry, 60: 1-24, 2002.

Sowerby A., Emmett B., Beier C., Tietema A., Penuelas J. Estiarrte M., Meeteren J. M., Hughes S., Freeman C., Microbial community changes in heathland soil communities along a geographical gradient: interaction with climate change manipulations. Soil Biology & Biochemistry, 37:1805-1813, 2005.

Tarafdar J. C., Jungk A., Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils, 3: 199-204, 1987.

Tóth, J. A., K. Lajtha, Zs. Kotroczó, Zs. Krakomperger, B. Caldwel, R. D. Bowden, M. Papp, The effect of climate change on soil organic matter decomposition. Acta Silvatica et Ligniaria Hungarica Vol. 3: 75-85. pp., 2007.