

**Original Article****Enzyme Activity – Indicator of Soil Biological Dynamics****VIDICAN Roxana, Vlad STOIAN\****University of Agricultural Science and Veterinary Medicine Cluj-Napoca, 3-5 Manastur St., 5400372 Cluj-Napoca, Romania*Received 19 October 2015; received and revised form 30 October 2015; accepted 22 November 2015  
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**Abstract**

Substrate utilization rate is a value of functional diversity specific for every ecosystem. The microbial groups are responsible for the exploitation of nutrient resources and conversion of organic matter, releasing into the soil the necessary enzymes for decomposition and mineralization processes. Monitoring the microbial enzymatic activity in the soil provides a number of useful indicators in determining the quality and equilibrium of soil. The enzymatic activity is concentrated in the rhizosphere of plants, the scale of the interpretation of the enzymatic processes ranging from a micro-level to a macro-(landscape) level. Enzymes provide good indications on biogeochemical cycles of elements, with a higher intensity of activity near to the soil surface. The study of soil enzymes provide effective analytical units for microbial processes, nutrient reserves and composition of microbial communities.

**Keywords:** soil enzymes, microbial communities, soil dynamics, heterogeneity, biogeochemical cycles.

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**1. Introduction**

The microbial community is responsible for the materialization of organic matter in the soil and involves a high number of metabolic processes, circulating the nutritive elements and stabilizing the soil's structure [1, 2].

For understanding the functioning of ecosystems, it is required to relate to species and functional diversity, through establishing the connections between abundance and composition [3]. Functional microbial diversity is strongly related to the presence/absence of utilizing specific substrates and the utilization degree [4, 5]. Diminishing a specific group's dimension is not equivalent with the reduction of a process in the soil, the emerged space in the ecological niches being exploited by microbial groups with similar functions [6].

Enzymes are proteins with measurable soil activity, the enzyme tests conducting to rapid, simple and highly accurate results [7].

Enzyme's sensitivity is correlated with the genetic expression and the environmental changes, the microbial composition determining the catalyzing potential of the substrates [8].

Knowing the enzymes origin offers a better understanding of the microorganisms involved in the biogeochemical cycles and the energy and nutrients transfer paths in the soil's nutritive network [9, 10].

The soil's enzymatic activity is the expression of sum between all activities of enzymes capable of catalyzing the same reaction, although with different origin and spatial location in soil [6, 11].

In the soil's enzymatic economy, the microbial activity is defined only by the enzymes associated with the living cells, without taking into calculation the extracellular enzymes or those from dead or damaged cells [12].

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The mineralization–decomposition biological processes may be analyzed: at molecular level - highlights the enzymatic characteristics of the organic matter decomposition, at organism level – adjustment of enzymatic expression and growth kinetics, at community level – involves the metabolism, microbial succession and competition processes [13].

The purpose of this study is to assess the potential of testing enzymes for determining the microbial diversity, establishing the connections with the functional diversity. At the same time, the enzymatic activity is evaluated, for describing and better understanding the soil's fertility, with applications in defining the soil-plant system.

## 2. Kinetics and classification of enzymes

The enzymes are proteins capable of increasing the speed of reactions, with low energy consumption, considered as activators of biological systems and biochemical transformations in the soil. In soil, the extracellular enzymes are released from the decomposed organic matter and adsorbed onto colloids, acting on specific substrates [14].

The enzymatic activity is based on the existence of cofactors, small sized molecules required for completing the catalytic structure of enzymes [15]. The enzymes which require cofactors are active only at intracellular level, demanding the simultaneous presence of cofactors in the substrate [16]. The catalization of reactions is specific to each enzymatic group, at the reaction's ending these remaining unmodified, the entire activity depending on pH, temperature and presence/absence of inhibitors/activators [17].

At high temperatures or extreme pH-s takes place denaturation of enzymes [18].

The kinetics of enzymes in solutions is described by the Michaelis-Menten (Eq. 1.) equation and highlights the measuring potential of enzyme's affinity for the substrate [19, 20].  $V$  - speed of reaction,  $S$  – substrate,  $V_{max}$  – maximum speed of the enzymatic catalysis, achieved at the enzyme's saturation on a substrate with infinite concentration,  $K_m$  - Michaelis constant, equal to the substrate's concentration at half of the maximum speed.

$$V = V_{max} [S] / K_m + [S] \quad (\text{Eq. 1.})$$

The actual classification of enzymes is performed based on the executed function and contains six classes: EC 1 – oxidoreductases, EC 2 – transferases, EC 3 – hydrolases, EC 4 – lyases, EC 5 – isomerases and EC 6 – ligases [21]. Each class contains sub-classes and sub-subclasses, the

enzymes name being given by the substrate with which it reacts.

## 3. Origin and spatial distribution

The most important sources of enzymes in the soil are the microbial cells, the enzymatic proteins being a major component of the cells cytoplasm [22]. The enzymatic activity is a good indicator of soil quality and microbial activity, at the basis of this phenomenon being the sensitivity of microorganisms to soil management techniques and environmental conditions [23, 24].

Microbial degradation and hydrolysis of the organic compounds takes place after the enzymes are released in the cell's exterior, thus starting the catalysis processes of decomposition and mineralization of nutrients [25]. The soils rich in enzymatic proteins are characterized by higher energetic transfer and circulation of nutrients, with benefic effects on the environment quality and increased outputs [26]. The origin of the same enzyme may differ as location in both the cells which produce it as well as the soil's extracellular matrix [15]. A series of enzymatic proteins are considered bounded, in a natural form, and are acting in the soil as part of complexes established with organic and inorganic colloids [27].

The primary producers of enzymes from the rhizosphere are considered the microorganisms, which play a role in the formation of compounds which are absorbed and used by both the plants and microbial groups [28].

Microbial activity in the rhizosphere is stimulated by the release of extracellular enzymes at roots level or through rhizodeposition, the enzymatic activity increasing in these areas compared to the extra-rizospheric soil [29]. Most of the extracellular enzymes have a reduced mobility in the soil, which determines their activity in the vicinity of the secretion area, the systems thus created having gradual concentrations, with a maximum reached in the area of plant roots [30]. As the microbial population decreases, along with distancing from the rhizosphere, the observed enzymatic activity also decreases, related with the more and more reduced quantity of radicular exudates, the mass flow and diffusion of used substrates by microorganisms [31, 32].

The heterogeneous distribution of enzymes in soils is due to their capacity to bond in clay-humus-enzyme type complexes or on particles of organic matter [33]. The clay's particles dimensions allow the attachment, growth and protection for the microorganisms and as well the extracellular enzymes, offering an increased stability to these

complexes for the various conditions of soil management techniques [34].

The distribution scale of enzymes in soil may vary from a few microns - to millimeters (micro-scale), to a few meters (meso-scale) and at the level of high dimensions ecosystems (macro-scale). Heterogeneity at a reduced scale, connected with microbial abundance, is due to the preferential trajectories of water flow in the soil, these systems ensuring the development of substrate for microorganisms [35]. Extreme microorganisms, with severely preferences for aerobic/anaerobic conditions, may use preferential transfer paths for nutrients and a wide range of substrates [36, 37].

At a medium vertical and horizontal distribution scale of enzymatic activity are defined the productivity premises, soil quality and ecosystem stability [38, 39]. The vertical distribution of enzymatic activity is affected negatively by the soil compaction level and the amount of microbial biomass, along with the quantity of organic carbon [40].

The horizontal distribution of enzymatic activity is tightly connected with the vegetation successional stage; in the first stages the enzymes specific to the carbon cycle are active, while once the succession changes, the enzymatic activity related to the nitrogen cycle increases [13].

The interpretation of macro-scales for indicators of enzymatic activity are a form of information aggregation in regards to the biogeochemical cycle, subject to the climatic gradients and long distances crossed by substrates [41]. The spatial dependency of enzymatic activity is directly proportional with climatic, topographic, geologic and vegetal conditions of units [42].

#### 4. Monitoring of the enzymatic activity

The enzymes offer indications on the nutrient cycles in soil, most important for the carbon cycle being xylanase, amylase, cellulase, lipase, glucosidases, and invertase; for the nitrogen cycle relevant are considered to be: proteases, amidases, urease, and deaminases; for phosphorus-phosphatases, and for sulphur – arylsulfatase [43, 44, 45, 46].

In general, the enzymatic activity decreases proportionally with the depth, being correlated with the microbial activity and the carbon and organic nitrogen contents in soil [47, 48]. The soil's enzymes have reduced catalysis speed and high Michaelis constants, which indicates a low affinity for the substrates they convert [49].

The intra- and extracellular localization of enzymes, correlated with the spatial variability and

the interactions with environment, produce a map of the enzymatic activity at a high scale [50]. Biotic enzymes are associated with viable cells and may be localized intracellular – in cytoplasm, in periplasmic space, or at the exterior surface of the cell [51].

The other enzymes are considered abiotic, and may exist in the adsorbed form in clay or in complexes with humic colloids [52]. The abiotic component offers indications over the semi-permanent changes, protection of the complexes in which it is integrated, promoting the stability of the soil matrix [53].

At the microbial community level, species capable to use the enzymes existent in the soil solution or in the organic-mineral complexes may be activated without the need of energetic consumption required for their synthetize [54, 55]. These aspects may be used in describing, at low scale, the functioning of the microbial communities.

Studying the enzymatic activity at vertical and horizontal meso-scale level offers analysis units for microbial processes and the reaction towards the management technologies overlapped to the type of soil [56, 57].

Synthetizing in soil quality indicators helps to calibrate the models for interpreting the enzymatic activity, independent to the type of soil [58].

Monitoring the enzymes at macro-scale is efficient in developing the soil quality indicators due to the microbial activity, concurrently offering information about the microbial groups, nutrient reserves and chemical properties [59, 60, 61].

#### 5. Conclusion

The enzymes are catalyzers of the reactions of nutrient cycles in soil, ensuring the degree of coherence of these processes. The enzymatic activity is an indicator of the soil quality as a result of direct dependence of soil biota dynamics and sensitivity to the applied management. Determining the functioning of enzymatic processes may be used as a measure of the microbial functional diversity and assessment of the microbial ecological characteristics.

#### References

- [1] Blaško R., L.H. Bach, S.A. Yarwood, S.E. Trumbore, P. Högberg, M.N. Högberg, 2015 Shifts in soil microbial community structure, nitrogen cycling and the concomitant declining N availability in ageing primary boreal forest ecosystems. *Soil Biology and Biochemistry*, 91, 200-211.
- [2] Bobuľská L., D. Fazekašová, L. Angelovičová, 2015, Vertical Profiles of Soil Properties and Microbial

- Activities in Peatbog Soils in Slovakia. *Environmental Processes*, 2(2), 411-418.
- [3] Abram F., 2015, Systems-based approaches to unravel multi-species microbial community functioning. *Computational and structural biotechnology journal*, 13, 24-32.
- [4] Zomorodi A.R., D. Segrè, 2015, Synthetic Ecology of Microbes: Mathematical Models and Applications. *Journal of molecular biology*.
- [5] Sherman C., Y. Steinberger, 2012, Microbial functional diversity associated with plant litter decomposition along a climatic gradient. *Microbial ecology*, 64(2), 399-415.
- [6] Nannipieri P., E. Kandeler, P. Ruggiero, 2002, Enzyme activities and microbiological and biochemical processes in soil. *Enzymes in the environment*. Marcel Dekker, New York, 1-33.
- [7] Bisswanger H., 2014, Enzyme assays. *Perspectives in Science*, 1(1-6), 41-55.
- [8] Nannipieri P., L. Giagnoni, G. Renella, E. Puglisi, B. Ceccanti, G. Masciandaro, F. Fornasier, M. C. Moscatelli, S. Marinari, 2012, Soil enzymology: classical and molecular approaches. *Biology and Fertility of Soils*, 48(7), 743-762.
- [9] Ermakov V. V., 2015, Geochemical ecology and biogeochemical criteria for estimating the ecologic state of biospheric taxons. *Geochemistry International*, 53(3), 195-212.
- [10] Bell C., M. Stromberger, M. Wallenstein, 2014, New insights into enzymes in the environment. *Biogeochemistry*, 117(1), 1-4.
- [11] Kang H., S. Kang, D. Lee, 2009, Variations of soil enzyme activities in a temperate forest soil. *Ecological Research*, 24(5), 1137-1143.
- [12] Shukla G., A. Varma (eds.), 2010, *Soil enzymology*. Springer Science & Business Media.
- [13] Benckiser G., S. Schnell (eds.), 2006, *Biodiversity in agricultural production systems*. CRC Press.
- [14] Ștefanic G., D.I. Săndoiu, L. Dincă, 2014, *Metode de analiză și interpretare a stării de fertilitate a solului*. Printech-București, ISBN 978-606-23-0220-7
- [15] Huang P. M., Y. Li, M. E. Sumner, 2012, *Handbook of Soil Sciences. Resource Management and Environmental Impacts*. CRC Press, Boca Raton, FL.
- [16] Paul E.A. (2014). *Soil microbiology, ecology and biochemistry*. Academic Press.
- [17] Bettelheim F., W. Brown, M. Campbell, S. Farrell, O. Torres, 2012, *Introduction to general, organic and biochemistry*. Cengage Learning.
- [18] Svendsen A. (ed.), 2003, *Enzyme Functionality: Design: Engineering, and Screening*. CRC Press.
- [19] Wang G., W.M. Post, M.A. Mayes, 2013, Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecological Applications*, 23(1), 255-272.
- [20] Bárta J., P. Šlajsová, K. Tahovská, T. Pícek, H. Šantrůčková, 2014, Different temperature sensitivity and kinetics of soil enzymes indicate seasonal shifts in C, N and P nutrient stoichiometry in acid forest soil. *Biogeochemistry*, 117(2-3), 525-537.
- [21] Drauz K., 2012, *Enzyme catalysis in organic synthesis: a comprehensive handbook (Vol. 1)*. John Wiley & Sons.
- [22] Bugg T. D., 2012, *Introduction to enzyme and coenzyme chemistry*. John Wiley & Sons.
- [23] Štursová M., P. Baldrian, 2011, Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. *Plant and soil*, 338(1-2), 99-110.
- [24] Neogi S., P. Bhattacharyya, K.S. Roy, B.B. Panda, A.K. Nayak, K.S. Rao, M.C. Manna, 2014, Soil respiration, labile carbon pools, and enzyme activities as affected by tillage practices in a tropical rice-maize-cowpea cropping system. *Environmental monitoring and assessment*, 186(7), 4223-4236.
- [25] Li X., L. Hou, M. Liu, X. Lin, Y. Li, S. Li, 2014, Primary effects of extracellular enzyme activity and microbial community on carbon and nitrogen mineralization in estuarine and tidal wetlands. *Applied microbiology and biotechnology*, 99(6), 2895-2909.
- [26] Blagodatsky S., R. Grote, R. Kiese, C. Werner, K. Butterbach-Bahl, 2011, Modelling of microbial carbon and nitrogen turnover in soil with special emphasis on N-trace gases emission. *Plant and soil*, 346(1-2), 297-330.
- [27] Dunn C., T. G. Jones, A. Girard, C. Freeman, 2014, Methodologies for extracellular enzyme assays from wetland soils. *Wetlands*, 34(1), 9-17.
- [28] Huang P. M., 2005, *Soil abiotic and biotic interactions and impact on the ecosystem and human welfare*. Science Publishers.
- [29] Brzostek E.R., A. Greco, J.E. Drake, A.C. Finzi, 2013, Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen

availability in temperate forest soils. *Biogeochemistry*, 115(1-3), 65-76.

[30] Koranda M., J. Schnecker, C. Kaiser, L. Fuchslueger, B. Kitzler, C.F. Stange, A. Sessitsch, S. Zechmeister-Boltenstern, A. Richter, 2011, Microbial processes and community composition in the rhizosphere of European beech—the influence of plant C exudates. *Soil Biology and Biochemistry*, 43(3), 551-558.

[31] Plaxton W., H. Lambers (eds.), 2015, Phosphorus Metabolism in Plants, *Annual Plant Reviews*, 48.

[32] du Jardin, Patrick. "Plant biostimulants: Definition, concept, main categories and regulation." *Scientia Horticulturae* 196, 3-14.

[33] Sun Z., E.W. Bruun, E. Arthur, L.W. de Jonge, P. Moldrup, H. Hauggaard-Nielsen, L. Elsgaard, 2014, Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. *Biology and Fertility of Soils*, 50(7), 1087-1097.

[34] Acosta-Martínez V., R. Lascano, F. Calderón, J.D. Booker, T.M. Zobeck, D.R. Upchurch, 2011, Dryland cropping systems influence the microbial biomass and enzyme activities in a semiarid sandy soil. *Biology and Fertility of Soils*, 47(6), 655-667.

[35] Yang X., M.C. Richmond, T.D. Scheibe, W. A. Perkins, H. Resat, 2014, Flow partitioning in fully saturated soil aggregates. *Transport in Porous Media*, 103(2), 295-314.

[36] Moëgne-Loccoz Y., P. Mavingui, C. Combes, P. Normand, C. Steinberg, 2015, Microorganisms and Biotic Interactions. In *Environmental Microbiology: Fundamentals and Applications* (pp. 395-444). Springer Netherlands.

[37] Rumpel C., I. Kögel-Knabner, 2011, Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil*, 338(1-2), 143-158.

[38] Pagel H., J. Ingwersen, C. Poll, E. Kandeler, T. Streck, 2014, Micro-scale modeling of pesticide degradation coupled to carbon turnover in the detritusphere: model description and sensitivity analysis. *Biogeochemistry*, 117(1), 185-204.

[39] Zhang C., S. Xue, G.B. Liu, Z.L. Song, 2011, A comparison of soil qualities of different revegetation types in the Loess Plateau, China. *Plant and Soil*, 347(1-2), 163-178.

[40] Hobley E., G.R. Willgoose, S. Frisia, G. Jacobsen, 2013, Environmental and site factors controlling the vertical distribution and radiocarbon ages of organic carbon in a sandy soil. *Biology and fertility of soils*, 49(8), 1015-1026.

[41] Schmidt H., M. Thom, K. Matthies, S. Behrens, U. Obst, S. Wieprecht, S.U. Gerbersdorf, 2015, A multi-disciplinarily designed mesocosm to address the complex flow-sediment-ecology tripartite relationship on the microscale. *Environmental Sciences Europe*, 27(1), 1-11.

[42] Allison S.D., 2005, Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters*, 8(6), 626-635.

[43] Karaca A., S.C. Cetin, O.C. Turgay, R. Kizilkaya, 2011, Soil enzymes as indication of soil quality. In *Soil Enzymology* (pp. 119-148). Springer Berlin Heidelberg.

[44] Riah W., K. Laval, E. Laroche-Ajzenberg, C. Mougin, X. Latour, I. Trinsoutrot-Gattin, 2014, Effects of pesticides on soil enzymes: a review. *Environmental chemistry letters*, 12(2), 257-273.

[45] Hernández T., C. García, C. Rad, S. González-Carcedo (eds.), 2012, *Soil enzymology in the recycling of organic wastes and environmental restoration*. Springer.

[46] Nieder R., D.K. Benbi, 2008, *Carbon and nitrogen in the terrestrial environment*. Springer Science & Business Media.

[47] Lal R., B.A. Stewart (eds.), 2010, *Food security and soil quality*. CRC Press.

[48] Li, W., 2015, *Contemporary Ecology Research in China*. Springer-Verlag Berlin Heidelberg.

[49] Singh B.P., A.L. Cowie, K. Y. Chan, 2011, *Soil health and climate change* (Vol. 29). Springer Science & Business Media.

[50] Smeets E., R. Weterings, 1999, *Environmental indicators: Typology and overview* (p. 19). Copenhagen: European Environment Agency.

[51] Ritz K. (ed.), 2011, *The architecture and biology of soils: Life in inner space*. Cabi.

[52] Hillel D., 2005, *Encyclopedia of Soils in the Environment*. Amsterdam: Elsevier.

[53] Pankhurst C., B.M. Doube, V.V.S.R. Gupta, 1997, *Biological indicators of soil health*. Cab International.

[54] Dion P., C.S. Nautiyal, 2008, *Microbiology of extreme soils*. Springer.

[55] Ibanez J. G., Hernandez-Esparza M., Doria-Serrano C., Fregoso-Infante A., Singh M. M., 2010, *Environmental chemistry: fundamentals*. Springer Science & Business Media.

- [56] Chapin III F.S., P.A. Matson, P. Vitousek, 2011, Principles of terrestrial ecosystem ecology. Springer Science & Business Media.
- [57] He Z., H. Zhang (eds.), 2014, Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment. Springer.
- [58] Isermann K., 2003, Handbook of processes and modeling in the soil-plant system. in: R. Nieder, & D. K. Benbi (eds.). Food Products Press, The Haworth Reference Press.
- [59] Khan M.S., A. Zaidi, J. Musarrat (eds.), 2009, Microbial strategies for crop improvement. Berlin: Springer.
- [60] Uphoff N., A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga, J. Thies (eds.), 2006, Biological approaches to sustainable soil systems. CRC Press.
- [61] Liu T.X., 2008, Soil ecology research developments. Nova Publishers.

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