ASSESSMENT OF SOIL EXTRACELLULAR ENZYMATIC ACTIVITY FROM DIFFERENT LAND-USE AREAS OF ARGEȘ COUNTY, ROMANIA

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Abstract: Soils constitute a complex environment inhabited by numerous types of organisms and play a crucial role in the biogeochemical cycling of elements in nature. Within this environment, various types of heterotrophic microorganisms are involved in the mineralization of detrital organic matter through enzymatic processes and the recycling of constituent elements. The extracellular enzymes synthesized by these microorganisms are highly sensitive to environmental conditions, including changes induced by anthropogenic pressure. In this study, we assessed the dynamic intensity of four extracellular enzymes involved in the biogeochemical cycles of C, N, and P in multiple types of areas along the upper course of the Argeș River. Enzymatic activity was present throughout the study period at all stations. The spatial distribution of enzymatic activity varied spatially, with differences observed between the analyzed areas for each enzyme. Additionally, only alkaline phosphatase exhibited higher intensity in the summer season, while the others were more active in the spring and autumn seasons.

Keywords: Arges County, soil mineralization, extracellular enzymes, soil preservation.

INTRODUCTION

Soil constitutes the upper layer of the lithosphere. It is a complex environment that serves as the substrate for numerous organisms and the site of processes such as the mineralization of organic matter and the recycling of nutrients. Soils play an essential role in the biogeochemical cycles that involve the transfer of matter and energy within ecosystems (Golui *et al*., 2023; Ponge, 2015; Luo *et al*., 2017).

Soils support various essential physico-chemical and biological processes crucial for ecosystem functioning. There are different types of soils, classified based on their composition, which, in turn, is influenced by factors such as climate, the type of vegetation covering them, the substrate structure representing the soil

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base, or the quantity and quality of detrital organic matter present in them (You *et al*., 2023). In addition to natural factors, soil composition is often influenced by anthropogenic factors, such as deforestation, intensive agricultural land use, or the accumulation of chemical compounds from industrial emissions or road traffic (Santorufo *et al*., 2021).

Numerous decomposition and mineralization processes are carried out by various types of heterotrophic microorganisms present in different soil types. The mineralization of organic matter resulting from the death of living organisms produces inorganic compounds that can be utilized by plants to support their growth and development. Mineralization processes are essential for the functioning of biogeochemical cycles that form the foundation of ecosystem existence, and they are also primarily responsible for maintaining soil fertility (Vilkiene *et al*., 2016; Lu *et al*., 2021; Luo *et al*., 2017).

The first stage of the mineralization process involves the enzymatic hydrolysis of large organic macromolecules. Through hydrolysis, these macromolecules are transformed into smaller molecules that can be absorbed by microorganisms and used as a food source. Enzymatic hydrolysis is carried out by extracellular hydrolytic enzymes, synthesized by various types of microorganisms.

The intensity of enzymatic activity in the soil depends on a variety of factors, including the chemical composition of the soil, temperature, humidity, and pH. In addition to these, a key factor is the availability and composition of the organic substrate, represented by detrital organic matter. In the absence of substrate, enzymatic activity is entirely absent (Batista *et al*., 2022; Gomez *et al*., 2020; Ndabankulu *et al*., 2022).

However, it is important to understand that enzymatic activity can vary considerably depending on local conditions and environmental factors. Therefore, field studies are necessary to determine specific levels of enzymatic activity in different soil types (Nikolova *et al*., 2023).

This study aimed to **analyze the dynamics of mineralization processes occurring in soils of various land-use areas within the same geographical region**. These processes can provide information about the functionality of recycling mechanisms and the transfer of matter and energy through terrestrial ecosystems (Akinyemi *et al*., 2020; Bayranvand *et al*., 2021). Land surfaces of different uses and varying degrees of anthropogenic pressure were selected. The extracellular enzymatic activity (EEA) was evaluated as an indicator of changes occurring in the soil. The intended goal was to **obtain information that could contribute to a better understanding of the transformations occurring in ecosystems** in the studied region and to develop appropriate **measures for the conservation of natural areas.**

MATERIALS AND METHODS

We established eight sampling stations (Fig. 1) across various types of ecosystems, with two stations allocated for each type of area: mountain forest, submountainous hills, urban green areas, and lands used for agricultural activities. Within the same area, we varied the placement of the stations in order to assess the dynamics of the studied enzymes in a broader diversity of soil typologies.

The selection of the study sites aimed to capture existing **differences in decomposition processes in soils of various types**, both **natural and subjected to anthropogenic pressure**, within the studied region. Samples were collected from the upper soil layer, up to a maximum depth of 10 cm.

In the mountainous area, the first station (S1) was positioned upstream of the Vidraru Dam, near the Capra River, in a forested area. The second station (S2) was located near the Poenari Castle, close to a busy road, Transfăgărășan, at a distance of over 50 meters from the forest edge.

In the hilly zone, one station was placed in a wooded area (S3), near the commune of Catanele. Another station was situated in a meadow, near the commune of Corbeni (S4).

Two urban parks in Curtea de Argeş, Meșterul Manole and San Nicoară, were considered as green areas for sampling. Samples were collected from grass-covered areas. In the case of Meșterul Manole Park, the station (S5) was placed between trees, approximately 1 meter from their base and over 50 meters from road traffic. In San Nicoară Park, the station (S6) was positioned more than 10 meters from the base of trees and approximately 7 meters from road traffic.

For soil samples from agricultural spaces, two arable lands were selected from the Vâlele (S7) and Băiculești (S8) communes. These lands are predominantly used for intensive agriculture, mainly cultivating maize, according to their owners.

After collection, the samples were transported to the laboratory and processed. 1g of soil was dissolved in sterile water and then centrifuged. The determination of **enzymatic activity** was carried out by **assessing substrate consumption** (Pacesila *el al*., 2014).

The activity of **four hydrolytic enzymes** was analyzed in this study: **α-glucosidase (α-GLC)**, **β-glucosidase (β-GLC)**, **alkaline phosphatase (ALP)**, and **alanine aminopeptidase (AAP)**. These enzymes are involved in breaking down macromolecular substances present in the composition of detrital organic matter. Among these substances are starch, cellulose, organophosphoric compounds, and oligopeptides, making these enzymes crucial players in the biogeochemical cycles of C, N, and P (Trivedi *et al*., 2016; Malobane *et al*., 2020; Erdihan *et al*., 2022; Brianvand *et al*., 2021).

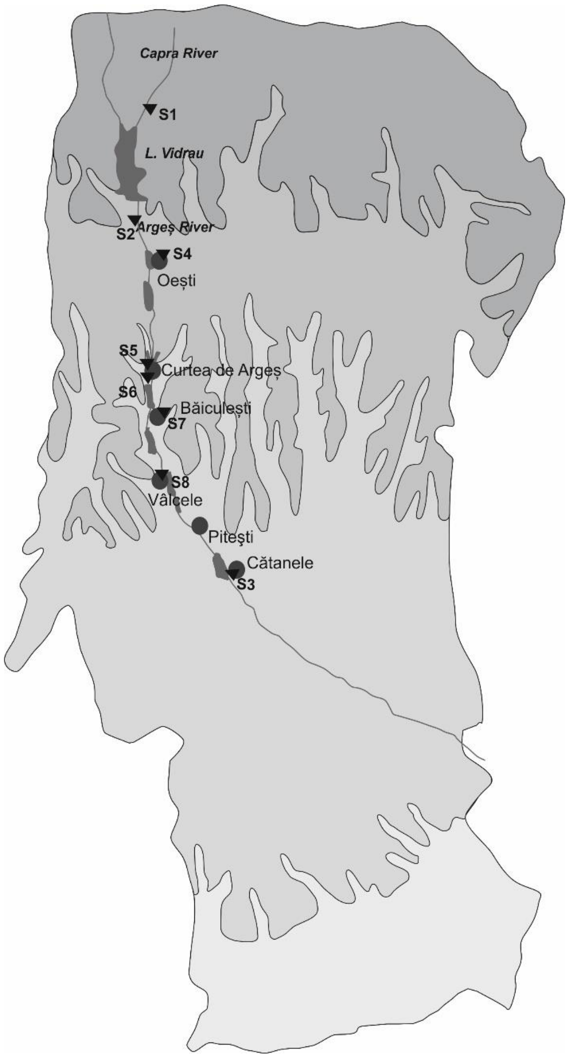

Figure 1. Sampling stations location (S1– S8).

RESULTS

α-GLC exhibited maximum average values in agricultural zones, S7, 43.97 nmol p-nitrophenol g^{-1} h^{-1}, and S8, 48.21 nmol p-nitrophenol g^{-1} h^{-1} (Fig. 2). α-GLC hydrolyzes the 1-4 glycosidic bonds in polysaccharides, serving as an enzyme involved in the hydrolysis of starch, a compound used by plants to store energy. Cultivated plants contain significant starch resources, which enter the soil after plant death, where they are decomposed by extracellular enzymes. However, α-GLC was less intense in S1 despite the presence of plant-derived substrates, including dead leaves (starch is stored in chloroplasts within leaves).

Additionally, α-GLC was more intense in April 2018, 42.45 nmol p-nitrophenol g^{-1} h^{-1}, and less intense in September of the same year, 21.71 nmol p-nitrophenol g^{-1} h^{-1} (Fig. 3). The product of starch hydrolysis, in which this enzyme participates, is glucose, a significant energy source for microbial communities (Ma *et al*., 2020).

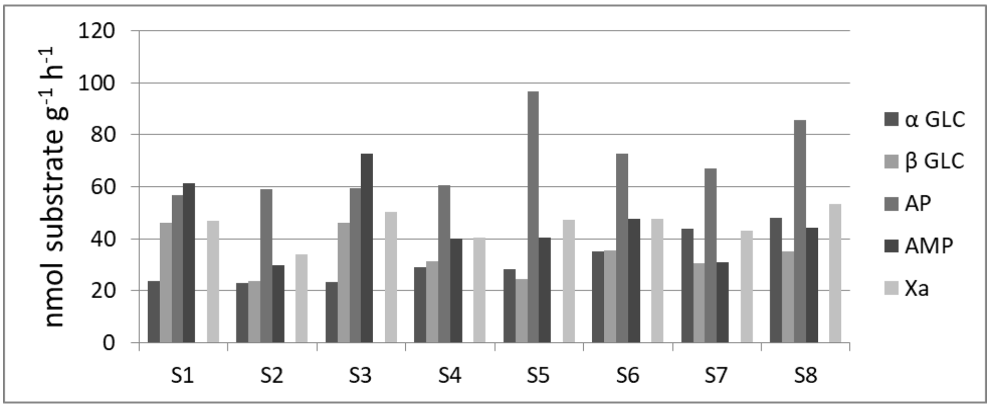

Figure 2. Spatial distribution of the studied enzymatic activity. S1–S8 – sampling stations. α-GLC – alpha glucosidase, β-GLC – beta glucosidase, ALP – alkaline phosphatase, AMP – alanine alinopoptidase, Xa – enzymatic activity average at each sampling station.

The highest intensity values of **β-GLC** were observed in forested areas, S1, 46.2 nmol p-nitrophenol g^{-1} h^{-1}, and S3, 46.03 nmol p-nitrophenol g^{-1} h^{-1}. This enzyme is involved in the final hydrolysis stage of cellulose, a structural polysaccharide in higher plants, leading to the formation of glucose (Adetunji *et al*., 2017). The presence of a plant origin detrital substrate rich in cellulose, in the litter composition, promotes the synthesis of this enzyme by microbial communities. The lowest value was found in S2, located at the roadside, likely due to a lack of abundant substrate.

Seasonally, β-GLC (Fig. 3) was more intense in October 2017, with an average intensity value of 41.62 nmol p-nitrophenol g^{-1} h^{-1}. This autumn month is characterized by leaf fall and the death of part of the herbaceous vegetation, leading to the accumulation of cellulose-like substrate in the soil. The month with lower β-GLC activity values was September 2018, with an average of 16.63 nmol p-nitrophenol g^{-1} h^{-1}.

ALP is an enzyme that releases phosphorus from organic compounds in the form of orthophosphate (Li *et al*., 2021). It is synthesized by microorganisms, especially when inorganic phosphorus is deficient, and inhibited by high concentrations of orthophosphate, its reaction product (Zhou *et al*., 2021). It was more intense in S5, despite its proximity to road traffic, where the substrate consumption had an average value of 67.22 nmol p-nitrophenol g^{-1} h^{-1}. The proximity to road traffic exposes the soils to released pollutants, including to heavy metals that have inhibitory effects. The pollutants negatively influence EEA (Bartkoviak *et al.*, 2020). This aspect may suggest a certain adaptability of microbes in those soils, to produce ALP more resistant to the action of these types of inhibitors.

The lowest average value of the intensity of this enzyme was observed in S1, 56.96 nmol p-nitrophenol g^{-1} h^{-1}. ALP is inhibited by lower pH values among other factors. Forest soils are often more acidic, and at their level, the mineralization of organic phosphorus is achieved to a greater extent with the help of acid phosphatase (Huang *et al.*, 2022; Vikram *et al.*, 2022). Forest soils in the Argeș region also have acidic pH values (Enescu & Dincă, 2018).

ALP was more intense in October 2017, with a value of 97.99 nmol p-nitrophenol g^{-1} h^{-1}. It was less intense in June when the average substrate consumption had a value of 52.95 nmol p-nitrophenol g^{-1} h^{-1}.

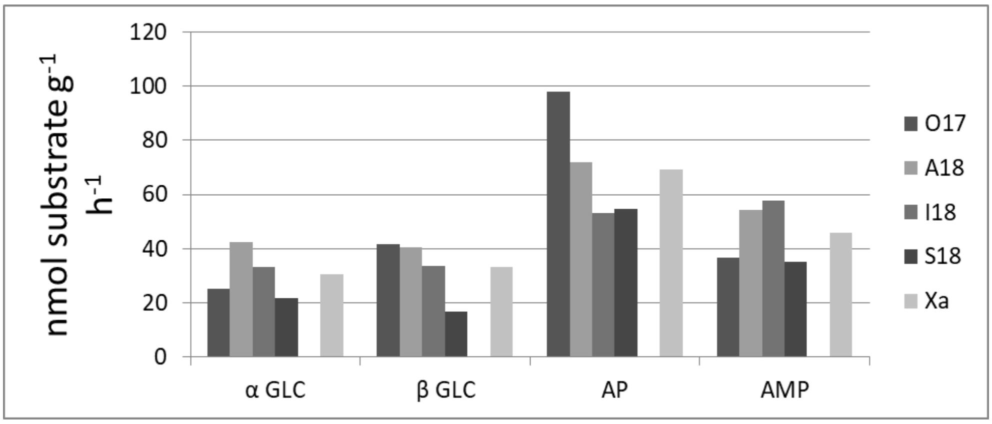

Figure 3. Seasonal dynamics of extracellular enzymatic activity. O17 – October 2017, A18 – April 2018, J18 – June 2018, S18 – September 2018. α-GLC – alpha glucosidase, β-GLC – beta glucosidase, ALP – alkaline phosphatase, AMP – alanine alinopoptidase, Xa – seasonal enzymatic activity average.

AMP is an enzyme involved in the degradation of protein macromolecules, hydrolyzing oligopeptides. In soils, the substrate consists of protein molecules of animal, plant, fungal, or bacterial origin (Norman *et al.*, 2020). In the conducted study, the highest AMP values were found in forested areas, S1, 61.5 nmol p-nitroanilide g^{-1} h^{-1}, and S3, 72.79 nmol p-nitroanilide g^{-1} h^{-1}. Conversely, the lowest value was found in S2, 29.97 nmol p-nitroanilide g^{-1} h^{-1}, located at the roadside and covered with herbaceous vegetation.

Unlike other enzymes, AMP had higher values in June 2018, 57.85 nmol p-nitroanilide g^{-1} h^{-1}, the beginning of the summer season characterized by higher temperatures than the other seasons. The least intense activity was recorded in September of the same year, 35.14 nmol p-nitroanilide g^{-1} h^{-1}.

DISCUSSIONS

The intensity of hydrolytic EEA is influenced by dynamic environmental factors, including substrate presence, temperature, pH, humidity, or human activities (Bueis *et al*., 2018; Furtak *&* Gałązka, 2019).

A key factor for enzymes to perform their activity is **substrate availability** (Nannpieri *et al*., 2018). In its absence, enzymatic catalysis of hydrolytic reactions does not occur, and enzymes become inactive.

Temperature variation can also increase the catalytic capacity of an enzyme, or decrease it to inactivation. Each enzyme has an optimal temperature range in which it performs its activity, with values outside this range significantly diminishing its activity. The same applies to **pH**; significant variations in pH can decrease or deactivate EEA (Fanin *et al*., 2022; Pavani *et al*., 2017).

Another important factor is **humidity**. Hydrolytic reactions take place in the aquatic environment, and water can also serve as a transport medium connecting enzymatic molecules with substrate molecules (Długosz *et al*., 2023; Yao *et al.*, 2022).

Anthropogenic changes, such as housing construction or intensive agriculture, have a direct impact on EEA by altering the structure of microbial communities and nutrient availability (Yuan *et al*., 2022; Furtak *&* Gałązka, 2019). In general, microorganisms adapt to these changes and synthesize enzymes that can function under varied environmental conditions.

Soils represent an essential non-renewable resource in the existence of terrestrial ecosystems. The changes they undergo have considerable influences on both the communities of organisms that depend on them and human communities. **The analysis of EEA is an important primary tool in assessing the health and functionality of soils**, namely their ability to recycle nutrients necessary for plants and other types of primary producers. It can serve as an initial alert to the impact that human activity has on soils (Datta *et al*., 2021; Jat *et al*., 2022). Thus, other categories of complex and detailed analyses can be subsequently conducted to determine the overall state of ecosystems and the **potential application of conservation measures**.

Given the higher enzymatic activity in forested areas, particularly for enzymes like β-glucosidase (β-GLC), conservation efforts should prioritize the **protection of existing forest ecosystems**. Avoiding deforestation and promoting **sustainable forestry practices** can help maintain the detrital substrate essential for enzyme synthesis (Moghimian *et al.*, 2017; Silva-Olaya *et al.*, 2021).

Moreover, **proper management practices in urban green areas**, such as avoiding excessive soil compaction and minimizing chemical pollutants, can support soil health. Maintaining green buffer zones along roadsides can mitigate the impact of pollutants and contribute to enzyme activity (Shrestha *et al.*, 2021; Chiriac & Murariu, 2022; Andrei & Luca, 2022).

Regarding the agricultural practices, promoting a sustainable approach consistsing of **environment friendly methods of farming** can help maintain soil structure and organic matter content, supporting enzymatic processes. Crop residue management and organic farming methods can contribute to the availability of detrital organic matter, sustaining enzyme activity (Zhang *et al*., 2020).

Monitoring and regulating industrial activities, especially those contributing to chemical emissions and pollutants, are crucial for preventing negative impacts on EEA. **Implementing measures to control emissions** near sensitive areas, such as forested zones, can protect soil health and enzyme activity (Yeboah *et al.*, 2021).

Not least, **assessing and regulating land-use changes** can prevent disruptions to enzyme activity. **Balancing urbanization and agricultural expansion with conservation efforts** is crucial for maintaining overall ecosystem health.

CONCLUSIONS

In most cases, the studied areas did not differ significantly in terms of overall average EEA intensity values. Differences were observed, however, for each enzyme individually. All enzymes recorded different values in the analyzed stations, thus indicating differentiation of environmental factors influencing EEA activity for each specific area.

Additionally, all enzymes were present in all studied stations throughout the entire study period. This suggests that each area harbors microbial communities with high diversity actively involved in the biogeochemical cycles of C, N, and P. In other words, in all the analyzed regions, soils have fulfilled their ecological function by providing nutrients and energy to green plants and other types of primary producers. The differentiation between areas was more pronounced in the intensity with which enzymes carried out their activity rather than their presence or absence. Ecologically, these differences in results and enzymatic activity significance are noteworthy. α-GLC was more intense in urban areas, while β-GLC and AAP had higher values in forested areas where the presence of plant-derived substrate was more abundant. ALP was more active in both urban and arable zones.

The increase in temperature did not lead to an increase in EEA intensity, although there are studies suggesting this aspect. Except for AAP, which was more active in the summer season, a period with higher temperatures, the other enzymes had higher values in the autumn or spring seasons.

The enzmatic intensity determined for sampling stations differed in terms of relief type, vegetation cover, or land use. This aspect may be partially due to the location of study stations in the same geographical region, resulting in their exposure to the same climatic conditions. Although anthropogenic pressure is high in the region (Dunea, 2022), it does not seem to significantly influence EEA, with some enzymes recording the highest values in urban or agricultural areas. However, monitoring biological indicators, including enzymatic ones, in a context of interdisciplinary research, is necessary to explore the complex interactions influencing soil health and to prevent potential issues that may irreversibly impact the natural environment. Moreover, supporting research initiatives focused on understanding the impact of climate change on microbial enzymatic activity and developing innovative solutions for sustainable soil management are desireble acting directions.

Regarding the conservation measures that could follow the results of the present research, they should address the specific needs of each land-use area, considering the unique enzymatic activity patterns observed. A holistic approach that integrates ecological, social, and economic aspects is essential for effective soil conservation and sustainable land management. Raising awareness among the local population, policymakers, and stakeholders about the importance of soil health and enzymatic activity is also essential. Educational programs can promote sustainable practices and encourage community involvement in conservation efforts.

Establishing long-term monitoring programs for soil health, including EEA assessments, can provide valuable data for adapting conservation strategies over time. Continuous research and periodic assessments will aid in identifying trends and potential emerging issues.

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