COMPARATIVE ANALYSIS OF PROTEASE AND UREASE ACTIVITY IN SOILS FROM LIVINGSTON ISLAND (ANTARCTICA)

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Abstract: Soil formation and quality is determined, among others, by nutrients availability. The soil enzymes, catalyzing key biochemical reactions, are responsible for biotransformation of matter in soils and hence for providing nutrients. The activities of protease and urease enzymes are important for the release of simple carbon and nitrogen needed for the growth and reproduction of soil organisms. The aim of the present study was to evaluate the protease and urease activities of soil samples, collected from an extreme environment (Livingston Island, Antarctica), as biomarkers of soil quality and to compared them with activities in soils, collected from the moderate climatic zone. The enzyme activities were determined spectrophotometrically by the amount of the hydrolysis products (tyrosine and ammonia) of the substrates (casein and urea). Our results indicated that the enzyme activities vary between the different soil types, depending on the composition of the organic substances, the diversity of microbial species and the intensity of the biological processes.

Key words: *soil enzymes, protease activity, urease activity, soil quality*

Introduction

Soil is a complex living system and is often defined as a non-renewable resource because its formation is a very slow and complex process. Soil is the most important element for the existing of all terrestrial living organisms and thus for maintaining the environmental quality (Binkley & Fisher, 2012). These features of soil are determined mainly by the nutrients availability. The soil enzymes catalyzing key biochemical reactions are responsible for biotransformation of matter in soils and hence for providing nutrients (Kujur & Patel, 2014). All soils contain enzymes. Their origin is primary due to microbial activity. Soil inhabiting microorganisms continuously synthesize various enzymes, which can accumulate, can activate certain metabolic processes or inactivate and/or degrade in the soil (Dick et al., 1994). In this way they are closely related to the stabilization of the soil structure, degradation of organic matter and the dissolution of various compounds (Allison & Vitousek, 2005), mineralization, nutrient cycles (Dick et al., 1994) and transfer of materials and energy (Kujur & Patel, 2014). Microbial degradation of organic matter in the soil plays an important role in the global C and N cycles (Davidson & Janssens, 2006). The activities of protease and urease enzymes are important for the release of simple carbon and nitrogen needed for the growth and reproduction of soil organisms (Nasreen et al., 2012).

Proteases occur naturally in all organisms. They catalyze the hydrolysis of peptide bonds in proteins to their constituent monomers and are important in the N cycle (Sardans et al., 2008). In soil these enzymes are present as extracellular products of the bacterial activity and usually they are associated with organic and inorganic colloids (Nannipieri et al., 2003). The activity of proteases results in NH_4^+-N release in the soil and thus plays a significant role in N mineralization. This is an important process that regulates the amount of available N for plant growth (Makoi & Ndakidemi, 2008).

Urease is an enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_4^+ ions. In soil its origin is basically microbial and its activity is extracellular (Dick et al., 1994). It is essential for hydrolysis of nitrogen compounds, which are delivered to the soil by plants (Blonska & Lasota, 2014) and to a lesser extent by the animals and microorganisms (Salazar et al., 2011). The mediated conversion of organic to inorganic nitrogen by urease leads to loss of the N from the soil by evaporation of NH_3 in the atmosphere (Das & Varma 2010). For this reason, the urease activity has received a particular attention, because it is one of the key factors for the regulation of N supply to plants. According to many authors, the urease activity could be used as an indicator of soil quality

and ecosystem stability (Gil-Sotres et al., 2005).

Soil enzyme activities may vary greatly depending on the variation in organic matter, microbial community, microbial activity associated with soil biological processes, which are affected by biotic and abiotic factors. The aim of the present study was to evaluate the protease and urease activities of soil samples, collected from extreme environments (Livingston Island, Antarctica) and to compare them with soils, collected from the moderate climatic zone. We expected the soil enzymatic characteristics to provide information about the status of key biochemical reactions involved in rate limiting steps of biotransformation of soil nutrients in extreme environment.

M**aterial and methods**

Soil samples: Soil samples were collected from the surface soil layer (0-15 cm) from 4 locations on Livingston Island (Antarctica) and 4 locations from a moderate climatic zone (Bulgaria, Europe) (Table 1.). After purification of the crude waste, samples were sieved through a 2 mm mesh and stored at 4°C prior to analyses.

Table 1. Sampling location and soil description

Soil sample	GPS coordinates	Climatic zone	Habitat type	Soil
S1	S62°38'09.2", W60°21'19.9"	polar	open	*Bare soil with some rare moss tufts around*
S2	S62°38'14.1", W60°21'35.6"		open	*Soil under moss cover*
S3	S62°38'0.65" W60°21'14.5"		open	*Soil under mixed cover of moss and grass, containing significant quantity of vegetation remnants and mussel shells; resting place of birds*
S4	S62°38'48.9", W60°22'17.9"		open	*Soil under mixed cover of moss and grass, mixed with vegetation remnants*
S5	N43°16'75", E26°56'8.7"	moderate	open	*Soil under mixed grass cover*
S6	N42°41'5.82", E23°20'1.97"		closed	*Soil under broadleaf tree cover*
S7	N 42°41'7.69", E23°20'5.03"		closed	*Soil under broadleaf tree cover*
S8	N 42°41'9.99", E23°20'7.23"		closed	*Soil under broadleaf tree cover*

Soil characteristics: The pH of the soils was determined in aqueous extracts (in the ratio 1:2.5) with a digital pH meter HANNA. The contents of ammonia and nitrate nitrogen in the soil samples were assessed by the method of Nessler and by the cadmium reduction method, respectively, using the HANNA *Hi- 83200* meter.

Protease activity assay: The activity of proteases in soil samples were determined after the method described by Speir & Ross (1975). Soil samples (2g) were incubated for 24 hours at 30°C with 10 ml of 0.1 M TRIS buffer, pH 8, containing 0.2% casein as substrate. After precipitation with trichloroacetic acid the mixture was centrifuged. In the resulted supernatant the tyrosine content was determined spectrophotometrically at 730 nm after the addition of Folin-Ciocalteu reagent. The activities of proteases were expressed as milligrams of tyrosine, released per g of soil per 24 h (mg tyrosine g^{-1} soil 24 h^{-1}).

Urease activity assay: The urease activity in soil samples was determined after the method described by Alef & Nannipieri (1995)**.** Soil samples (1g) was mixed with 5 ml of 0.1M phosphate buffer, pH 7.0, containing 0.2M urea as substrate and incubated for 1 hour. After incubation, 10 ml of 2 M potassium chloride was added, and the mixtures were stored at 4°C for 10 minutes to stop the enzymatic reaction. The content of ammonia was determined by the method of Nessler. The

formed yellow coloration was proportional to the ammonia concentration and was read spectrophotometrically at 420 nm. Urease activity was expressed in terms of milligrams of ammonium nitrogen released per g of soil per 1 h (μg $N\text{-}NH_4^+$ $g^{-1}1h^{-1}$).

Statistical analyses: Data from soil analyses were subjected to simple correlation analysis to test the statistical significance of soil physicochemical properties and soil enzyme activities between the soil samples. Pearson's product moment correlation coefficient was used to study relationships among the soil characteristics and enzyme activities. Cluster analysis was performed by the unweighted pair group method on calculated Euclidean distances. All calculations were carried out using the package STATISTICA v. 4.

Results

The data from analyzes of the pH and the content of inorganic nitrogen forms as $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ of the soil samples from the examined habitats are presented in Table 2.

Table 2. Soils sample characteristics (pH and ion concentrations of nitrogen ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$)

№	Climate zones	Habitat type	pH	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$
				(μg g^{-1} soil)	(μg g^{-1} soil)
S1	polar	open habitat	6,87	3,6	3,41
S2			7,16	1,5	2,79
S3			8,08	3,2	5,68
S4			7,21	1,5	2,79
S5	moderate	open habitat	7,11	3,9	9,11
S6		closed habitats	6,83	11,9	18,21
S7			6,70	10,8	17,54
S8			6,51	13,4	16,17

The mean pH value of the Antarctic samples was in general higher although not statistically significant than those from the moderate climatic zone (7.33 ± 0.26 vs 6.78 ± 0.12, p = 0.07). All the soils from the open habitats had higher pH values, although not statistically significant, compared to those from the closed habitats (Table. 2) regardless of whether originating from Antarctica or from moderate latitudes. The mean value of pH from the examined open habitats soils was 7.29 ± 0.023, whereas the mean value of pH of the examined closed habitats soil were 6.68 ± 0.09. Only the pH value of the S1 sample (6.87) was with lower pH value and was similar to the mean pH values from the closed habitats (6.68 ± 0.09).

The content of inorganic nitrogen forms showed similar relationships. In all soil samples, derived from closed habitats, significantly higher concentrations of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ were determined. The mean value of $N\text{-}NO_3^-$ from the examined soils from Antarctic habitats was 2.45 ± 0.55 μg g^{-1}, whereas the mean value of $N\text{-}NO_3^-$ from the examined moderate zone soils were 10.0 ± 2.10 μg g^{-1} soil. The analysis of the S5 sample, which was gathered from the moderate climatic zone showed a lower $N\text{-}NO_3^-$ amount (3.9 μg g^{-1} soil) that was similar to the values of S1 (3.6 μg g^{-1} soil) and S3 (3.2 μg g^{-1} soil) samples from the Antarctic zone. In regard to $N\text{-}NH_4^+$ amount in the tested soils, the results suggested that the samples from the polar zone were much poorer (3.67 ± 0.68 μg g^{-1} soil) than those from the moderate zone (15.26 ± 2.09 μg g^{-1} soil). Even the polar soil with the highest ammonia content (S3: 5.68 μg g^{-1} soil) had two times less $N\text{-}NH_4^+$ than the soil from the moderate zone with the lowest content (S5: 9.11 μg g^{-1} soil). Here, also, it is noteworthy that in closed habitats the observed ammonia concentration was significantly higher (min 16.17; max 18.21 μg g^{-1} soil) than in the studied open habitats (max 9.11 μg g^{-1} soil).

The obtained results for protease activity in the analyzed samples are presented in Fig. 1.

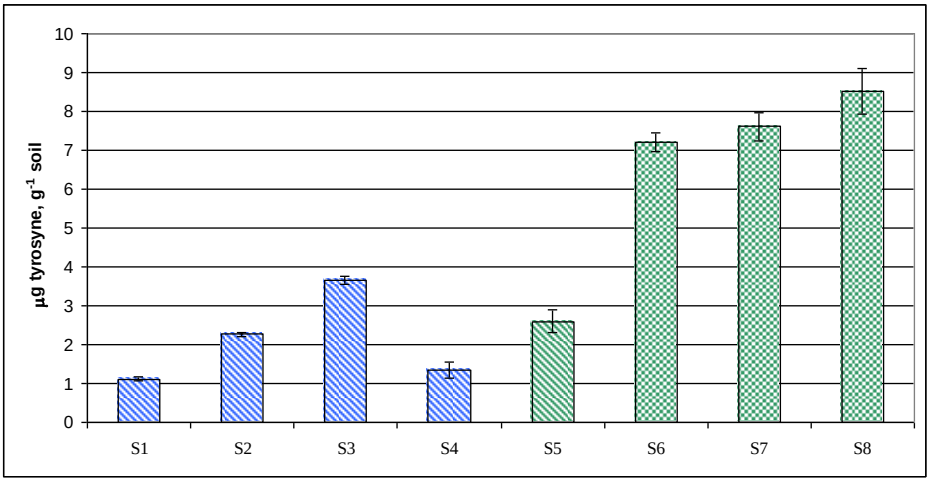

Figure 1. Protease activity (with casein as a substrate) in soil samples, collected from the Antarctic zone (S1-S4) compared to those collected from the moderate zone (S5-S8).

The results from the analysis of protease activities demonstrated that in soils, obtained from all Antarctic habitats (S1-S4: 2.1 ± 0.55 mg tyrosine g^{-1} soil 24 h^{-1}) were similar to S5, a sample from the open habitat of the moderate climatic zone (S5: 2.6 mg tyrosine g^{-1} soil 24 h^{-1}). However, the protease activities of the closed habitats S6, S7 and S8 of the moderate zone were significantly higher (7.76 ± 0.38 mg tyrosine g^{-1} soil 24 h^{-1}).

The urease activity in the studied soil samples expressed by the amount of ammonia produced during urea hydrolysis is presented in Fig. 2.

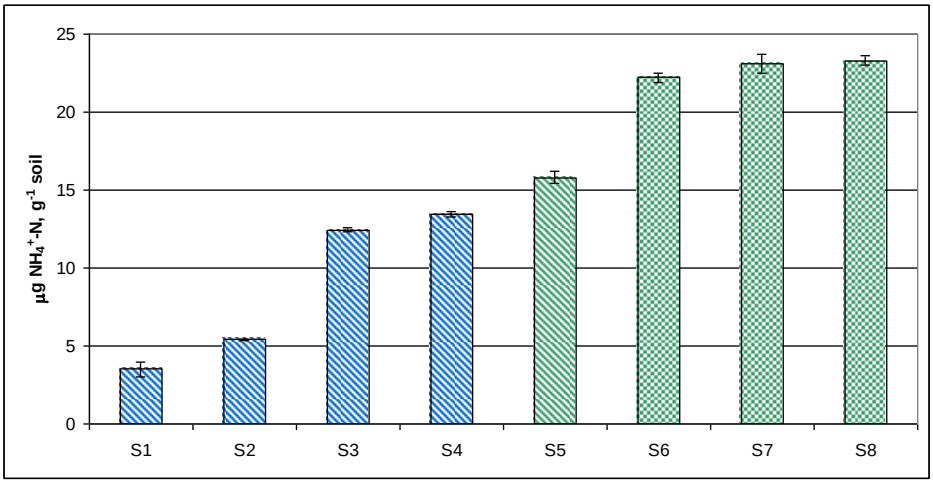

Figure 2. Urease activity (with urea as a substrate) in soil samples, collected from the Antarctic zone (S1-S4) compared to those collected from the moderate zone (S5-S8).

The mean urease activity of all polar soil samples was 8.7 ± 2.5 µg N-NH_4^+ g^{-1} soil 1 h^{-1}. The lowest urease activity was present in the polar sample S1 (3.5 µg N-NH_4^+ g^{-1} soil 1 h^{-1}), followed by polar sample S2 (5.4 µg N-NH_4^+ g^{-1} soil 1 h^{-1}). The values from S3 and S4 samples were about two times higher: 12.45 and 13.45 µg N-NH_4^+ g^{-1} soil 1 h^{-1}, respectively. These activities were close to the measured activity in sample S5 (15.2 µg N-NH_4^+ g^{-1} soil 1 h^{-1}), a sample from open habitat in the moderate climatic zone. All samples from the closed habitats of the moderate climatic zone had a significantly higher urease activity (22.87 ± 0.34 µg N-NH_4^+ g^{-1} soil 1 h^{-1}).

Correlation analysis of the studied parameters is presented in Table 3. Significant positive correlations were present between the determined protease and urease activity and the content of

nitrates and ammonia in the analyzed samples.

Table 3. Correlations between soil characteristics and enzyme activities (marked correlations were significant at $p < 0.05$)

Parameters	Means	Std.Dev.	pH	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	Protease activity
pH	7.058	0.477				
$N\text{-}NO_3^-$	6.225	4.938	-0.653			
$N\text{-}NH_4^+$	9.462	6.833	-0.569	**0.956**		
Protease activity	4.281	3.008	-0.493	**0.958**	**0.944**	
Urease activity	13.650	8.364	-0.401	**0.862**	**0.852**	**0.910**

The similarities between the soil samples based on their characteristics and enzyme activity were further studied by the Euclidean distance metrics. Clustering was performed using the unweighted pair group average (UPGMA) method. The dendrogram (Fig. 3) indicated the presence of two main groups of soil samples differing significantly in the studied characteristics. The first cluster included the soil samples S6, S7 and S8 and the remaining samples formed a second group. The second cluster was divided into two sub-groups of samples - S1 and S2 on the one hand, and S4, S3, S5 on the other.

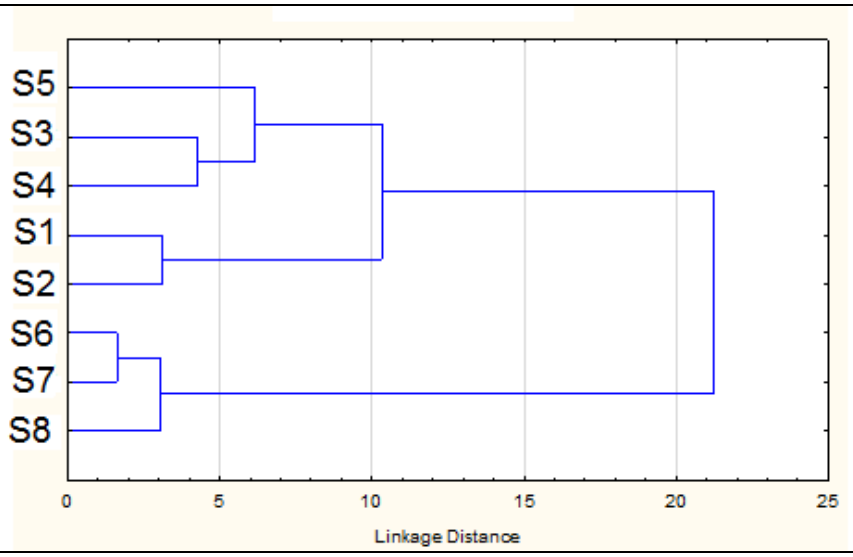

Figure 3. Cluster analysis of the soil samples based on their characteristics and enzyme activities

The obtained cluster dendrogram confirmed the similarity among the open habitats, regardless of their origin, and their difference from the closed habitats, which in turn grouped close together.

Discussion

Our results indicated the presence of variation in the enzyme activity among the studied soil samples from Livingston Island (Antarctica). The highest protease activity was found in sample S3 where we also found the highest $N\text{-}NH_4^+$ concentration and a considerable amount of $N\text{-}NO_3^-$ in comparison to the other samples. In addition, in this sample the most alkaline pH value among all tested soils was measured. The urease activities of the studied Antarctic soils demonstrated large differences with deviation from 2.5 to 14 μg $N\text{-}NH_4^+ g^{-1}$soil $1\ h^{-1}$. The lowest urease activity (3.5 μg $N\text{-}NH_4^+\ g^{-1}$soil $1\ h^{-1}$) was detected in the soil sample (S1) from a bare scree area. A higher urease activity (5.4 μg $N\text{-}NH_4^+\ g^{-1}$ soil $1\ h^{-1}$) was detected in soil sample (S2) from area with mosse and lichen cover. The highest urease activities (12.45 and 13.45 μg $N\text{-}NH_4^+\ g^{-1}$ soil $1\ h^{-1}$) was proved in

soil samples (S3 and S4) from a site with moss and grass cover. Likely these variations in urease activities depended on the type of vegetation on the surface.

The urease activity of the studied samples from Livingston Island was in general lower than the activity in the soils of the moderate climate zones used for comparison. This was particularly apparent for the closed habitats. However, in some samples (S7 and S8) the urease activity values were close to that measured in the soil sample from the open habitat of the moderate zone. The same relationship was observed with respect to the protease activity. Thus, it could be concluded that the activity of enzymes in soil samples were dependant mainly on the type of habitat and to a minor extent on the climate variables, as supported by the tests under identical laboratory conditions. Open habitats were characterized by relatively low organic matter content, and soil microorganisms. Indeed we found low concentrations of $N\text{-}NH_4^+$ in all tested soil samples. However when comparing the concentration of $N\text{-}NH_4^+$ we observed in a sample (S3 sample) almost two times higher levels. Likely, this finding was due to the presence of vegetation cover (grass and moss), which in turn is connected to soil microorganism's activity. The root exudates and litter activate the mineralization process and matter transformation in the soil (Bolter et al., 2002). In regard to the measured $N\text{-}NO_3^-$ concentrations, they were higher in the samples S1 and S2 in comparison to the other samples tested. Thus, it could be assumed that the activity of the enzymes tested under identical laboratory conditions, depend mainly on the type of habitat (vegetation cover) and to a minor extent on climate characteristics. Open habitats are characterized by relatively low organic matter content and soil microorganisms. Indeed, we found low concentrations of $N\text{-}NH_4^+$ in all tested soil samples. However, when comparing the concentration of $N\text{-}NH_4^+$ we observed almost two times higher levels in the S3 sample. It is known that the heterotrophic microorganisms develop better in sites with vegetation cover. The root exudates and litter activate the mineralization process and matter turnover in the soil (Bolter et al., 2002). In regards to $N\text{-}NO_3^-$ concentrations, these were higher in the S1 and S2, compared to the other tested samples. The enzyme activity can be affected by a number of natural factors, i.e. temperature and moisture contents, pH, vegetation cover and presence of organic matter (Blonska & Lasota, 2014). The observed correlations between enzyme activity and the amount of organic matter in Antarctic soils (see also Sokolovska et al., 2015) suggested the close relationship between enzyme activity, soil microbial activity and the turnover of organic matter (Kandeler al., 1999) in the studied soils, and thus indicated the state of soil development (Emmerling et al., 2002; Nannipieri et al., 2003). The terrestrial environments of Antarctica are limited in organic matter (Convey 1996). They exhibit low complexity food web structures which are dominated by microorganisms. Most of the energy and matter are assimilated by primary production in the form of detritus because of the absence of herbivores (Heal and Block 1987). Soil bacteria play a crucial role in this turnover. Soils on Livingston Island were found to have a relatively high bacterial abundance (Kenarova et al., 2013) with ammonifying bacteria predominating in the composition of the soil microbial communities (Sokolovska et al., 2015). The limiting factor for the activity of this functional group of bacteria appeared to be low air humidity and not the temperature regime (Sokolovska et al., 2015).

Acknowledgements

This study was supported by a research grant RD-08-66/02.02.2016.

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