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Microbial activity in soils from the Făgăraș Mountains

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Abstract. The aim of this paper was to assess the microbial activity and detection of some enzymes from soils of mountain zones in order to understand the complex processes that occur in these habitats. The sampling sites are located in three zones from the Făgăraș Mountains (woodside, forest and grassland). Six soil samples from these zones were subjected to microbiological and enzymological studies. The physical-chemical analyses were carried out using a portable multiparameter (pH, Eh, electrical conductivity and salinity). The following four ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, nitrifiers and denitrifiers. The presence of all ecophysiological bacterial groups was registered in all the soil samples. The studied bacterial groups present fluctuations according with the sampling zones. The descending ranking of their abundance in the soil samples was: aerobic mesophilic heterotrophs, ammonifiers, nitrifiers, denitrifiers. Based on the bacteria number of each ecophysiological group, the bacterial indicators of soil quality (BISQ) were calculated. All of the four enzymatic activities analysed in soils (phosphatase, catalase, actual and potential dehydrogenase) were registered at every sampling site. Based on the absolute values of enzymatic activities, the enzymatic indicator of soil quality (EISQ) was obtained. The EISQ and BISQ values indicate a good intensity of these activities in all the analysed zones.

Keywords: enzymatic activity, enzymatic and bacterial indicator of soil quality, the Făgăraș Mountains microbial activity.

Introduction

Forest soils are in a continuous dynamic with the plant communities due to the different substrate inputs (leaves litter, rhizodeposition), forming a natural pedodiversity, caused by this development (Zhang *et al*., 2019). These are in a tight correlation with the forest growth and the variation of natural conditions (relief, rock, climate, vegetation) (Samec *et al*., 2018). The soil fauna represents an important component of the soil biota, including three branches: macrofauna, mezofauna and microbiota. These exhibit important roles in the nutrient cycles, which finally reflect in the total energy flow of ecosystems (Wu and Wang, 2019). Beneath each spot of earth in the forest there is a living network, interconnected, considered by some researchers as "the hidden intelligence" by which underground symbiotic relations (mycorrhiza) with the fungi are established, sending messages of alarm and resources share (Ågren and Weih, 2012). The ecosystem processes are shaped according to the plant layer composition, the tree species, the successive stages and the environmental factors which influence the redistribution of nutrients (C, N and P) between plants and soils (Ågren and Weih, 2012). The soil composition determined by the C:N:P nutrients ratio shows the soil fertility and the regulation of the growth/development of plants, indicating the nutrients composition at plants level (Bui and Henderson, 2013; Fan *et al*., 2015).

Being an integrant part of the forest ecosystems, the soil microorganisms fulfil important roles in regulating the carbon and nitrogen cycles and in determining the responses of the terrestrial ecosystems subjected to environmental changes (Ren *et al.,* 2018; Yao *et al*., 2018). Thus, small changes at microbiota level can lead to significant changes of the nutrients transformation in the soil-plant system (Bragazza *et al*., 2015; Deng *et al*., 2016; Ren *et al*., 2018). The soil microorganisms can modify the diversity and productivity of plants and affect the sustentation of soil fertility. Also, the plant and soil properties significantly affect the microbial communities in soil (Yao *et al*., 2018). The microbial communities of soil can quickly respond to the changes in the plant-soil interactions (Yao *et al*., 2018).

The soil nutrients are correlated to the enzyme activities, considered one of the best representations of the soil activity and health (Lucas-Borja *et al*., 2016; Yao *et al*., 2019). The activity of soil enzymes is often used as bioindicator to assess the soil quality. These are involved in microbial decay, in the basic biochemical processes (C, N and P cycles, decomposition of organic matter hydrolysis of esters etc.) and react quickly to internal and external changes of the local environmental conditions (Ananbeh *et al*., 2019; Guo *et al*., 2019; Yao *et al*., 2018). The soil enzymes participate to the biochemical reactions and thus can regulate the nitrogen cycle in soil level. The biogeochemical cycle of nitrogen (N cycle) is one of the most important functions of soil performed by microorganisms, from soil (Pereg *et al*., 2018). Nitrogen (N) is an essential element for plant growth (Pu *et al*., 2019), but it is also responsible for the existence of life in the biosphere, because it is included in the structure of all proteins and nucleic acids and in many other biomolecules (Cabello *et al.*, 2019). It is widely present in nature, but it is in a great measure found in forms directly inaccessible to plants or animals. The microorganisms use reactions from the N cycle in assimilating, respiratory or dissimulating purpose. Ammonium assimilation consists in its incorporation in the carbon skeletons, usually by glutamine synthesis (Cabello *et al.*, 2019). The bacteria which fix the molecular nitrogen, reduce N_2 to NH_4^+ (ammonium), while other bacteria (ammonificators) decompose the organic compounds with nitrate to NH_3. The nitrifying bacteria oxidize $NH_4^+$to NO_2^- (nitrites) and then NO_3^- (nitrates), the preferred form of N for plants. The denitrification process occurs under anaerobic conditions, mediated by the denitrifying bacteria which perform the reduction of nitrates (NO_3^-) to nitrites, NO, N_2O and N_2, turning back as N in the atmosphere and completing the N cycle (Pereg *et al.*, 2018; Abbas *et al.*, 2019).

The human impact affect the N cycle at global scale, with implications on environment and health. Actually, over half of the fixed nitrogen which enters the ecosystems has anthropogenic origin, while the human activities modify, also, the composition and dynamic of the microbial populations in the terrestrial environment (Cabello *et al.*, 2019).

Materials and methods

Soil sampling. In the summer of 2018, in the Făgăraș Mountains (Viștișoara Valley, Natura 2000 site ROSCI0122) 6 soil samples were sterile taken from forest, woodside and meadow zones. These were transported on ice to the lab, where they were subjected to microbiological and enzymological analyses.

Physical and chemical analyses. Using a portable multiparameter, pH, redox potential (Eh), electrical conductivity and salinity in aqueous solutions were measured (1:50).

Microbial analyses in soil samples. The following ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, nitrifiers and denitrifiers. The number of aerobic mesophilic heterotrophs was determined on plates nutrient agar medium (Atlas, 2010). After incubation the number of colonies in each Petri dish was counted, the average of the parallel samples values was calculated from the most significant dilution and it was multiplied with the reverse value of the respective dilution. The probable number of three groups of studied bacteria (ammonifying, nitrifying and denitrifying) was determined through MTM (multiple tubes method) according to Cușa, 1996. For ammonifiers a peptone medium was used, for nitrifying bacteria (nitrate and nitrite bacteria) in aerobe condition, the Barjac culture medium was used. For the determination of most probable number of bacteria that produce denitrification the Allen culture media was used. The most probable number was calculated according to the statistical table of Alexander (1965). Based on the bacteria number of each ecophysiological groups, the bacterial indicators of soil quality (BIQS) were calculated.

Enzymatic activities in soil samples. Activities of the following four enzymes in soil were measured: actual and potential dehydrogenase, phosphatase, catalase (Alef and Nannipieri, 1995; Carpa *et al*., 2014). Dehydrogenase activity (actual and potential) was determined after 24h incubation of the soil samples at 37ºC with TTC solution, and expressed by the amount of the formed 2,3,5-triphenylformazan (mg formazan / g soil). Enzymatic activity of dehydrogenases was determined by spectrophotometry using an Able Jasco V530 spectrophotometer at 440 nm wavelength. Phosphatase activity was determined after 24h incubation of the soil samples at 37ºC with phenyl phosphate disodic solution, and it is expressed in mg phenol/ 2.5 g soil. Phosphatase activity was determined by using an Able Jasco V530 spectrophotometer measuring the absorbance at 620 nm wavelength. Catalase activity was determined after 1h incubation of the soil samples at room temperature with H_2O_2 solution. The residual H_2O_2 is determined by titration with $KMnO_4$ in the presence of H_2SO_4. Catalase activity was expressed in mg split H_2O_2/ 1.5 g soil. The analytical data serves as the base for calculating the enzymatic indicator of soil quality (EISQ) (Muntean *et al*., 1995-1996).

Result and Discussions

The function of the terrestrial ecosystems depends on the interaction between above-ground and below-ground communities (Bardgett and Wardle, 2010). Below-ground community components, such as bacteria, provide necessary nutrients for the plants and, in turn, plants supply the necessary resources for them. The soil sampling zones belong to the Natura 2000 Site the Făgăraș Mountains, ROSCI0122 (198 618 ha in the central part of the country) (Fig. 1).

In this protected area are found a series of plant and animal species which are rare, vulnerable or even endemic. The habitats are very diverse, from floodplains and meadows to forests, subalpine shrubs and alpine meadows. The flora is well represented, being recorded over 900 plant species. Of the dominant species we can mention: *Campanula persicifolia, Corydalis cava, Geranium robertianum, Agrostis tenuis, Festuca rubra, Fagus sylvatica, Picea abies, Sphagnum* sp. and *Polytrichum* sp.

The samples were prelevated from three zones in the Făgăraș Mountains: two samples from the woodside (1 and 2), two samples from forest (3 and 4) and two samples from the meadow (5 and 6) (Fig. 1).

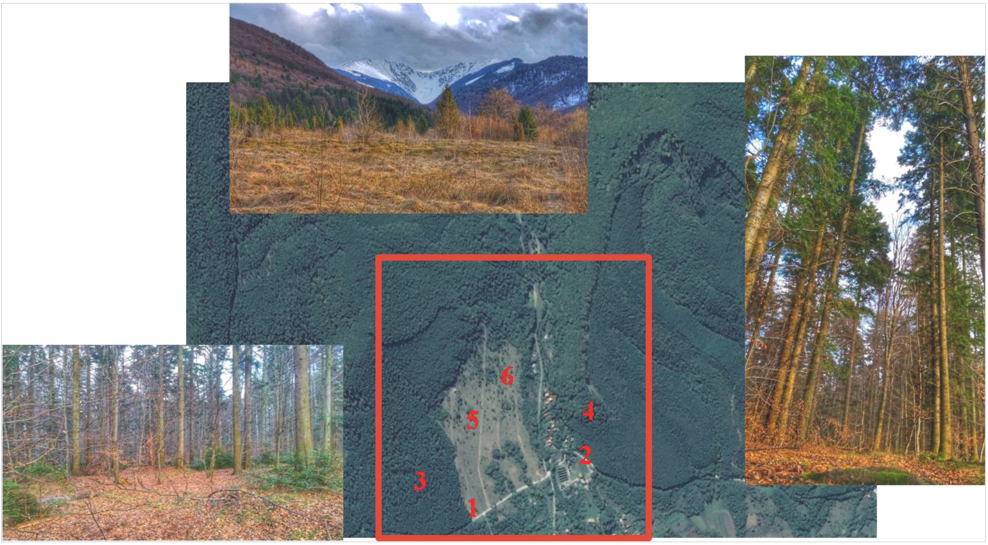

Figure 1. Soil sampling location (1, 2- woodside; 3,4- forest; 5, 6- meadow)

Physical and chemical analyses. All the analysed soils presented a strongly acid character (Blaga *et al*., 2005), exhibiting pH values between 4.29 in the woodside samples and 4.9 in the meadow samples (Fig. 2).

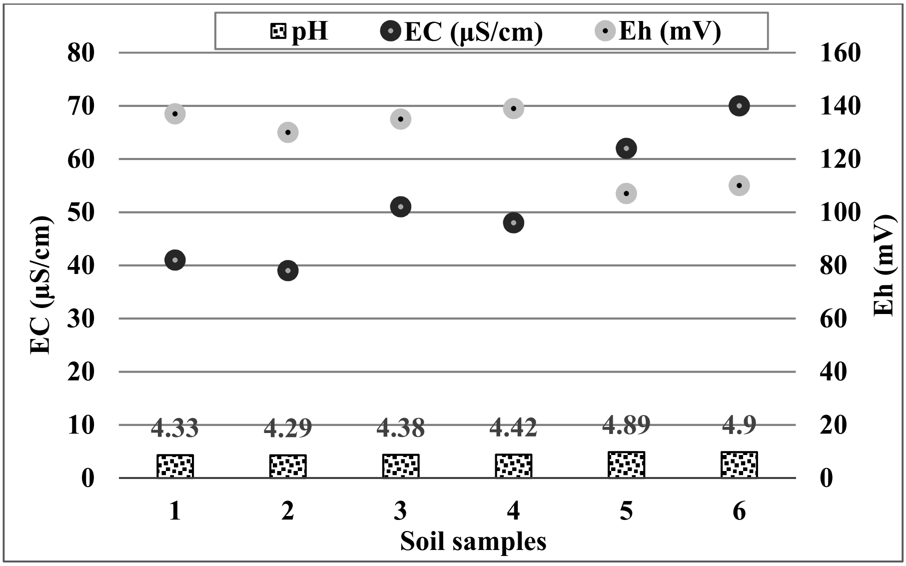

Figure 2. Physical and chemical parameters

The redox potential (Eh) presented values inversely proportional to pH, reaching 137 mV in the woodside zone and 110 mV at the meadow samples. The electrical conductivity (μS cm^{-1}) followed slightly rising trend, following the pH one (Fig. 2). No salinity was detected in these soil samples.

Microbial analyses. Soils are highly dynamic systems that are influenced by the interaction between organic and inorganic components and biota. Bacteria represent the main soil organisms in establishing the soil micro-food web (Wardle, 2002). Heterotrophic bacteria are microorganism that require organic carbon supply for their metabolism. Heterotrophic microorganisms in terrestrial systems have an important role in organic matter decomposition.

In the soils from the Făgăraș Mountains aerobic heterotrophic bacteria were the most numerous of all the ecophysiological groups studied. The highest number was detected in the meadow soils, reaching an average of 4565683 bacteria/ g soil /grassland zone (Tab. 1).

Ammonification consists in mineralization of protein nitrogen to NH_3 (ammonia) by decomposing organic matter or nitrite reduction; stage preceded by fixation of molecular nitrogen under the action of over 100 genera of different bacteria. Ammonifying bacteria were significantly higher in woodside compared to other samling sites. The highest level of ammonifying bacteria was founded in forest (sample 4)(8500 bacteria/g soil). In the other zones the presence of these bacteria was lower, specially in the woodside zone, where values of 2800 and 6800 bacteria/g soil (Tab. 1) were recorded. This fact may be due to the composition of the organic matter which require different time spans for decomposion, this process being one slower and with continuous activity.

Table 1. Probable number of bacteria in soil samples from the Făgăraș Mountain

Soil samples	Heterotrophs	Ammonifiers	Nitrate bacteria	Nitrite bacteria	Denitrifiers	BISQ
1	197667	6800	6800	400	1700	3.76
2	677533	2800	3600	1800	1200	3.83
3	825000	7200	6100	3600	3200	4.12
4	2340333	8500	6000	4500	2900	4.02
5	5158333	7200	1400	200	2400	3.88
6	3973033	8100	9100	2100	2000	4.22

The next stage of the nitrogen biological cycle is the nitrification, composed of two stages: first the oxidation of NH_3 to nitrites (NO_2^-) and than to nitrates (NO_3^-). In the analyzed soil samples the presence of nitrifying bacteria was much more reduced than the presence of ammonifying bacteria. The highest presence of nitrate bacteria was noticed at meadow (sample 6) (9100 bacteria/g soil). In the forest samples the nitrate bacteria activity was comparable to the one in the meadow. The smallest value of nitrate bacteria was obtained at the forest zone (600 bacteria/g soil) (Fig. 3).

The presence of nitrite bacteria in the soils from the Făgăraș reached almost half of the values for nitrate bacteria. Detection was minimal in the samples of meadow soil (2000 bacteria/g soil) (Fig. 3).

Denitrification is the last stage of nitrogen cycle. This, if very intense, can be harmful to soil, because leads to loosing the nitrogenous compounds assimilable by plants and, finally, to reducing the soil fertility (Muntean, 2009). The presence of denitrifying bacteria was the lowest of all the studied groups. The highest value was 3200 bacteria/g soil, in the forest samples. The lowest values were recorded in the woodside samples (Fig. 3).

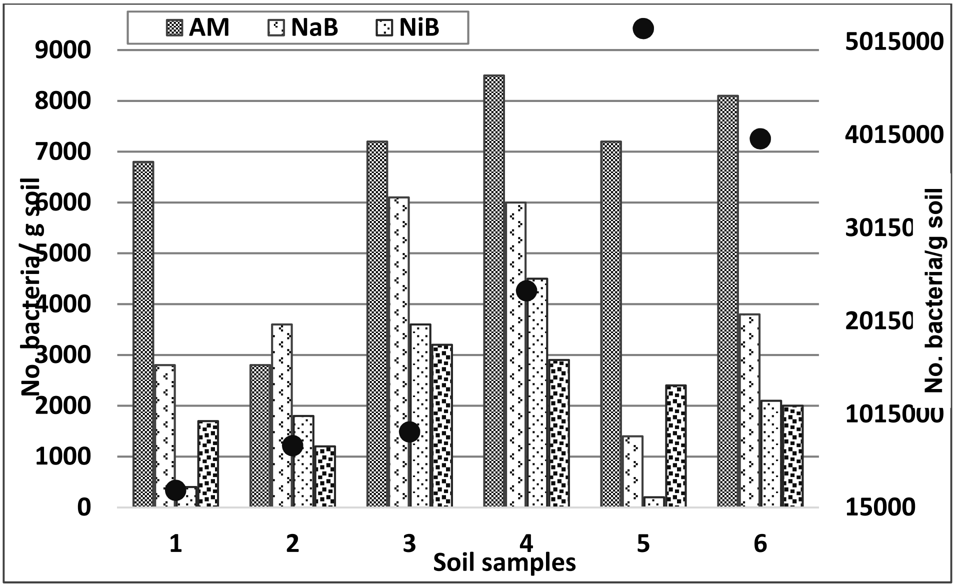

Figure 3. Bacterial determination in soil samples from the Făgăraș Mountain

Enzymatic analyses. The soil enzymes participate in important ecosystem processes, as decomposition of organic matter, formation of humus in soil and, naturally, in the nutrients cycle. These enzymes are known as sensitive indicators of natural and anthropogenic changes in ecosystems, used to evaluate the impact of different pollutants (Wang *et al.*, 2018). The following enzyme activities were detected in all the soil samples: actual and potential dehydrogenase, phosphatase activity and catalase activity (Tab. 2).

Table 2. Enzymatic activities in soil samples from the Făgăraș Mountain

Soil samples	**Dehydrogenases activity (mg formazan/g soil)**		**Phosphatase activity** (mg phenol/ 2.5 g soil)	**Catalase activity** (split H_2O_2 / 1.5 g soil)	**EISQ**
	Actual	Potential			
1	0.525	0.873	1.109	48.52	0.593
2	0.464	0.961	1.071	49.11	0.600
3	0.818	1.274	1.221	4976	0.621
4	0.771	1.361	1.167	49.91	0.632
5	1.196	1.813	1.150	43.34	0.563
6	1.074	1.749	1.100	43.60	0.563

Dehydrogenase activity is an indicator of the variability of live microbiota. Actual dehydrogenases activity (ADA) and potential dehydrogenases activity (PDA) were detected in all the analysed soil samples, with a few variations according to the prelevation zone. PDA presents values almost double compared to the actual one for the simple reason that a nutritive supplement rich in glucose was added, before incubation. The highest dehydrogenase activity was recorded for the meadow (samples 5 and 6), where the vegetation was more abundant (Fig. 3). Here ADA reached values of 1.196 mg formazan/g soil while PDA reached values of 1.813 mg formazan/g soil.

Phosphatases are the expression of organic phosphorus mineralization in all the ecosystems. The phosphorus cycle is one of the most important biological processes in the soil environment. The highest phosphatase activity was noticed in the forest samples, where a value above 1.18 mg phenol/ 2.5 g soil was recorded. In general, it was noticed an average activity on the studied zones; the lowest activity was found in woodside (sample 2), from woodside zone, 1.07 mg phenol/ 2.5 g soil (Fig. 4).

Catalase is the enzyme which accumulates in soils, keeping its activity for a long time. Through the activity of catalase the decomposition of H_2O_2 to H_2O and O_2 occurs.The enzyme is present in all the aerobic microorganisms. Regarding the areas studied an increased activity was noticed, slightly uniform, with a small difference regarding meadow samples (5 and 6), from the mountain meadow, where 43.6 and 43.34 split H_2O_2/1.5 g soil were recorded.

The enzymatic activity of microorganisms is used as a biological indicator of soil fertility and also has an important role in understanding the functioning of ecosystems.

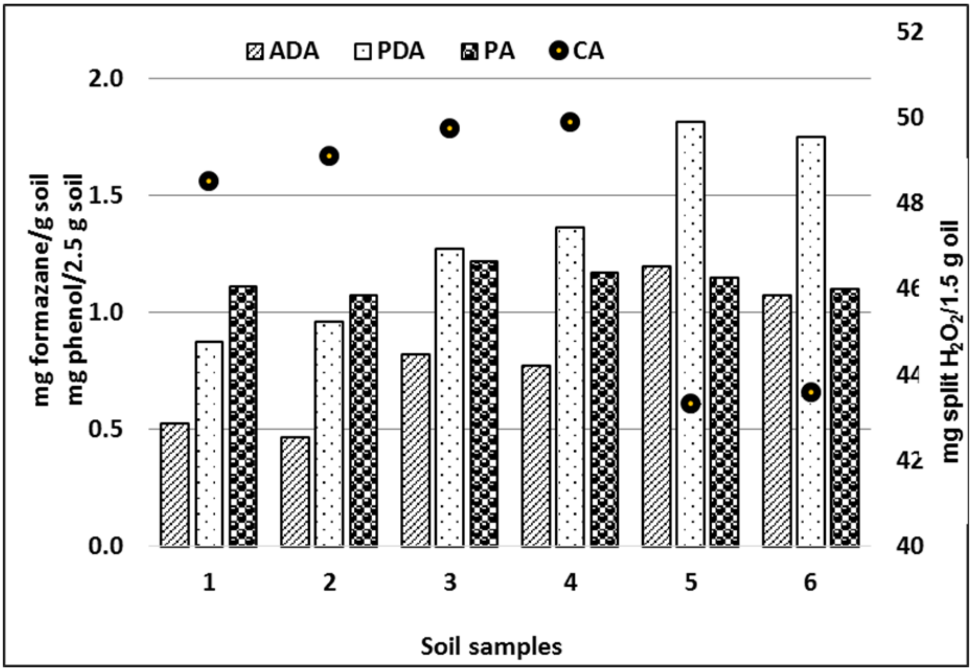

Figure 4. Enzymological activities in soil samples from the Făgăraș Mountains

Based on the microbiological and enzymological activities detected in soil samples, the bacterial and enzymatic indicators of soil quality (BISQ and EISQ) were calculated (Fig. 5). For this study the EISQ values varied between 0.59 and 0.63. Normally this indicator has values between 0 and 1 (Muntean, 1995-1996). We can affirm that a medium activity is noticeable at soil level, as well as a good ecosystem function.

The bacterial indicator of soil quality presented quite close values, ranging between 3.7 and 4.2. This indicator also evidences a good activity in the analysed mountain soils (Fig. 5).

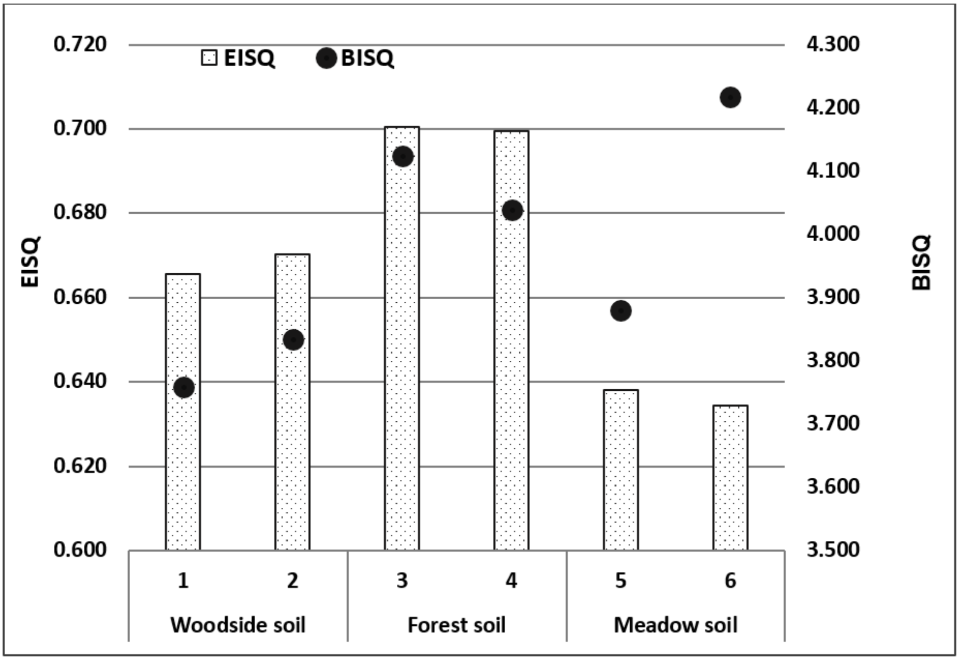

Figure 5. Bacterial and Enzymatic indicator of soil quality

Conclusions

Results of physico-chemical parameters analyses of soil samples revealed: a pH close to strong acidity; the redox potential ranging between 107-139 mV; conductivity detected in all sampling sites (values between 39 and 70 µS cm^{-1}).

A correlation between the microbial activity to each sampling sites and specific vegetation was observed (with differences between woodside, forest and grassland zones). The number of different bacteria (heterotrophic, ammonifying, nitrifying, denitrifying bacteria) is influenced by the amount of nutrients in the soil, season and zone. The highest values were registered in the sampling sites form forest zone. The bacterial indicator of soil quality (BISQ) presents variations according to the sampling sites.

An appreciable enzymatic potential of soils was registered in all the studied soils. The highest values of the enzymatic indicator of soil quality (EISQ) were registered in the forest soil (EISQ = 0.632).

The high enzymatic and bacterial potential registered in the forest soil, could be correlated with putative organic materials originating from the end of the vegetation period.

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