RELATIONSHIPS BETWEEN SOIL PHYSICO-CHEMICAL PROPERTIES AND BACTERIA COUNTS IN OAK FOREST SOIL

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Abstract

Variation of the number of Actinomycetes and nitrifying bacteria and of some physical and chemical soil properties associated with environmental changes of the oak forest soil were investigated during the spring and autumn of the year 2013. Soil samples were collected from 2 locations Z1 (zone 1) and Z2 (zone 2), under one site, namely Cefa-Ateas forest, Oradea forest district, located at 30 kilometers from Oradea, Bihor County. The variability of selected soil properties, relationship between physico-chemical and microbiological soil properties and the statistical significance of means differences between zones, seasons and interactions were studied using the statistical method: one-way analysis of variance (ANOVA) and principal component analysis (PCA)

The results show that ecological factors such as: relative moisture, soil hydrolytic acidity, sum of exchangeable bases, mobile phosphorus, humus, C:N ratio, are important factors that influence the soil microbial populations of Actinomycetes and nitrifying bacteria in the studied forestry ecosystem. Relationship between soil microorganism's counts, physical and chemical properties of the soil was significant.

Also, the results of revealed significant differences between zones of Actinomycetes, mobile phosphorus content, C:N% ratio and sum of exchangeable bases. Relative moisture, humus and ammonia nitrogen variables are responsible of season groups discrimination, but with different abundance.

Keywords: microorganisms, soil, parameters, variability, forest.

INTRODUCTION

Soil microorganisms represent the largest and most diverse biotic group in soil and their activities have the importance role in transformation on plant nutrients to available form and also have metabolisms related to soil fertility improvement.

Soils bacteria have an important role in the nutrient cycling and influence decomposition and nutrient mineralization in the terrestrial ecosystems (Kathiresan, 2002). Distribution of microorganisms in forest soils is mostly determined by vegetation and soil physical and chemical properties.

Microbial communities degrade most of the organic material that settled on the forest soils. The organic matter decomposition rate depends of physical factors, substrate quality and the type of microbial community. The degradation of certain compounds by specific microorganisms, lead to a succession of microbial community until all the substrate is completely decomposed.

Studies on examining the factors that influence the soil microbial communities in various ecosystems are substantial (Hossain and Sugiyama, 2011; Nusslein and Tiedje, 1999).

Many studies have also reported that the soil physical and chemical properties are known to influence the abundance and quality of soil microorganisms. Birkhofen et. all., 2012, explained the relationship between soil properties and soil biota across large spatial

scales and different land-use type and showed that after accounting for heterogeneity resulting from large scale differences among sampling locations, soil properties still explain significant proportions of variation in soil micro flora.

The distribution of microorganisms in forest soils is mostly determined by vegetation and soil chemical characteristics. For example, the study conducted by Hackl, E. (2004), compared the bacterial communities on six forests under different pine and oak vegetation. The results shown that Gram-positive bacteria communities, especially *Actinomycetes*, were more abundant under conifer forests than underoak coverage. These results suggest that bacterial communities are adaptive to the soil chemistry.

Relatively less information is available on the relationships between soil physico-chemical properties and counts of microorganisms in oak forest soils. The present work aims to study the variation of soil *Actinomycetes* and nitrifying bacteria in oak forest soil and the factors influencing their ecophysiology.

MATERIALS AND METHODS

Soil samples were collected from 2 locations Z1 (zone 1) and Z2 (zone 2), under one site, namely Cefa-Ateas forest, Oradea forest district, located at 30 kilometers from Oradea, Bihor County. The site covers more than 203.2 hectares. Forest soil is haplic luvisol. The soil samples were taken in spring and autumn, on March 15-19 and October 1-5, year 2013, from the experimental plots. In March the oaks were still not foliaged, during the October the trees already were after leaf-fall. In both studied locations (zone 1 and zone 2) we collected three mixed soil samples from the top soil (0-20 cm), and each one was consisting of 5 individual, randomly collected subsamples. The collected soils were sieved through a 2 mm mesh screen to remove plant roots, rocks, and macrofauna. After sieving, soil samples were analyzed to characterize their physical, chemical and microbiological properties.

Physical and chemical properties of the soil were estimated adopting the following methods: soil moisture content (Ur, %) was determined by weight loss at 65^0C for 24 h; the pH of the soil was measured in a soil water suspension (1 : 2, soil : water); hydrolytic acidity (Acid., me/100 g soil) and sum of exchangeable bases (SBS, me/100 g soil) were determined by Kappen procedure; ammonium nitrogen ($N\text{-}NH_4$, ppm) was determined with Nessler reagent method, mobile phosphorus (P mob., ppm) content was determined by Egner-Riehm-Domingo procedure, by extraction the ammonium lactate acetate; humus content (humus, %) was determined by using Walkley-Black method; percentage of C and N (C:N, %) content in soil dry mass was determined using elementer analyzer.

The quantitative variation of two ecophysiological bacterial groups have been studied: *Actinomycetes* (Act., ufc/g soil) and nitrifying bacteria (Nitrif. b., ufc/g soil). Total number of *Actinomycetes* and nitrifying bacteria were determined using the dilution method. The soil samples (10 g) were suspended in 90 ml distilled water. Dilutions (of 10^{-6}) were prepared from the soil samples using distilled water and these were dispersed with a top drive shaker for 5 min. Plate count method was used to estimate total number of *Actinomycetes* on agar with glucose and asparagines. To estimate the number of nitrifying bacteria the most probable number method (MPN) was used. Nitrate and nitrite-forming bacteria were cultured in a liquid culture medium containing Winogradsky's salt solution. (Dragan-Bularda, 1986). After incubation the most probable number of nitrifying bacteria was calculated according to the statistical table of Alexander (1965).

The samples were processed using one-way variance of analysis (ANOVA) (n=3, triplicates; P=0.05), in order to determine the statistical significance of means differences. Differences were done between means of four groups built up by mixing season sampling (SPR as spring and AUT as autumn) and sampling zoning (Z1 and Z2). ANOVA results

were generated with GraphPad Prism version 5.00 software (GraphPad Software, San Diego, CA, www.graphpad.com). Groups comparisons and clusters identification were done with principal component analysis, PCA, and hierarchical cluster analysis, HCA (G.P. Quinn, 2002; Herve Abdi, 2010) using PAST version 2.17c software, (Palaeontology Statistics, Copyright Øyvind Hammer and D.A.T. Harper (February 2013), http://folk.uio.no/ohammer/past/) (Øyvind Hammer, 2005).

RESULTS AND DISCUSSION

The main objective of the paper is to evaluate the spatial variability of the physical-chemical and microbiological properties of soil under oak forest from two zones relatively close to each other (less than 100 m). In order to determine the statistical significance of means differences between zones, seasons and interactions one-way analysis of variance (ANOVA) and principal component analysis (PCA) statistical methods were used.

In the following are presented the results comparisons between zones for spring and autumn season and between seasons for the two investigated zones (fig. 1-4).

Table 1

Descriptive statistics, means (standard deviations), and pair-wise comparisons with Bonferroni-corrected post-hoc tests (P = 0.05).

Mean (SD)	SPR_Z1	AUT_Z1	SPR_Z2	AUT_Z2	p
Act. (ufc/g)	17227.13a (1844.636)	15908.29a (2834.665)	726.51b (82.427)	528.04b (242.516)	< 0.0001
Nitrif. b. (ufc/g)	575.12c (194.375)	601.31c (190.286)	104980.81b (19661.549)	164990.93a (40488.912)	< 0.0001
Acid. (me/100 g soil)	2.29d (0.166)	3.36c (0.015)	5.02b (0.285)	5.71a (0.044)	< 0.0001
Ur (%)	17.91b (1.19)	18.48a,b (0.358)	15.83c (1.029)	20.12a (0.744)	<0.0001
N-NH4 (ppm)	11.92a (1.056)	8.78b (0.862)	10.72a (0.426)	12.08a (0.15)	< 0.0001
P mob. (ppm)	60.71a (3.788)	39.54b (3.852)	28.59c (3.054)	12.22d (2.205)	< 0.0001
C:N %	13.34a (4.423)	12.15a (0.76)	11.62a (1.293)	11.57a (0.223)	**0.694**
pH	6.29a (0.081)	6.15a (0.038)	5.67b (0.22)	5.69b (0.045)	< 0.0001
SEB (me/100 g soil)	26.13a (1.381)	24.01b (0.209)	21.82b,c (0.935)	23.54c (0.225)	<0.0001
Humus (%)	6.53a (0.366)	4.83b (0.022)	6.19a (0.146)	5.16b (0.033)	< 0.0001

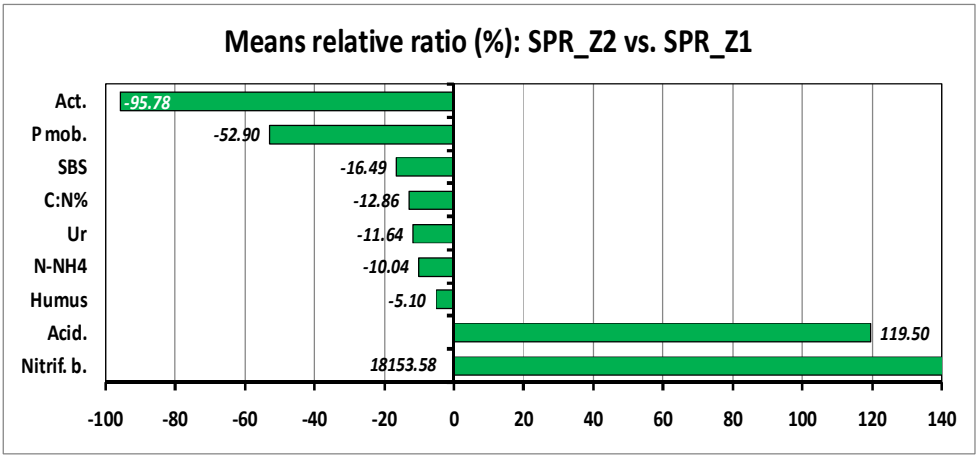

Fig. 1 Results comparisons between zones Z2 and Z1, for spring season.

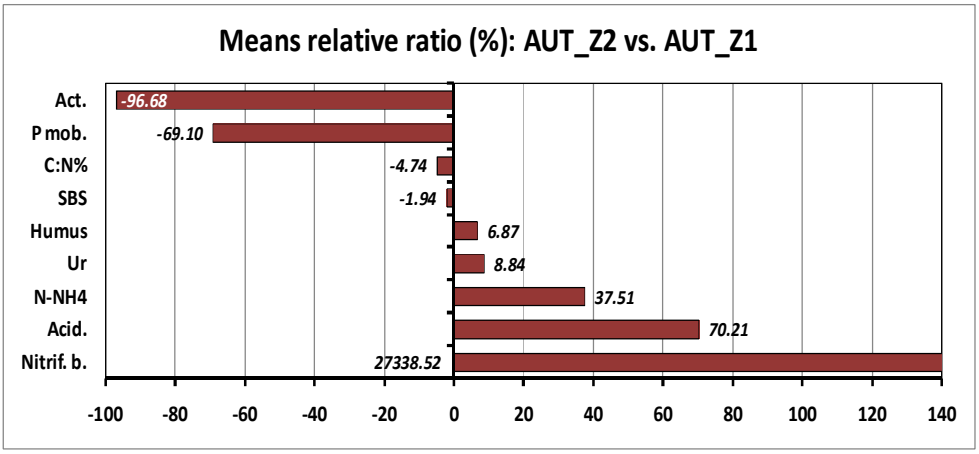

Fig. 2 Results comparisons between zones Z2 and Z1, for autumn season.

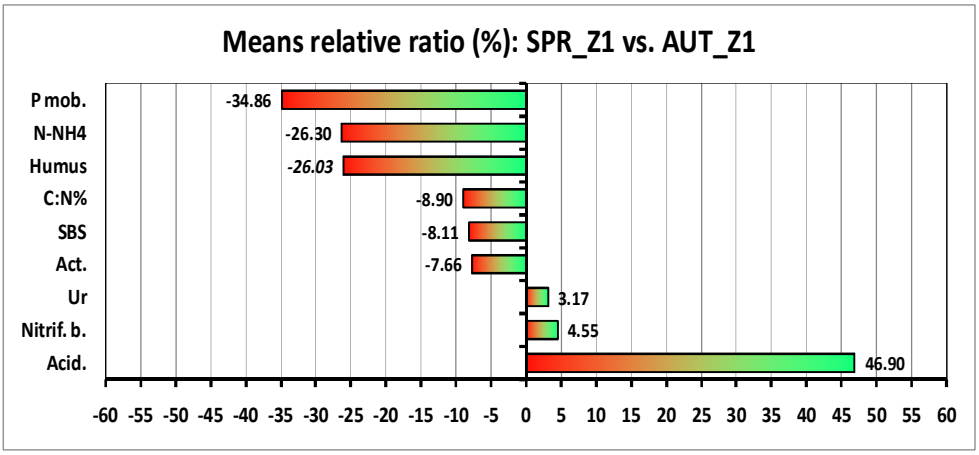

Fig. 3 Results comparisons between seasons autumn and spring, for Z1 zone.

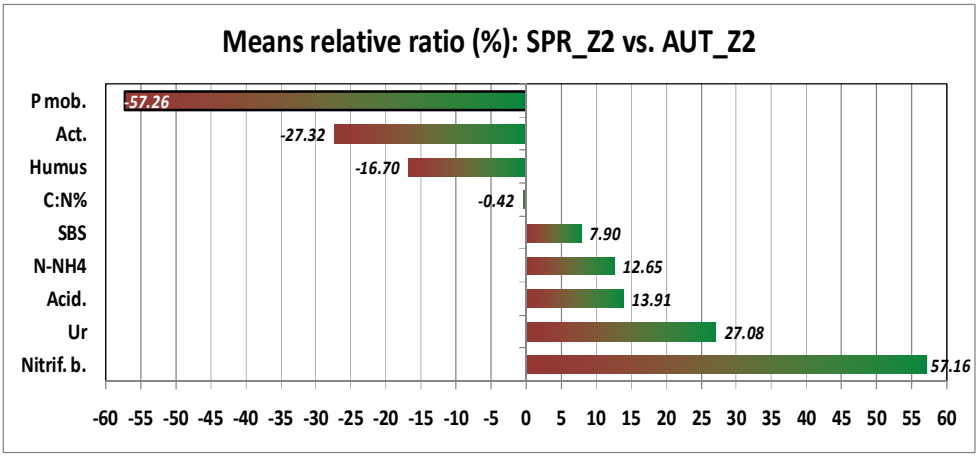

Fig. 4 Results comparisons between seasons autumn and spring, for Z2 zone.

Multivariate analysis

Four sample groups: SPR_Z1, AUT_Z1, SPR_Z2 and AUT_Z2, were considered, along nine variables: nitrifying bacteria (Nitrif. b., ufc/g), *Actinomycetes* (Act., ufc/g), hydrolytic soil acidity (Acid., me/100 g soil), relative moisture (Ur, %) mobile phosphorus (P mob., ppm), C:N%, humus (%), ammonium nitrogen (N-NH4, ppm) and sum of exchangeable bases (SBS, me/100 g soil) in order to generate the principal component analysis, PCA. The first two variables are somehow dependent by the last physical-chemical ones. They are continuous variables with different units, thus the PCA was conducted by the correlation matrix and between groups model.

Figure 5 presents the PCA biplot with the principal components: Component 1 (PC1) with 60.896 % of cumulative variance and Component 2 (PC2) with 20.758 % of cumulative variance. These two principal components gather a total amount of 81.654% of variance explained that emphasizes a successful PCA.

The four studied groups are not-overlapped and suggest possible clusters. Zone Z1 season groups, SPR_Z1 and AUT_Z1, have positive PC1 scores and are in contrast with Z2 season groups, SPR_Z2 and AUT_Z2 with negative PC1 scores.

The variables that are responsible for this contrast are: nitrifying bacteria, hydrolytic acidity, *Actinomycetes*, mobile phosphorus, C:N% and sum of exchangeable bases, and they have high contributions in PC1 built up process.

Variables soil acidity and nitrifying bacteria have negative loadings for PC1, the rest has positive loadings for PC1 (see table 2). These facts emphasizes that the SPR_Z1 and AUT_Z1 (e.g. the Z1 groups) presents a abundance of *Actinomycetes* and the highest values of P mob., C:N% and SBS content, compared with the SPR_Z2 and AUT_Z2 (e.g. Z2 groups).

This variation in the soil properties from zone 1 to zone 2 might be related with the local environmental factors such as micro-climate, vegetation and other ecological factors.

Also, the nitrifying bacteria variable is highly mutual correlated with variable acidity, trough negative loadings for PC1, so they can be considered as associated in a variable group. The *Actinomycetes* variable is highly mutual correlated with variables mob. P, C:N% and SBS, through positive loadings for PC1, thus can be associated as another variable group.

These results indicate the importance of the soil physical and chemical properties in driving the bacterial populations in the study area.

Microorganisms abundance is controlled by various soil conditions and since many soil properties are interrelated with one another, it is difficult to draw distinct lines if division where one type of property dominates the behaviour of the soil (Ann McCauley et al., 2005).

In the same way, the highly mutual correlated variables humus and N-NH_4 with positive loadings and high contributions for PC2 (see table 2), can be associated in a separate variable group. Along with stand alone variable Ur – with negative PC2 loadings and high contribution to PC2 – these three variables contrast the season groups (i.e. spring and autumn), disregard the zone. Spring groups: SPR_Z1 and SPR_Z2 have positive PC2 scores and content abundance of humus and N-NH_4 variables compared with AUT_Z1 and AUT_Z2 – with negative scores and Ur content abundance compared with the spring groups. Thus Ur, Humus and N-NH_4 are responsible of season groups discrimination, but with different abundance (or dominance of content). Most soil functions are significantly influenced by the hydrolytic acidity, relative moisture, organic matter, nutrients content. These factors are essential for soil microorganisms and their diversity. In this way, the seasonal variations seem to influence the chemical and microbiological properties of oak forest soil.

Table 2

Contributions of the variables (%) to the principal components.
Values in **bold** are dominant for the principal component; values in *italics* have negative principal component correlation, otherwise have positive principal component correlation.

Variable contribution (%)	Component 1 (PC1)	Component 2 (PC2)
Acid. (me/100 g soil)	***18.111***	0.250
Pmob. (ppm)	**17.538**	1.084
C:N%	**16.735**	2.124
Act. (ufc/g soil)	**16.140**	*6.137*
Nitrif. b. (ufc/g soil)	***15.602***	4.036
SBS (me/100 g soil)	**12.497**	*0.015*
Humus (%)	2.771	**43.822**
Ur (%)	*0.336*	***11.719***
N-NH4 (ppm)	*0.269*	**30.813**

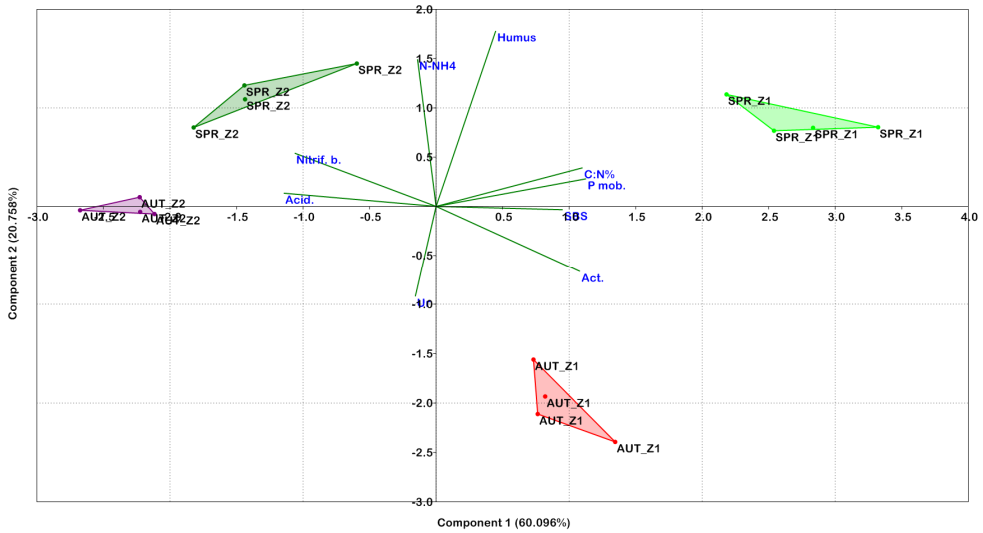

Fig. 5 Biplot of principal component analysis (PCA). Vectors of variables (emerging from center) and samples with corresponding abbreviation. First component explains 60.896 % of variance and second component 20.758 %.

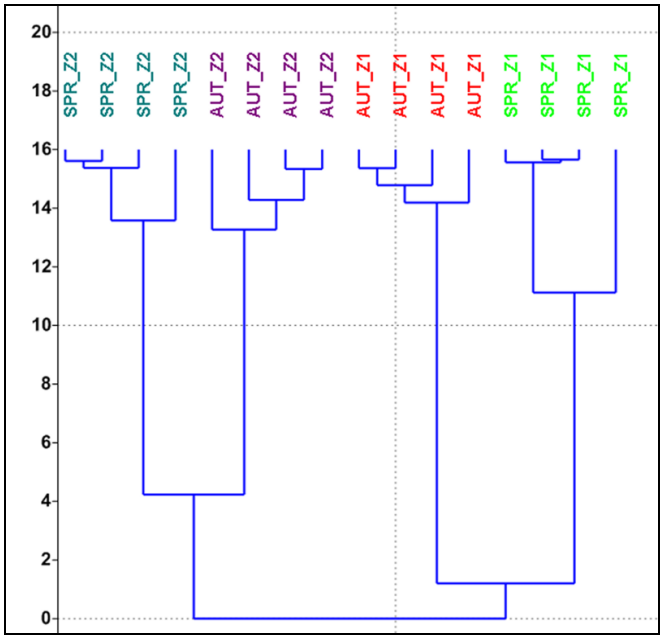

Fig. 6 Dendrogram of the hierarchical cluster analyses of the studied groups (described by season and zones). Vertical axis consists of Euclidian similarity distance for cluster discrimination.

From the four sample groups occurred the possible cluster formation. The loadings of the two PCA axes were the input for the hierarchical cluster analysis (HCA with:

Euclidian distance, two-paired, paired linkage) (Herve Abdi, 2010: Øyvind Hammer, 2005).

HCA was successful around 5.00 cut-off similarity distance value. The dendrogram (fig. 6) presents four clusters that overlap over the four sample groups (fig. 6).

Around the value 1.00 cut-off similarity distance, there are present only two clusters: first built up by the SPR_Z2 and AUT_Z2 (e.g. zone Z2 groups) and second by SPR_Z1 and AUT_Z1 (e.g. zone Z1 groups.

We can confirm that all considered variables can generate two zoning clusters (i.e. disregarding the seasons), not only the former four clusters.

CONCLUSIONS

The results of this paper show significant differences between spring and autumn season and between zones in the counts of microorganisms and in the variation of the physical and chemical properties of the soil, results prescribed by the one-way variance of analysis (ANOVA) and principal component analysis, PCA.

The one-way variance of analysis (ANOVA) results revealed significant differences between zones for nitrifying bacteria, hydrolytic acidity, *Actinomycetes*, mobile phosphorus, C:N% and sum of exchangeable bases.

Also the results show significant differences between seasons for relative moisture, ammonium nitrogen and humus.

The nitrifying bacteria variable was correlated with hydrolytic acidity and *Actinomycetes* were correlated with variables: mobile phosphorus, C:N% ratio and sum of exchangeable bases.

The hydrolytic acidity, mobile phosphorus, C:N% ratio and sum of exchangeable bases are important factors that control survival and growth of nitrifying bacteria and *Actinomycetes*.

As compared with the physical and chemical soil properties the number of monitored microorganisms groups proved to be more variable at the investigated zones.

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