Seasonal Dynamic and Vertical Distribution of Microorganisms and Nutrients in Soils of Mostar Pit (Bosnia and Herzegovina)

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Summary

The aim of this paper is to determined dynamics and vertical distribution of nutrients and microbial communities in calcocambisol and terra rossa in natural lawns in Mostar district (Bosnia and Herzegovina). The main research was focused on total number of bacteria, actinomycetes, fungi, free-living nitrogen fixers, ammonifiers and *Azotobacter* sp., as well as on the chemical properties of soils. Microbiological and chemical properties of soils were determined during the 2011 in different depths using the standard methodology. Chemical analyses of the examinated soil showed the acid pH value, poor to moderate humus content, as well as total N and available P and K. Results of research confirm the strong influence of season of sampling, soils depth and their interaction on microbial activity. Number of microorganisms was controlled by pH value, humus and total nitrogen content, soil moisture and temperature. In both soil types, total bacterial number was the highest in spring, while number of fungi and actinomycetes was the highest in autumn. *Azotobacter* sp. was only detected in surface layer of soils. These results confirm that understanding of microbial, physical and chemical properties of soil and their interaction, which can be controlled by human activities, is necessary for improvement of soil fertility.

Key words

microorganisms, calcocambisol, terra rossa

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Introduction

Soil is the most suitable environment for life and development of microorganisms, plants and animals. From soil populations, microorganisms are the most important because they play significant role in humification and mineralization, and they provide cycling of nutrients. Without microbes, soil could be characterized as "senseless geological substrat" (Govedarica and Jarak, 1995). Composition of microbial populations and its number is controlled by biotic and abiotic factors, from which the most important are soil reaction (pH), organic matter content, moisture, temperature, oxygen content, soil types etc. Biogeneity of soil is one of the parameters of soil fertility. The values of this parameter are higher in soils with high organic matter content, neutral pH and optimal availability of water and air (Jarak and Colo, 2007). With temperature increase nutrients are available for soil organisms (Sharma et al., 2006).

In subsurface layer, because of low oxygen content, microbial activity decreases and delays nutrient cycling (Govedarica et al., 1993). Previous researches show that decreasing of moisture lead to decreasing of microbial activity from 15 to 66%, depending on season and soil depth (Sardans and Penuelas, 2005). On the other side, microorganisms by their activity in humification, oxidation, reduction etc. change the physical and chemical properties (Tangjang and Arunachalam, 2009), as well as soil fertility.

The aim of this paper was determination of seasonal dynamics, vertical distribution and microbial activity in soils under natural lawns, as well as relationship between available nutrients and abundance of microorganisms.

Material and methods

Samples for chemical and microbial analyses were taken from calcocambisol and terra rossa, in natural lawns of Mostar pit (Bosnia and Herzegovina). Sampling was performed on different soil depth (0-30 cm and 30-60 cm) in March, July, October and December of 2011. Microbial characteristics were determined by dilution method according to total bacterial number, abundance of actinomycetes, fungi, free-living nitrogen fixers, ammonifiers and *Azotobacter sp.* Total number of bacteria was determined on 0.1xTSA, ammonifiers on meat pepton agar. On Fyodor`s agar was determined free-living nitrogen fixers number, as well as the number of *Azotobacter sp.* Actinomycetes number was determined on starch-ammonia agar and fungi on rose bengal streptomycin agar (Peper et al., 1995). Microbial number was expressed in CFU g^{-1}. Chemical analyses involed determination of soil reaction (pH-1M KCl), $CaCO_3$ content (volumetrically), total nitrogen (Semimikro Kjeldahl, Bremner, 1996), humus content (Simakov and Cipljenikov, 1960), and available phosphorous and potassium (Al-method, Egner et al., 1960). Soil temperature was measured directly by soil termometer in depths. Moisture content was measured gravimetrically after 2 h-drying of samples on 105°C.

Results and discussion

Abundance and activity of microorganisms was controlled by many factors, from which physical and chemical characteristics of soils are among the most important factors (Jarak and Colo, 2007).

Table 1. Soil reaction, $CaCO_3$ and humus content in calcocambisol and terra rossa

Soil types	Seasons	Depth (cm)	pH-KCl	$CaCO_3$ (%)	Humus (%)
Calcocambisol	Spring	0-30	6.75	17.01	4.21
		30-60	7.08	21.24	2.68
	Summer	0-30	6.28	12.78	3.15
		30-60	6.62	17.12	1.85
	Autumn	0-30	6.65	15.42	3.78
		30-60	6.92	18.83	2.19
	Winter	0-30	6.37	13.91	2.64
		30-60	6.73	17.53	1.26
Terra rossa	Spring	0-30	6.14	13.66	2.12
		30-60	6.73	16.53	1.31
	Summer	0-30	5.51	9.94	1.67
		30-60	6.08	13.25	0.83
	Autumn	0-30	6.06	11.93	1.89
		30-60	6.55	14.96	0.96
	Winter	0-30	5.65	10.18	0.24
		30-60	6.14	13.55	0.38

Soil reaction has significant influence on abundance of some microbial groups, as well as on humification and mineralization process and soil fertility. Some microorganisms require neutral pH reaction (bacteria), other acid (fungi) or alkaline (actinomycetes). In our research season dynamics of soil reaction were presented in Table 1.

Calcic cambisol belongs to neutral, while terra rossa to slightly acidic to neutral soils. Soil acidity was the highest in terra rossa during the summer (5.5). This can be linked with high concentration of feric oxides and hydroxides in liquid phase of soil (data not shown). In autumn, pH values were higher and in spring pH values of soils were the highest. In both soils pH value was higher in layer 30-60 cm, which can be linked with carbonates presence in this layer (Table 1). Aydinalp and Cresser (2008) confirm that pH value increases in subsurface layer.

Humus content was higher in calcocambisol compared with terra rossa. According to Muckenhausen (1975) these soils belong to slow (1-2) or intermediate (2-4) soils. The highest humus content was noticed in spring. In summer was observed decrease of its content, in autumn was noticed increase and in winter its content was the lowest. Humus content in layer 30-60 cm was lower than in surface layer (Table 1). Similar dynamic was noticed in total nitrogen content (table 2). These data were previously confirmed by Aydinalp and Cresser (2008), who suggested that content of total N decreases in subsurface layer.

Available phosphorus was presented in Table 2. Its content in both soil types was the highest in autumn. In subsurface layer its content was lower than in surface layer, especially in terra rossa with values from 14.26 to 3.64 mg/100 g soils. Similar results were observed by Aydinalp and Cresser (2008).

Different content of available potassium was registred in both soil types. The highest content of it was observed in autumn, while in winter it was the lowest. In subsurface layer its content is lower than in layer 0-30 cm (Table 2). Similar conclusion was previously described by Aydinalp (1996) and Aydinalp (2001).

Table 2. Total nitrogen, available P and K in calcocambisol and terra rossa

Soil types	Seasons	Depth (cm)	Total N (%)	P_2O_5 (mg/100 g)	K_2O (mg/100 g)
Calcocambisol	Spring	0-30	0.26	16.36	17.42
		30-60	0.13	7.84	8.24
	Summer	0-30	0.19	15.52	18.21
		30-60	0.09	6.78	8.76
	Autumn	0-30	0.23	17.25	19.3
		30-60	0.11	8.76	9.57
	Winter	0-30	0.12	14.84	15.83
		30-60	0.07	6.1	7.77
Terra rossa	Spring	0-30	0.14	13.63	14.26
		30-60	0.06	4.88	7.01
	Summer	0-30	0.09	12.45	15.18
		30-60	0.04	4.1	7.54
	Autumn	0-30	0.12	14.26	16.1
		30-60	0.05	5.75	8.0
	Winter	0-30	0.06	11.61	12.84
		30-60	0.03	3.64	6.64

Table 3. Temperature and moisture in calcocambisol and terra rossa

Soil types	seasons	Depth (cm)	Temperature (°C)	Moisture (%)
Calcocambisol	Spring	0-30	14.2	26.1
		30-60	12.1	19.8
	Summer	0-30	24.5	22.3
		30-60	22.3	21.4
	Autumn	0-30	15.6	32.9
		30-60	13.5	25.7
	Winter	0-30	4.8	15.2
		30-60	6.3	11.5
Terra rossa	Spring	0-30	13.9	25.2
		30-60	12.1	18.6
	Summer	0-30	24.0	21.4
		30-60	21.8	20.2
	Autumn	0-30	15.2	31.6
		30-60	13.3	24.1
	Winter	0-30	4.4	14.4
		30-60	5.7	10.4

Soil temperature was the highest in summer and the lowest in winter. Amplitude of temperature in spring and autumn was very low (about 1.5^{0}C). Soil temperature decreased with depth, except in winter (Table 3).

Moisture content in surface layer of both soils type was the highest in autumn and the lowest in winter. In subsurface layer soil moisture was lower than in surface layer (Table 3).

Number of microorganisms is one of the parameters of soil biogeneity and it is controlled by soil properties and rate of anthropogenic activities (Redzepovic et al., 1991). Soil microbes significantly affect physical, chemical and biological properties of soil (Tangjang and Arunachalam, 2009).

Based on obtained results, both soil types in Mostar pit were characterized by moderate biogeneity in surface layer, lower biogeneity in subsurface layer, which is in coordinance with other papers (Redzepovic, 1985; Redzepovic et al., 1991; Vukmirovic et al., 1985; Sikora, 1990; Sikora, 1996).

Seasonal dynamic of bacterial number showed large oscillation and was influenced by time of sampling (Table 4).

The highest total number of bacteria in surface layer of both soil types was noticed in spring and the lowest in summer (Table 4). Vertical distribution of bacteria was controlled by soil temperature, moisture and availability of nutrients. Total bacterial number was higher in surface layer compared with subsurface layer.

Fungi in soil play important role in degradation of plant polymers. The highest number of fungi was recorded in autumn in both soil types, while the lowest was noticed in winter (Table 4). In subsurface layer, number of fungi was lower compared with surface layer, which is in accordance with previous researches (Bossio et al., 2005; Kennedy et al., 2005).

Actinomycetes are aerobic, alkalophilic microorganisms. They require dry soils, thus their number is the highest in sur-

Table 4. Seasonal abundance of total number of bacteria, fungi and actinomycetes in calcocambisol and terra rossa

Soil types	Seasons	Depth (cm)	Total number of bacteria ($CFUx10^6g^{-1}$)	Fungi ($CFUx10^3g^{-1}$)	Actinomycetes ($CFUx10^4g^{-1}$)
Calcocambisol	Spring	0-30	41.2	18.5	37.8
		30-60	22.6	8.5	18.8
	Summer	0-30	12.3	21.3	22.5
		30-60	5.6	10.1	10.5
	Autumn	0-30	28.7	28.7	47.3
		30-60	14.2	13.7	5.7
	Winter	0-30	18.7	14.2	10.2
		30-60	10.3	6.8	5.7
Terra rossa	Spring	0-30	35.3	16.4	34.2
		30-60	18.9	7.2	16.5
	Summer	0-30	10.2	18.9	11.1
		30-60	4.8	8.9	9.4
	Autumn	0-30	22.9	25.6	43.4
		30-60	12.8	12.6	21.6
	Winter	0-30	15.3	11.8	5.5
		30-60	8.6	4.9	4.4

Table 5. Seasonal abundance of *Azotobacter* sp., ammonifiers and free-living nitrogen fixers in calcocambisol and terra rossa

Soil types	Seasons	Depth (cm)	*Azotobacter* sp. ($CFUx10^2g^{-1}$)	Ammonifiers ($CFUx10^4g^{-1}$)	Free-living nitrogen fixers ($CFUx10^4g^{-1}$)
Calcocambisol	Spring	0-30	15.3	46.1	47.7
		30-60	-	22.8	25.2
	Summer	0-30	3.9	17.4	18.4
		30-60	-	8.9	9.7
	Autumn	0-30	11.6	37.3	41.2
		30-60	-	19.0	23.4
	Winter	0-30	7.5	23.5	28.4
		30-60	-	12.1	15.2
Terra rossa	Spring	0-30	12.4	39.8	41.3
		30-60	-	19.3	21.3
	Summer	0-30	2.2	11.3	12.6
		30-60	-	5.9	6.6
	Autumn	0-30	9.5	30.5	33.6
		30-60	-	14.6	17.5
	Winter	0-30	6.1	17.2	20.5
		30-60	-	8.7	11.1

face layer of soils in autumn. Similar trend was recorded in our research. The highest number of actinomycetes was noticed in autumn in both soil types, while the lowest number was observed in winter (Table 4). Lower abundance of actinomycetes in terra rossa can be linked with low pH value. Number of actinomycetes decreased with soil depth, which was confirmed by other authors (Wu et al., 2005).

Number of *Azotobacter* sp., ammonifiers and free-living nitrogen fixers is presented in Table 5. In spring, the number of ammonifiers was the highest in both soil types, regardless of the soil depth. These data show that presence of ammonifiers in soil was influenced by time of sampling, soil temperature and moisture. According to Alexander (1977) number of ammonifiers is controlled by type of substrates, environmental factors, soil type, rhizosphere, as well as nutrient availability (Blazinkov et al., 2005).

The highest abundance of free-living nitrogen fixers was noticed in spring in both soil types, while in summer it was the lowest (Table 5). This can be linked with mediteranean climate, characterized with optimal temperature and precipitation during the spring and high temperatures with absence or low precipitation in summer. Also, lower abundance of free-living nitrogen fixers in terra rossa can be linked with unfavorable chemical properties of this soil type.

Azotobacter sp. presence was only noticed in surface layer of both soil types (Table 5). In both soil types its presence was the highest in spring and lowest in summer. Absence of *Azotobacter* sp. in depth of 30-60 cm was previously described (Alexander, 1977; Kalinovic, 1975; Sikora, 1996). *Azotobacter* sp. requires wet and aerated soils and represents one of the parameters of soil fertility (Govedarica, 1986). Good presence of *Azotobacter* sp. in soils can be connected with good aeration of soils and root exudates, which are sole carbon and energy source. Previous research suggest that rhizosphere is main zone of nitrogen fixation (Killham, 1994).

Conclusion

Results of this research show that microbial activity of soils was moderate in surface layer, while in subsurface layer was poor. Improvement of unfavorable chemical properties is necessary for better activity of microbial populations and intensity of microbial transformation in soils. Understanding of microbial, physical and chemical properties of soil and their interaction, which can be controlled by human activities, is necesary for improvement of soil fertility.

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