

## **Microbiological characteristics of cambisols from Western Balkan Mountains (Bulgaria)**

**Kostadinka Nedyalkova, Radka Donkova and Hristo Valchovski\***

*Department of Soil Microbiology, N. Poushkarov Institute of Soil Science, Agrotechnology and Plant Protection, Sofia*

**E-mail:** h\_valchovski@abv.bg

### **Abstract**

Changes in microbial population density and basal soil respiration of Cambisols from Western Balkan Mountains due to the influence of vegetation cover (grassland, pine forest and beech forest) were studied, and their relations with soil properties were examined. It was found that microbial counts were the highest in the soil under grassland, followed by soils under beech forest and pine forest. Bacterial groups (ammonifying, spore forming, oligonitrophilic and mineral nitrogen utilizing) prevailed in the grassland. Fungi were predominant in the soil under pine forest. Basal soil respiration, as assessed by CO<sub>2</sub> evolution, was the highest in the beech forest (93.80 mg CO<sub>2</sub>/100g/24h), followed by the grassland (67.96 mg CO<sub>2</sub>/100g/24h) and the pine forest (64.39 mg CO<sub>2</sub>/100g/24h). Positive correlation between basal respiration and soil organic carbon content was found. The study clearly confirmed the impact of vegetation cover on microbiological parameters of Cambisols from Western Balkan Mountains.

**Key words:** microbial population density, soil respiration, CO<sub>2</sub> evolution, Cambisol, Balkan Mountains

Vegetation cover is one of the factors that greatly influence microbial communities and their activity. Vegetation affects soil microclimate, structure, pH, organic matter content, root exudates, quality and quantity of plant residues etc. (Raich et al., 2000). Thus plants exert selective influence on soil rhizosphere by stimulating particular microbial population growth and activities.

Fierer and Jackson (2006) reported that bacterial diversity and richness differed by ecosystem type and were to a great extent defined by soil pH. Bacterial diversity was the highest in neutral soils and lower in acidic soils.

Grassland soils possess the richest soil biodiversity. They are usually included in arable crop rotations in order to restore soil carbon levels and soil biodiversity, and for disease-suppressing

services, as well (Garbeva, 2004).

Plant species can influence the diversity and activity of microbial communities in soil (Grigulis et al., 2013). Forest soils are stable (often very old) environments with species richness of soil biota (Hagvar, 1998). Deciduous forest soils have a good aeration and allow ion exchanges, favouring high soil biodiversity. Coniferous forest soils, in contrast, have lower biological activity, since the acidic conditions restrict microbial activity. In coniferous soils fungal population is dominated, with fungal to bacteria biomass ratios reaching 1000:1 (Coleman and Crossley, 1996). It was shown that in tropical dry evergreen forest soils microbial populations were significantly correlated with organic carbon, total nitrogen, extractable P and Ca, soil respiration and  $\beta$ -glucosidase

enzyme activity (Sudhakaran et al., 2014).

Vegetation affects soil respiration, as well. It was shown that the availability of C substrates to microorganisms, plant root density, soil organism population levels, soil physical and chemical properties, soil drainage are among factors influencing respiration rates (Raich et al., 2000).

The aim of this work was to find out how vegetation cover and soil properties influence population density of soil microorganisms and respiration rate of Cambisols from Western Balkan Mountains.

## Materials and methods

The research site was located in Western parts of Balkan Mountains in the region of Iskar Gorge near Lukovo village. Cambisols under prevalent types of vegetation in the explored region were investigated. Samples were collected from surface soil layers (0-20 cm depth) from soils under grassland (former arable land, abandoned 20 years ago), pine forest and beech forest.

Population density of main microbial groups involved in organic residue mineralization and nutrient cycling in soils were determined by plate counts technique (Grudeva et al., 2007). Soil suspension dilutions were placed onto selective agar media and after incubation colony numbers of ammonifying, spore forming, oligonitrophilic, oligotrophic and mineral nitrogen utilizing bacteria, actinomycetes, fungi, and cellulolytic soil microorganisms were determined. Population density was presented as numbers of colony forming units per gram of dry soil (CFU/g).

Basal respiration (BR) was assessed by CO<sub>2</sub> evolution from soil (Alef and Nannipieri, 1998). It was measured after 24-hour incubation of soil wetted up to 60% of water-holding capacity. Respiration rates are presented as mg CO<sub>2</sub>/100 g soil/24h.

Chemical and physical soil properties were determined using standard methods. Soil samples were oven dried at 105°C to determine moisture content. Soil organic carbon (SOC) was determined by the method of Tyurin (Arinushkina, 1975).

Available inorganic nitrogen content (N) was determined by the method of Bremner (1965). Available phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) contents were estimated by acetate-lactate extraction according to the method of Ivanov (1984). Soil pH was measured potentiometrically in soil/water suspension (1:2.5 w/v). Mineral particles content was determined by wet sieving according to the method of Kachinskiy (1958). Data were processed using ANOVA (Duncan's test at 5% significance level). Relations between respiration and soil properties were assessed by correlation analysis.

## Results and discussion

### Soil chemical and physical properties

The Cambisols studied differed in chemical properties. The soil under grassland was characterized by neutral pH, relatively high available phosphorus (P<sub>2</sub>O<sub>5</sub>), available potassium (K<sub>2</sub>O) and organic carbon (SOC) contents. The soil under pine forest had acidic soil reaction, lower phosphorus, potassium and carbon contents and lower moisture level. The soil under the beech forest had slightly acid reaction, medium phosphorus and potassium contents, and relatively high carbon content (table 1).

Particle-size analysis showed no considerable differences in soil texture between soils under the grassland and the beech forest. The soil under pine forest had slightly higher clay content. As a whole, the investigated Cambisols had relatively low clay content (table 2). The physical clay fraction (sum <0.01 mm) varied between 18,5 and 24,9%.

### Soil microbiological properties

Data showed that population density of main groups of microorganisms in Cambisols studied was less than 1.0 x 10<sup>6</sup> CFU/g. An exception was made by ammonifying bacteria in the grassland only (table 3). Amounts of microbial groups differed depending on vegetation cover and soil properties. In this study, numbers of actinomycetes and cellulolytic microorganisms showed no significant differences among samples.

**Table 1.** Chemical properties of Cambisols under different vegetation cover

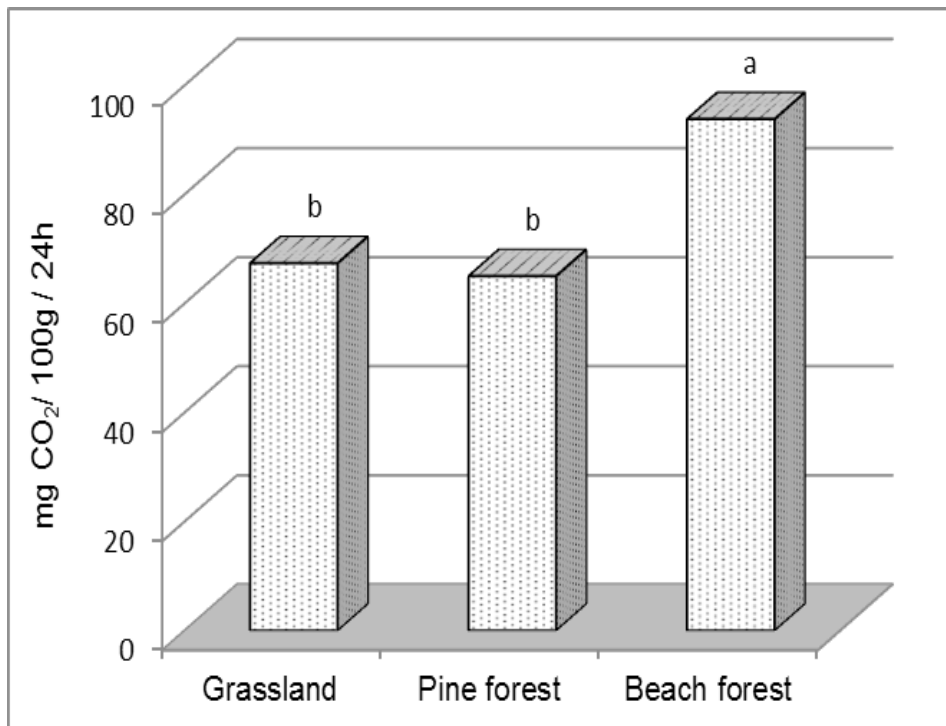
Vegetation cover	pH (H <sub>2</sub> O)	SOC (%)	Available N (mg/kg)	Available P <sub>2</sub> O <sub>5</sub> (mg/100g)	Available K <sub>2</sub> O (mg/100g)	Moisture (%)
Grassland	7.4	7.86	20.2	1.6	25.4	22
Pine forest	5.0	6.40	20.2	0.2	13.0	16
Beech forest	6.5	12.09	23.0	1.0	22.7	22

**Table 2.** Particle-size distribution in Cambisols studied

Vegetation cover	Particle size (mm)							
	Sum > 1	Sand		Silt			Clay	Sum
		1 - 0.25	0.25 - 0.05	0.05 - 0.01	0.01 - 0.005	0.005 - 0.001	< 0.001	< 0.01
(%)								
Grassland	0.0	57.5	8.1	15.9	4.8	4.4	9.3	18.5
Pine forest	0.0	59.7	1.4	14.0	5.0	5.8	14.1	24.9
Beech forest	0.0	61.8	8.1	11.2	6.5	4.7	7.7	18.9

**Table 3.** Microbial population density (CFU/g) x 10<sup>6</sup> of Cambisols from Western Balkan mountains. Different letters show significant differences (5% level) between values in each column

Vegetation cover	Ammonifying bacteria	Spore forming bacteria	Oligonitrophilic bacteria	Oligotrophic bacteria	Actinomycetes	Mineral nitrogen utilizing bacteria	Soil fungi	Cellulolytic microorganisms
Grassland	1.013 a	0.00030 a	0.328 a	0.215 b	0.133 a	0.686 a	0.0020 c	0.000673 a
Pine forest	0.453 b	0.00002 b	0.088 b	0.296 a	0.213 a	0.160 b	0.0186 a	0.000633 a
Beech forest	0.893 a	0.00001 b	0.145 b	0.262 ab	0.200 a	0.540 a	0.0078 b	0.000473 a



**Figure 1.** Basal respiration of Cambisols under different vegetation cover. Significant differences are shown by different letters.

In the soil under grassland, bacterial groups (ammonifying, spore forming, oligonitrophilic and mineral nitrogen utilizing) had the highest amounts, while soil fungi counts were the lowest. In the pine forest, population density of fungi was the biggest, but amounts of ammonifying, oligonitrophilic and mineral nitrogen utilizing bacteria were the smallest and considerably lower than in soils under the grassland and beech forest (table 3). In the beech forest, amounts of ammonifying, oligotrophyc and mineral nitrogen utilizing bacteria were similar to those in the grassland, while fungi numbers were much higher.

Data showed that population density of most of the microbial groups was bigger in the soils under the grassland and beech forest comparing to the soil under pine forest. This is obviously related to the type of vegetation cover, but it is also in relation with higher pH values and higher nutrient contents of those soils (table 1). The greatest root density and root exudates in the grassland contribute to the increment of microbial amounts there.

The lowest amounts of the majority of bacterial groups in the Cambisol under pine forest are in confirmation with other reports (Nustorova, 1995; Kuiters, 1990). It is known that needles contain phenolic compounds that suppress bacterial growth in soils with acidic pH and low clay content. In our study, the Cambisol under pine forest was characterized by acid pH and low nutrient, moisture and clay contents which contributed to lower bacterial growth. As mentioned before, fungi dominate in coniferous soils (Coleman et al., 1996) and this was proved in our study, as well. Soil fungi grew well in Cambisol under the pine forest. High counts of fungi in Cambisols of Serbia were also reported by Marinkovic et al. (2012).

Basal respiration (BR) represent CO<sub>2</sub> evolution by soil biota in which the role of soil microorganisms is dominant. In our study, BR ranged between 64.39 and 93.80 mg CO<sub>2</sub>/100g/24h. The highest value was measured in the soil under beech forest, followed by soils under the grassland and pine forest (Figure 1). It was found that basal respiration was correlated positively with soil organic

carbon content ( $r = 0.990$ ) and negatively with soil clay content ( $r = -0.769$ ).

Soil respiration rate in the pine forest was 31% lower than that of the beech forest which is in confirmation with results of other studies, as summarized by Raich et al. (2000). Authors reported that coniferous forests had 10% lower rates of soil respiration than broad-leaved forests. In regard to grasslands, 29% higher respiration rate than in forests was found. In our study, soil respiration rate in the grassland was similar to the one in the pine forest, and it was 28% lower than that in the beech forest. Such inconformity could be explained by special features of the grass vegetation, soil and the relief, microclimate etc., since numerous factors can influence soil respiration. We need additional examination of other soils and locations to ascertain the finding.

## Conclusion

Vegetation cover and soil properties considerably influenced microbial population amount and respiration of Cambisols from Western Balkan Mountains.

In general, the soil under grassland was characterized by higher microbial population density followed by soils under beech forest and pine forest. Populations of most bacterial groups (ammonifying, spore forming, oligonitrophilic and mineral nitrogen utilizing) prevailed in the soil under grassland. Amounts of fungi were the biggest in the soil under pine forest.

Basal soil respiration was the highest under the beech forest and decreased in the grassland and in the pine forest. It was positively correlated with soil organic carbon and negatively correlated with soil clay content.

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