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Microbiological Parameters of Technosols Monitored for Hydrophobicity

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Abstract

Soil hydrophobicity causes reduced water infiltration rate and has a negative impact on plant growth. Reports on hydrophobicity of Technosols are limited, and in Bulgaria studies have been initiated only recently. The present work aimed to monitor two Technosols (non-vegetated and afforested with *Pinus nigra*) located near Obruchishte (Maritsa-Iztok coal mines) for hydrophobicity level and to assess their microbiological status. In total, 24 soil samples from 12 sampling points and two soil depths (0-10 cm and 10-20 cm) were analyzed for hydrophobicity, moisture content, numbers of cultivable microorganisms, basal respiration and microbial biomass carbon. The hydrophobicity was measured by water-drop-penetration-time (WDPT) test. Microbial numbers were determined by plate counts technique. Sample incubation in closed vials was used to determine basal respiration and microbial biomass carbon. Among the studied samples, 42% possessed severe hydrophobicity, 37% were strongly hydrophobic and 21% were non-hydrophobic (hydrophilic). Both soils were characterized with low numbers of bacteria, actinomycetes and fungi (10^2 CFU/g), and low levels of basal respiration rate (0.13-6.54 mg CO_2-C/100g/24h) and microbial biomass carbon (1.57-18.86 mg C/100g). Values widely differed among sampling points and layer depths because of the high heterogeneity of the soil substratum. The hydrophobic samples contained a relatively higher amount of saprotrophic fungi than hydrophilic ones.

Keywords: hydrophobicity, Technosols, microbial numbers, basal soil respiration, microbial biomass carbon

Резюме

Почвената хидрофобност причинява намалена способност за инфилтриране на водата и оказва негативно влияние върху развитието на растенията. Публикациите във връзка с хидрофобните свойства на техногенни почви (Technosols) са ограничени, а в България изследванията по този въпрос започнаха неотдавна. Настоящата работа има за цел мониторинг на две техногенни почви (незалесена и залесена с черен бор, *Pinus nigra*) за хидрофобност и оценка на микробиологичния им статус. Почвите са разположени в близост до с.Обручище в района на въгледобивния басейн Марица-Изток. Анализирани са 24 почвени проби, взети от два слоя (0-10 см и 10-20 см) в 12 точки на пробовземане. Определени са нивото на хидрофобност, почвената влага, числеността на основните групи почвени микроорганизми, общата микробиологична активност (почвено дишане) и микробиалния въглерод. Почвената хидрофобност е определена чрез измерване на времето, необходимо за проникване на водната капка (WDPT, s) в почвата. Числеността на микроорганизмите е отчетена върху агарови среди. Почвеното дишане и въглерода в микробиалната биомаса са определени чрез инкубация в затворени съдове. Установено е, че 42% от почвените проби са екстремно хидрофобни, 37% са силно хидрофобни и 21% са хидрофилни. Двете почви се характеризират с ниска численост на бактериите, актиномицетите и плесенните гъби (от порядъка на 10^2 CFU/g), и ниски нива на микробиологична активност (0.13-6.54 mg CO_2-C/100g/24h) и микробиален въглерод (1.57-18.86 mg C/100g). Стойностите на тези показатели варират в широки граници в зависимост от точките на пробовземане и почвения слой поради високата хетерогенност на почвения субстрат. В хидрофобните проби относителния дял на плесенните гъби е по-висок в сравнение с хидрофилните почвени проби.

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Introduction

Soil hydrophobicity (soil water repellency) is a reduced water retention of soils (Doerr *et al.*, 2000). The negative impact on water infiltration leads to soil erosion, nutrient loss and decrease in plant growth and crop production. Hydrophobic properties are found in different soil types - sandy, loam, clay, peat and volcanic ash soils (Dekker *et al.*, 2005) but little information is available about hydrophobic Technosols and especially on their microbiological parameters. In Bulgaria those studies have been initiated recently.

In a recent study (Nedyalkova *et al.*, 2018), pioneer information on the level of hydrophobicity in the spring and microbial properties of samples of hydrophobic Technosols from the area of Maritsa-Iztok coal mines was reported. It is known that hydrophobicity, in general, is strongly dependent on the soil moisture and often is reversible (Doerr *et al.*, 2000). We intended to check the hydrophobicity level of the same soils in the hot (summer) season when shifts in soil hydrophobicity status are expected.

Among microbiological parameters, basal soil respiration and microbial biomass carbon content were widely used in ecological studies of different soil types (Nannipieri *et al.*, 1990; Alef, 1995) and were successfully applied for reclaimed mine soils (Ingram *et al.*, 2005).

The aim of the study was to monitor two Technosols for hydrophobicity level in the summer season and to assess soil microbiological status.

Material and Methods

Two Technosols located near the village of Obruchishte, Maritsa–Iztok coal mines (Bulgaria) were investigated for hydrophobicity level. They consisted of loam-textured Pliocene overburden sediments compiled more than 30 years ago during open-cast lignite mining activities and later were subjected to reclamation with coal ash.

Samples from a non-vegetated site and an afforested with pine trees (*Pinus nigra)* site, situated at about 30 m from each other, were collected in the summer (end of July) of 2017. At each site, 6 sampling points were chosen and samples from two layers - 0- (5)10 cm and 10-20 cm, were taken using a core sampler (3 cm-wide and 25 cm-long). In total, 24 soil samples (as listed in Table 1) were analyzed for hydrophobicity, moisture content, microbial amount, basal soil respiration and microbial biomass carbon content. All measurements were made in triplicates.

Soil hydrophobicity was assessed by water-drop-penetration-time (WDPT) test. Three water drops were placed onto the sample surface and the time for their complete infiltration into soil was recorded. The median value of the triplicate time was considered as hydrophobicity value of a sample. According to WDPT, samples were classified in the following classes (De Bano, 1981): non-hydrophobic or hydrophilic (WDPT<5 s), strongly hydrophobic (5<WDPT<600 s), and severely hydrophobic (WDPT>600 s). Soil moisture content was calculated after drying the samples at 105°C.

The amount of the main groups of cultivable microorganisms was determined by plate count technique. Ten-fold serial dilutions of samples were used to inoculate soil suspension on selective agar media. Soil bacteria were cultivated on Nutrient broth agar, actinomycetes – on starch-ammonium agar (Hutchinson's) medium and saprotrophic fungi – on Czapek's agar medium (Grudeva *et al.*, 2007). After incubation at 28°C, microbial colonies were counted and results were calculated as colony-forming units per gram of absolutely dry soil (CFU/g).

The respiration was measured in the laboratory after roots and macrofauna were removed from the samples, thus the CO_2 evolution rate (basal respiration) represented the total microbial activity in the soil (Nannipieri *et al.*, 1990). The soil was sieved (2 mm sieve), then fine roots were taken out and the samples were adjusted to 60% (w/w) moisture content. Basal soil respiration was determined in tightly closed vials after 24 h -incubation, as described by Alef (1995). After that samples were amended with glucose and incubated for another 4 hours at 22°C to determine microbial biomass carbon (C_{mic}). The CO_2 evolved was determined by titration. Microbial carbon was calculated according to the equation proposed by Anderson and Domsch (1978).

Mean values were compared by the least significant differences (LSD) at $p \leq 0.05$ under ANOVA. Correlation analyses were used to examine relations between the parameters.

Results

Among the studied samples, 42% possessed severe hydrophobicity, 37% were strongly hydrophobic and 21% were non-hydrophobic (hydrophilic). All but one samples from the non-vegetated soil were hydrophobic. Eight of the samples from *Pinus nigra* vegetated Technosols were severely and strongly hydrophobic and the remaining four samples were hydrophilic (Table 1).

Soil moisture ranged from 9.29 to 21.95 % in the non-vegetated soil, and from 13.64 to 31.58% in *Pinus nigra* vegetated soil. No correlation between moisture content and WDPT of samples was found.

In non-vegetated Technosol, bacteria numbers widely varied (0.07 to 6.04 CFU/g x 10^2) among soil layers of different hydrophobicity. The amount of actinomycetes (0 - 0.21 CFU/g x 10^2) was too low or absent in some sampling points. Saprotrophic fungi numbers (0.26 - 0.61 CFU/g x 10^2) did not change substantially among samples (Table 1).

In the *Pinus nigra* vegetated Technosol bacteria numbered between 0.19 - 12.33 CFU/g x 10^2, actinomycetes increased to 0 - 3,2 CFU/g x 10^2, and fungi reached 0.48 – 3.5 CFU/g x 10^2 considering both soil layers (Table 1).

The relative amount of bacteria, actinomycetes and fungi in all hydrophilic and all hydrophobic samples of both soils are shown in Fig. 1.

In non-vegetated soil, CO_2 production ranged from 1.51 to 6.54 mg CO_2-C/100g/24h in 0-10 cm layer and between 0.75-5.52 mg CO_2-C/100g/24h in 10-20 cm layer. In *Pinus nigra* vegetated soil, CO_2 values varied between 0.14-3.67 mg CO_2-C/100g/24h in top layer and between 0.13-2.21 mg CO_2-C/100g/24h in the lower layer. Mean values are presented in Fig.2.

Table 1. Hydrophobicity level of Technosol samples according the water-drop-penetration-time (WDPT), soil moisture content and microbial counts (CFU/g) in distinct sampling points. Values in columns followed by similar letters are not significantly different at $p<0.05$

№	Soil layer depth	Soil moisture (%)	WDPT (s)	Hydrophobicity level	Bacteria	Actinomycetes	Saprotrophic fungi
					(CFU/g) x 10^2		
Non-vegetated Technosol							
1	0-10 cm	18.34	205	strong	0.69 e	0.08 c	0.32 de
	10-20 cm	16.28	4	hydrophilic	0.48 e	0.14 b	0.25 e
2	0-10 cm	14.94	8895	severe	0.49 e	0.11 bc	0.37 cde
	10-20 cm	15.07	795	severe	3.19 c	0.21 a	0.47 bcd
3	0-10 cm	11.73	20	strong	1.63 d	0 e	0.26 e
	10-20 cm	21.95	323	strong	0.40 e	0.02 de	0.25 e
4	0-10 cm	11.11	17	strong	2.13 d	0 e	0.31 de
	10-20 cm	14.29	87	strong	0.53 e	0 e	0.61 ab
5	0-10 cm	9.89	9900	severe	6.04 a	0 e	0.37 cde
	10-20 cm	9.29	16	strong	4.10 b	0 e	0.65 a
6	0-10 cm	12.36	7820	severe	0.07 e	0.07 cd	0.26 e
	10-20 cm	13.64	3880	severe	0.73 e	0.07 cd	0.50 abc
Pinus nigra vegetated Technosol							
7	0-5 cm	23.46	4520	severe	5.67 c	1.00 d	2.25 b
	10-20 cm	31.58	21	strong	1.48 d	0.15 e	0.88 ef
8	0-5 cm	13.64	11510	severe	0.80 de	0.11 e	2.01 b
	10-20 cm	28.21	11510	severe	0.57 de	0.05 e	1.51 c
9	0-5 cm	22.10	34	strong	6.93 b	3.20 a	3.50 a
	10-20 cm	29.03	1	hydrophilic	5.60 c	1.87 c	1.41 cd
10	0-5 cm	14.94	11058	severe	0.46 de	0.09 e	1.10 ef
	10-20 cm	20.48	1	hydrophilic	0.17 e	0 e	0.48 g
11	0-5 cm	19.76	8	strong	12.00 a	2.27 bc	1.13 de
	10-20 cm	24.22	7102	severe	0.19 e	0.01 e	0.70 fg
12	0-5 cm	14.94	1	hydrophilic	12.33 a	3.00 ab	2.05 b
	10-20 cm	19.62	0	hydrophilic	5.27 c	1.80 c	2.16 b

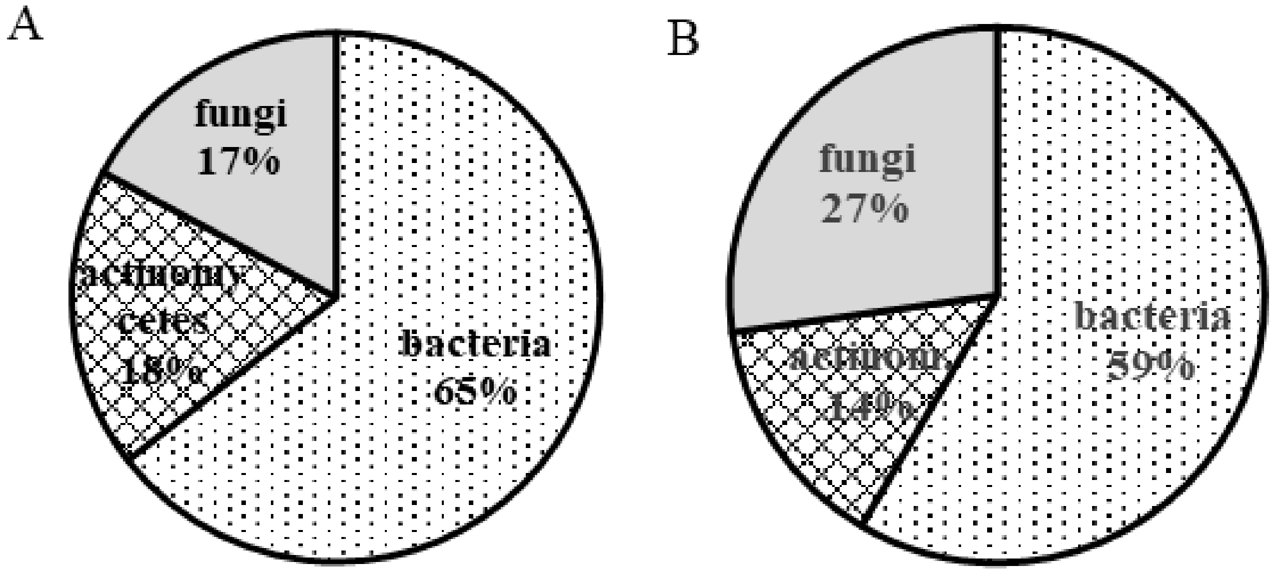

Fig. 1. Relative amounts of bacteria, actinomycetes and saprotrophic fungi in hydrophilic (A) and hydrophobic (B) soil layers

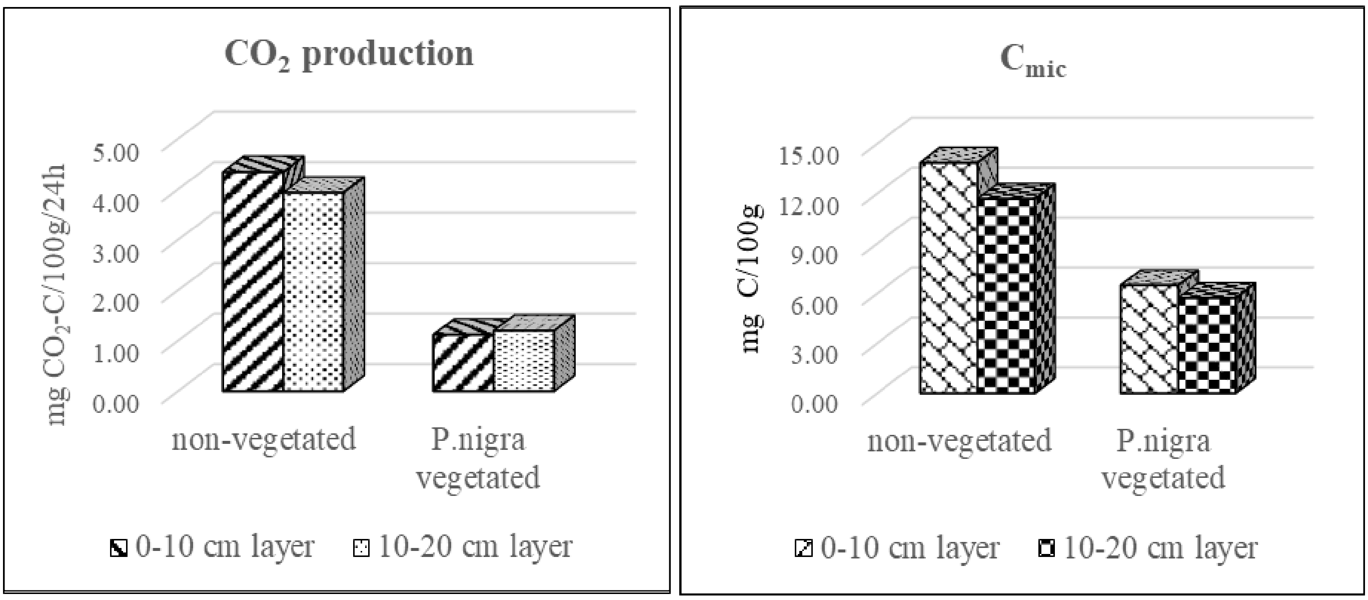

Fig. 2. Mean values of basal soil respiration (CO_2 production) and microbial biomass carbon (C_{mic}) in different soil layers of non-vegetated and *Pinus nigra* vegetated hydrophobic Technosols

Microbial biomass carbon (C_{mic}) content varied between 8.18-18.86 mg C/100g for 0-10 cm layer, and between 3.17-14.98 in 10-20 cm layer of non-vegetated soil. In the vegetated soil C_{mic} values of 1.67-14.38 mg C/100g in 0-10 cm layer and 1.57-14.38 mg C/100g in 10-20 cm layer were measured. Figure 2 shows the average values for both layers of each soil.

There was no significant correlation between hydrophobicity (WDPT) and CO_2 production, microbial biomass carbon or microbial numbers for all samples tested.

Discussion

The majority of soil samples studied possessed hydrophobic properties. Most of the samples from non-vegetated soil possessed strong and severe level of hydrophobicity. The highest WDPT values were registered in the *Pinus nigra* vegetated soil. This is in agreement with other studies in which hydrophobicity is associated with evergreen tree types (Doerr *et al*., 2000). Differences in WDPT values are attributed to the heterogenic composition of the samples and confirm the spatial variability of water repellency of lignitic mine soils reported by Gerke *et al*. (2001).

Comparing hydrophobicity levels of the spring (Nedyalkova *et al*., 2018) and summer (this study) soil samples, it could be pointed out that hydrophobicity decreased in 13 out of 24 samples at both plots.

Microbial number in the Technosols studied was, in general, very low. Values differed among sampling points and layer depths because of the high heterogeneity of the substrata. A decreasing trend of bacteria and actinomycetes numbers with increasing the WDPT values was noticed. Microbial counts in the *Pinus nigra* vegetated Technosol were higher than those in the non-vegetated soil which was, obviously, due to the influence of vegetation. It is well known that root exudates provide available nutrients for microorganisms and support microbial growth. In addition, the higher soil moisture content of the vegetated soil stimulated microbial growth.

The increase in the relative amount of fungi in all hydrophobic samples compared to the hydrophilic samples pointed to some relation of fungi with hydrophobicity but the relationship was not significant.

Values of basal respiration and microbial biomass carbon were low and due to sample heterogeneity both parameters varied widely. Vanhala *et al*. (2005) also pointed out the impact of heterogeneous soil environment in variations of basal soil respiration rate. In this study, the average soil respiration and C_{mic} values were higher in the non-vegetated soil and in the summer samples as compared to the spring samples (Nedyalkova *et al*., 2018). This could be explained by the higher temperature and hygroscopic moisture, both stimulating organic carbon mineralization in the July samples taken shortly after drizzle in the area.

Conclusion

The Technosols located near Obruchishte (Maritsa-Iztok coal mines) possessed hydrophobic properties. Most of the samples collected from non-vegetated and *Pinus nigra* vegetated soils studied were classified as strongly and severely hydrophobic. The highest level of hydrophobicity was measured in the soil under *Pinus nigra* trees. A trend of lowering of hydrophobicity level in the summer samples from both Technosols compared to the spring samples was noticed.

In general, both soils were characterized with low microbial counts, low basal respiration rate and microbial biomass carbon. Values widely differed among sampling points and layer depths because of the high heterogeneity of the substrata. The relative amount of saprotrophic fungi was higher in the hydrophobic samples than in the hydrophilic ones.

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