Relationships Between Microbial Parameters and Soil Properties in a Hydrophobic Spolic Technosol

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Abstract

Data on microbiology of water repellent (hydrophobic) soils in Bulgaria are scarce. This work aimed to find out relations between microbiological indicators, hydrophobicity level and other soil properties in a Spolic Technosol. Samples from 0-10 cm and 10-20 cm soil depth of a fly ash reclaimed soil covered with tuft vegetation and located near Obruchiste area were collected. Main microbiological (basal respiration - BR, microbial biomass carbon content -Cmic, amount of main microbial groups –CFU), chemical (organic carbon, available N, P and K contents) and physical (hydrophobicity level, moisture, sand and clay contents) properties were determined. It was found that the Technosol was hydrophobic in most of the monitoring points. Half of the samples were severely or extremely hydrophobic (assessed by water-drop-penetration time) and the hydrophobicity level was higher in the subsurface layer. The Spolic Techosol under tuft vegetation was characterized with low numbers of heterotrophic bacteria, actinomycetes and oligotrophic microorganisms (1-40 x 10^2 CFU/g) at 0-20 cm depth. As a whole, low BR rate (0.51-5.43 mg CO_2/g/ 24h) and low Cmic values (2.37-18.18 mg C/100g) were established for both layers. Weak relationships of microbial parameters with organic carbon, humic acid, fulvic acid and clay contents were noticed in this soil. Significant correlations of the amount of fungi and actinomycetes with available nutrient and moisture contents were found.

In general, a trend of positive relationships of fungi, actinomycetes and cellulolytic microorganisms with soil hydrophobicity was established. The relative parts of fungi and actinomycetes increased in the hydrophobic samples comparing to non-hydrophobic ones. Microbial biomass carbon tended to increase with increasing the hydrophobicity level. Data pointed on close positive relationships of microbial parameters studied with the hydrophobicity in this Technosol.

Our results add new information to the monitoring study of hydrophobic Technosols from Maritsa-Iztok coal-mining region (Bulgaria).

Key words: soil hydrophobicity, Spolic Technosol, basal respiration, microbial biomass, microbial amount

Introduction

Hydrophobic (water repellent) soils are characterized by reduced water retention and infiltration in depth. High hydrophobicity may lead to increased soil erosion and nutrient loss and may cause negative impact on plant growth and crop production (Doerr et al., 2000).

Scientific literature indicates complex interactions of water repellency with soil properties. In most cases soil organic matter, and especially its quality, is related to the formation of hydrophobic properties (Buczko et al., 2005; Buczko et al., 2006; Mataix-Solera, et al., 2007). Among large numbers of chemical and biological factors, Lozano et al. (2013) revealed the extractable lipid fraction of soil organic matter as a principal factor for hydrophobicity in forest soils. These authors found that fungal biomass was related to soil organic matter content which led to the suggestion that water repellency under *Pinus* trees could be mostly influenced by fungi.

Microorganisms contribute to organic matter formation in soils and their possible role as a factor in hydrophobicity development received a lot of attention. Relations between hydrophobicity and microbial soil properties have been investigated for many years. Evidence for contribution of fungi (Rillig, 2005; Lozano et al., 2013) actinomycetes (Lozano et al., 2014) and bacteria (Morales et al., 2010) to soil water repellency were reported. Studies showed that hydrophobicity was caused by hydrophobic organic compounds originated mainly from plants and microorganisms (Atanassova, Doerr, 2010; Atanassova et al., 2014; Atanassova, Doerr, 2015; Mao et al., 2014) and produced during residue decomposition or as a response to water stress conditions (Morales et al., 2010). Other microbial mechanisms contributing to hydrophobicity formation could be the production of hydrophobic substances and a reduction of soil porosity by fungal hyphae, gaseous and other by-products released in the process of residue mineralization (Morales et al., 2010).

In the first studies, water repellency was mostly associated with soil fungi which take part in organic matter decomposition (DeBano, 2000). Recently, a strong dependence of hydrophobicity with microbial community composition has been suggested (Lozano et al., 2014). DeBano (2000) revealed a connection with microbial hydrophobic exudates. Bodi et al. (2013) linked variation of hydrophobicity to the spatial variability of organic matter content and fungal biomass. In some studies, strong correlation between fungal biomass (ergosterol) and the level of hydrophobicity in agricultural soils (Feeney et al., 2006) was found.

Microbial effects in water repellency of different soils are still not clear (Zheng et al., 2016). Regarding Technosols, few studies on relationships between hydrophobicity, microorganisms and lipids are available (Petkova et al., 2017; Atanassova et al., 2017; Nedyalkova et al., 2018; Atanassova et al., 2020). The present study aimed to find out relations of microbiological indicators with hydrophobicity level and other soil properties in a Spolic Technosol under tuft vegetation.

Materials and Methods

The experimental site was located in Maritza-Iztok open cast coal mine region at the Obruchishte-3 spoil created more than 30 years ago. The plot consisted of loam Pliocene overburden sediments (yellowish-green and greyish-green clays) containing irregularly distributed coal particles. The soil is classified as Spolic Technosol (IUSS Working Group

WRB, 2015). To ameliorate soil acidity fly ash (obtained after coal incineration of in the thermal power station) had been added to the clay materials. The surface was covered by tuft vegetation. A grid of Δ^2m of ~ 40m^2 was constructed and samples at six points were taken from 0-10 and 10-20 cm depth using a 3 cm wide and 25 cm long core sampler.

Soil hydrophobicity (water repellency) was determined by measuring the time for water drop penetration (WDPT) according to Doer et al. (2002) and the level of hydrophobicity was classified as: wettable or hydrophilic – (<5s), slight (5-60 s), strong (60-600s), severe (600-3600s) and extreme (>3600s) (Dekker, Ritsema, 1996).

The amount of microorganisms was determined by plate counts technique on selective agar media. Heterotrophic bacteria were cultivated on Nutrient broth agar, actinomycetes – on starch-ammonium agar medium, saprotrophic fungi – on Czapek's agar medium, cellulolytic microorganisms – on Hutchinson's medium, oligotrophic microorganisms – on 1/100 diluted Nutrient broth (Grudeva et al., 2007). Microbial colonies formed were counted after incubation at 28°C and data were presented as colony-forming units per gram of absolutely dry soil (CFU/g).

Basal respiration of soil (CO_2 evolution) was measured in the laboratory after sieving (2 mm mesh) and removing roots and residues from the samples. The samples were adjusted to 60% moisture content and incubated 24 h at 25°C in tightly closed vials, following the procedure of Alef (1995). After basal respiration (BR) measurement, glucose was added and samples were incubated for another 4 hours at 22^{o}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, Domcsh (1978).

Soil chemical properties - total organic carbon (TOC), humic acids (HA), fulvic acids (FA), available nitrogen (N), phosphorus (P) and potassium (K) contents, and physical properties – hydrophobicity level (WDPT), moisture, sand and clay contents, were determined by conventional methods as described in Atanassova et al. (2018). Here we report their relations with microbial parameters only.

To examine relationships between microbial parameters and soil properties Pearson correlation coefficients were calculated. Multiple comparison between means was made by one-way ANOVA followed by Games-Howell post hoc test for unequal variances ($P<0.05$). Data were processed using SPSS 22 for MS Windows.

Results and Discussion

Soil hydrophobicity level in monitoring points differed among samples and between soil depths. Two of the samples in the surface layer were hydrophilic, three samples possessed slight hydrophobicity, two samples were strongly hydrophobic, one was severely hydrophobic and four samples possessed extreme hydrophobicity. WDPT values ranged between 8-11636 s for both layers. In general, higher hydrophobicity level in the 10-20 cm layer was found (Fig. 1).

Data on spatial variation of soil hydrophobicity in non-vegetated and *Pinus nigra* afforested Technosols from Maritza-Iztok mining region were reported in the study of Ivanov et al. (2019) in which extreme hydrophobicity was registered in the surface 0-10 cm layer of the soil under *Pinus* trees. The hydrophobicity variation in Spolic Technosols was related to the heterogenic composition of the soil and to randomly distributed coal particles and ashes in different layers (Atanassova et al., 2018). Similarly, variations in hydrophobicity (water repellency) for reclaimed lignite mine soils (Atanassova et al., 2017; Gerke et al., 2001) and for forest soils (Lozano et al., 2013) were reported.

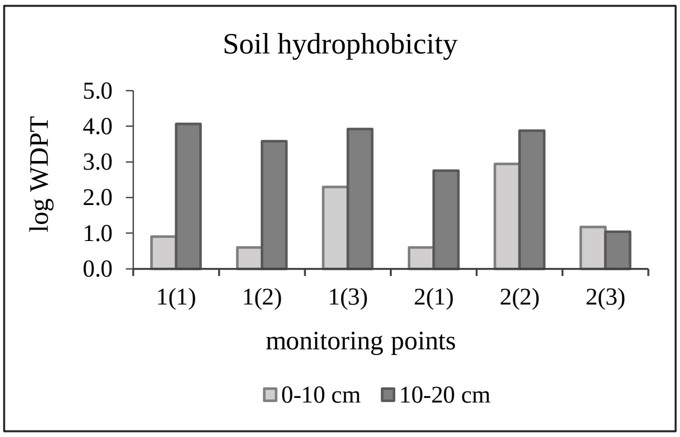

Fig. 1. *Soil hydrophobicity variation among monitoring points and soil depth in the Spolic Technosol from Obruchishte area in the Maritsa-Iztok coal mining region.*

The heterogenic properties of the Technosol determined variations in microbial parameters. The amount of microbial groups differed depending on sampling points and soil depth. For both soil layers, microbial numbers varied as follows: heterotrophic bacteria - 2.8-18.60 x 10^2 CFU/g, actinomycetes - 0.53-17.87 x10^2 CFU/g, saprotrophic fungi - 4.5-24.6 x 10^2 CFU/g, cellulolytic microorganisms - 12.53-37.87 x 10^2 CFU/g and oligotrophic microorganisms - 2.07-34.87 x 10^2 CFU/g (Fig. 2).

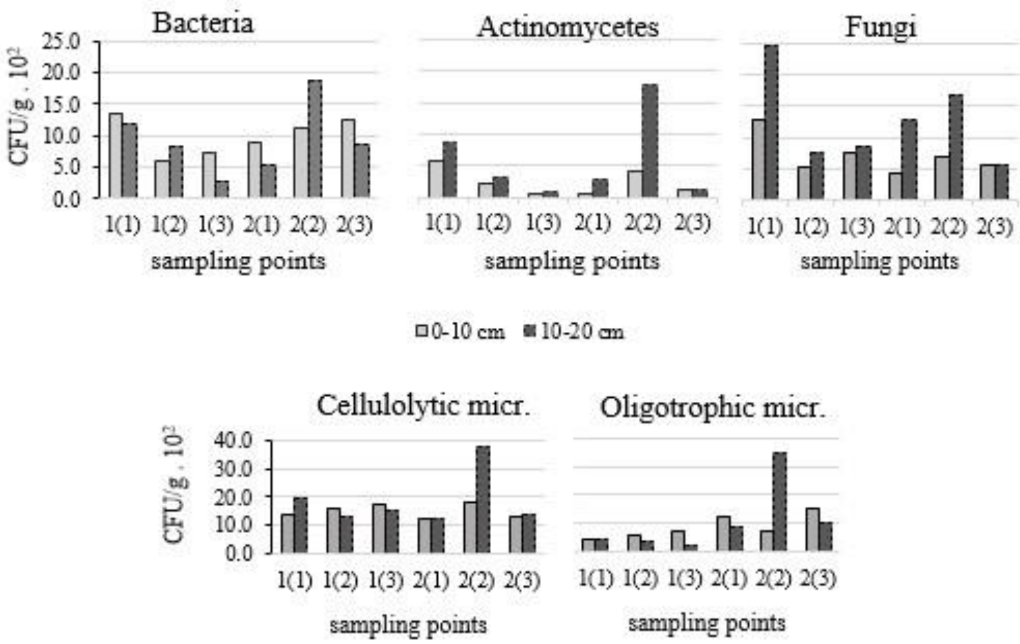

Fig. 2. *Amount of microbial groups studied in different monitoring points (0-10 cm and 10-20 cm depth) in the Spolic Technosol.*

In general, microbial amount in the Technosol was low comparing to native soils where up to 10^4-10^8 CFU/g could be found. Unfavorable physical properties and the scarce vegetation (tufts) of this soil are among reasons for the low microbial population size.

Fungi and actinomycetes had higher numbers in the 10-20 cm layer (Fig.2) in which higher hydrophobicity levels (Fig. 1) were detected, as well. The relative share of fungi and actinomycetes was higher in water repellent (hydrophobic) soil samples of both layers comparing to non- hydrophobic samples (Fig. 3). Data suggested close relationships of those microbial groups with soil hydrophobicity. In the scientific literature, fungi, actinomycetes and bacteria are often associated with hydrophobic soil properties (Rillig, 2005; Morales et al., 2010; Lozano et al., 2014). It is proved that microorganisms are able to produce hydrophobic substances as survival mechanism in response to recurring water stress (Morales et al., 2010). These substances (hydrophobins) coat soil particles and reduce soil porosity thus inducing low water retention and preventing water flow into the soil.

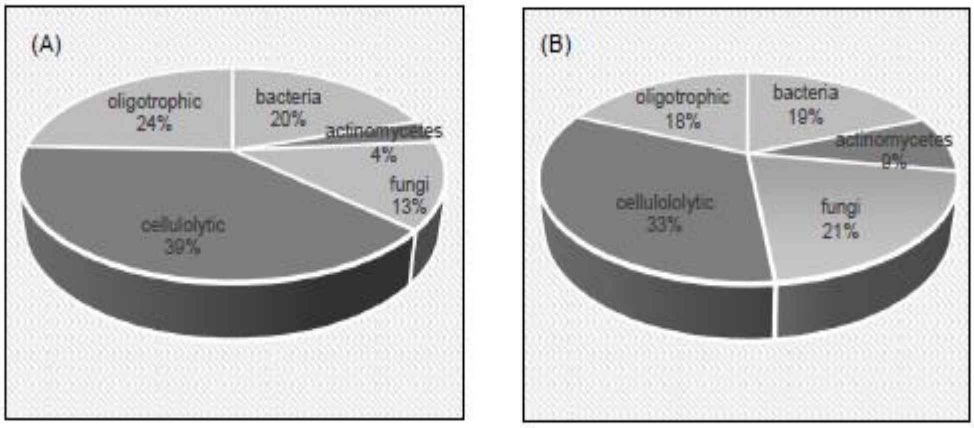

Fig. 3. *Relative part of microbial groups in non-hydrophobic (A) and water repellent (hydrophobic) (B) samples in both layers (0-20 cm depth) of the Spolic Technosol.*

Similarly to microbial numbers, low basal respiration and microbial biomass carbon values were determined in the Technosol. Basal respiration rate ranged from 0.51 to 5.43 mg CO_2/100g/24h. Microbial biomass carbon varied between 2.37 and 14.18 mg C/100g. As a whole, higher respiration and microbial biomass values were registered in hydrophobic samples comparing to hydrophilic ones (Fig. 4). Data obtained confirm findings for another Technosol from this area (Nedyalkova et al., 2018).

There was an increasing trend of microbial biomass carbon with increasing the hydrophobicity level. Some authors have found positive correlation between fungal biomass (ergosterol) and soil hydrophobicity (Young et al., 2012) but others obtained no clear trends for relationships between them (Feeney et al., 2006). Obviously, other factors (SOM, particle size distribution, water content) also influence those relationships and the causes of soil hydrophobic properties are more complex (Zheng et al., 2016).

Significant linear relationships for some microbial parameters and soil properties were determined. For 0-10 cm layer, cellulolytic microorganisms were significantly positively correlated with WDPT (r=0.975*). For 10-20 cm layer, very high positive correlation between fungi and actinomycetes (r=0.975*) and negative correlation of actinomycetes with moisture content (r=-0.960*) were detected.

Considering all samples in both layers, a trend of positive relationships with WDPT for fungi (r=0.746), actinomycetes (r=0.541) and cellulolytic microorganisms (r=0.691) was found. The numbers of actinomycetes (r=0.866**) and fungi (r=0.798*) were significantly positively correlated with available phosphorus content and between each other (r=0.937**). Negative corelations of actinomycetes with available K (r= -0.738*) and moisture (r= -0.777*) contents were registered. Results obtained pointed that fungi and actinomycetes were highly influenced by soil properties in this study.

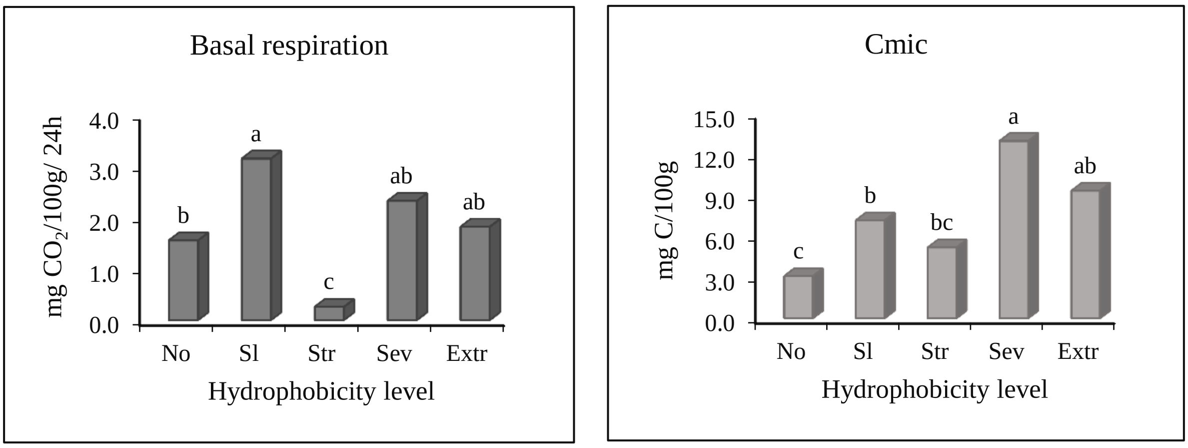

Fig. 4. *Basal respiration rate (CO_2/sample) and microbial biomass carbon content (Cmic/sample) in different hydrophobicity level samples (No - non-hydrophobic; hydrophobic: Sl- slight, Str- strong, Sev- severe, Extr –extreme) in the Spolic Technosol. Different letters above columns show significant differences between values.*

Basal respiration was in positive correlation with oligotrophic microorganisms (r=0.981** for 0-10 cm layer, r=0.628 for both layers). Weak relationships of microbial parameters with organic carbon, humic acids, fulvic acids and clay contents were noticed in the reclaimed soil.

Conclusion

Most of the samples from the Spolic Technosol under tuft vegetation were hydrophobic (water repellent) and half of them possessed severe or extreme hydrophobicity. Higher hydrophobicity level in the 10-20 cm layer was registered. Microbial parameters studied were in close positive relationships with the hydrophobicity in this soil.

The Spolic Technosol was characterized with low levels of microbial numbers, basal respiration and microbial biomass carbon. The microbial parameters had weak relationships with organic carbon, humic acid, fulvic acid and clay contents due to the heterogenic properties of the reclaimed soil. The amount of fungi and actinomycetes was significantly correlated with nutrient and moisture contents.

Our results add new finding to the monitoring of hydrophobicity in coal and fly ash reclaimed Technosols from the region of the largest coal mine producing complex in South Eastern Europe (Maritsa-Iztok coal mines, Bulgaria).

Acknowledgments

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