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Microbiome status and determinants in soils from the region of Maritsa-Iztok coal mine (Bulgaria) I. Natural soils – Smolnitsas (Pellic Vertisols)

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Abstract

Tsolova, V., Nedyalkova, K., Kolchakov, V. & Tomov, P. (2021). Microbiome status and determinants in soils from the region of Maritsa-Iztok coal mine (Bulgaria). I. Natural soils – Smolnitsas (Pellic Vertisols). *Bulg. J. Agric. Sci., 27 (4)*, 719–726

Soil microorganisms rapidly respond to anthropogenic changes and are useful indicators for perturbations in the soil matrix or the reclamation effect on mine spoils. This paper aimed to establish the microbiome status and determinants in natural and reclaimed soils affected by pyrogenic carbon emissions in the area of Maritsa-Iztok lignite basin and particularly to reveal the response of microorganisms to such anthropogenic influence. Pyrogenic carbon (PyC) is formed during the combustion of fossil fuels or vegetation and has multiple negative impacts on climate, public health and environment. Nowadays its influence on biological and chemical processes in soils is of paramount importance in view of the amplification of climate changes.

In this part of the study two typical for the region natural soils Smolnitsa type (Pellic Vertisol) were analysed. The first soil (profile 1) is situated far from the mining region and hence was chosen for a referent soil. Samples from genetic horizons of soils, as well as from the surface horizon of vulnerable soil (located between the two sources of PyC) were collected by pit trial technique and monitoring approach on 2-th of June 2018.

Data obtained showed that due to the inherent three dimensional variations of soil properties, the different crops and cultivation manner, microbial features (microbial biomass carbon and population density) of natural Pellic Vertisols predominately varied in depth but followed the typical for native soils trend of decreasing amounts down the profiles. In both soils, heterotrophic, oligotrophic and oligonitrophilic bacteria were the most numerous groups but in the deepest horizons only oligotrophs dominated. The oligotroph-pedoturbation observed in $A'''_{к}$ and AC_{k} horizons as an atypical population decline is a specific feature of studied Smolnitsas. Due to the high spatial variation of oligonitrophilic bacteria density this group could be used as bioindicator of soil anthropogenization including pyrogenic carbon (PyC) accumulation. Data suggests that additional inputs of PyC in soils surrounding coalfield could decouple SOM and PyC, supress microbiome synergy and dependency on PyC. Only some nutrients (organic carbon, phosphorus, potassium and easily mobile humic and fulvic acids) determined the microbial growth and density in studied soils while soil moisture, depth, carbonates and clay contents were not significant factors. Available nitrogen positively influenced microbiome status only in control soil with exception of oligotrophs for which soil pH was determinative. PH is not a microbial determinant in natural soils beyond the mentioned exception.

Keywords: Pellic Vertisols; microbial biomass; microbial density; pyrogenic carbon; soil nutrients

Introduction

Microorganisms are active agents in biochemical transformations in soils and play important role in many soil processes. They mediate vital ecosystem functions such as residue decomposition, transformation of nutrients, decontamination, energy transfer, soil aggregate formation, etc. They rapidly respond to changes in soil matrix due to anthropogenic interventions (Chodak & Niklińska, 2010).

Microbiological parameters have been used as indicators

of the intensity of biochemical transformation of organic matter in soils. Microbial biomass carbon content is valuable characteristic since it represents the living microbial component that is sensitive to changes in soil environment (Schloter et al., 2003). The amount of the microbial biomass is related to nutrient availability and fertility of soils. It constitutes 1-3 % of soil organic carbon but is the most labile carbon pool in the soil. It is easily available nutrient that has beneficial effect on plant growth (Moore et al., 2000). Population density of different microbial groups is another soil characteristic representing the living members of the community. It provides useful information about stages in soil formation, residue mineralization, particular soil properties and relations with other soil ingredients.

The characteristics and structure of microbiological populations in natural soils have changed long and simultaneously with the soil matrix and have reached a level of high stability and activity. That's why natural soils are commonly used as a reference in assessment of anthropogenic impact on soils or reclamation effect on mine spoils (Haney et al., 2008; Tsolova et al., 2014; Quadros et al., 2016).

This paper aimed to establish the microbiome status and determinants in natural and reclaimed soils affected by pyrogenic carbon emissions in the area of Maritsa-Iztok lignite basin and particularly to reveal the response of microorganisms to such anthropogenic influence. Pyrogenic carbon (PyC) forms during the combustion of fossil fuels or vegetation and has multiple negative impacts on climate, public health and environment (UNEP/WMO, 2011; U.S. EPA, 2012; Arctic Council, 2013; Schmidt & Noack, 2000). Nowadays its influence on biological and chemical processes in soils is of paramount importance in view of the amplification of climate changes.

In this part of the study, data on natural Smolnitsas (Pellic Vertisols) located outside and close to Maritsa-Iztok industrial complex are presented.

Materials and Methods

Description of studied sites

Smolnitsa is a basic soil type in the studied region and can provide knowledge about the most typical and ubiquitous model of microbiological changes in the modern soil environment. The first soil (Profile 1) is situated far from the Maritsa-Iztok mine region (Figure 1, top right) and represents a referent soil. It is located at 42°16′29.10″N; 26°15′38.87″E, near Skalitsa village, behind a farmyard, at the middle part of a hill with convex form (VV) and sloping gradient to the South-East. This soil is strongly leached Smolnitsa, non-eroded, light clayey, deep according to the national classification (Yolevski & Hadzhiyanakiev, 1976) and was sown with sunflower. According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015) it is classified as Pellic Vertisol (Mollic, Bathyhumic, Hypereutric, Clayic).

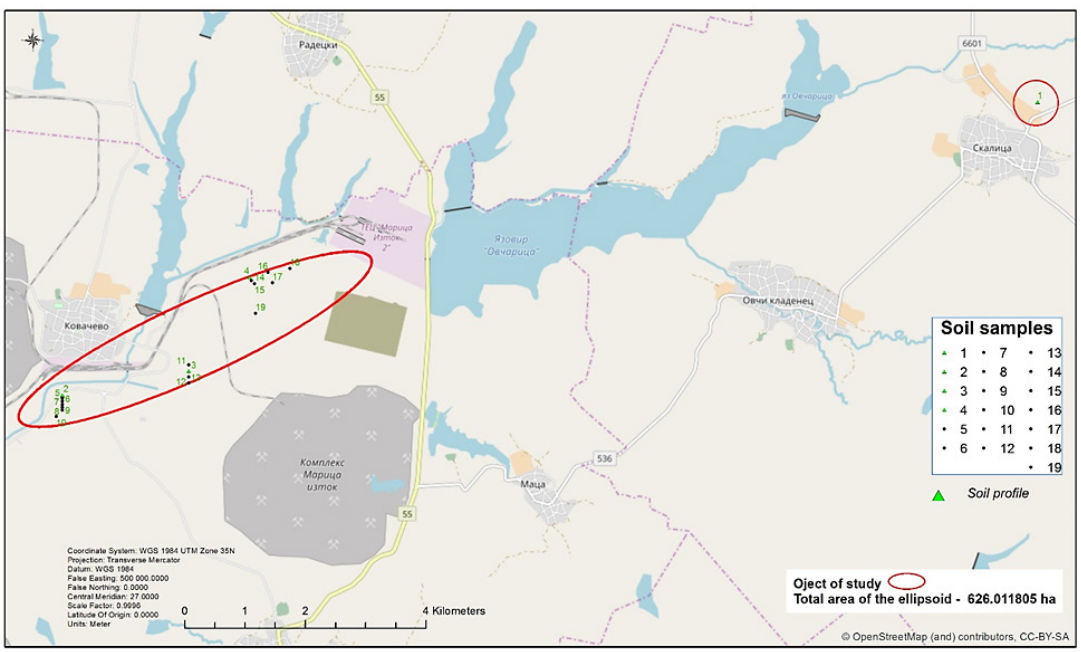

Fig. 1. Location of Pellic Vertisol profiles and sampling points in Maritsa-Iztok coal mining region

The second soil (Profile 2) is located close to the coal-mining region (Figure 1, inside the ellipsoid, on the left) and is vulnerable to pyrogenic carbon inputs. It is positioned near the village of Kovachevo, 450 m south of the railroad, and 5.8 km away from the "Maritsa-Iztok 2" Thermal Power Plant – TPP (Figure 1, in the right side of the elipsoid) at 42°13′45.30″ N and 26° 4′1.68″ E. Soil occupies the lower part of a Northern convex slope (VV) with gently sloping gradient, slightly oriented to the North-East. It is strongly leached Smolnitsa, non-eroded, heavy sandy-clayey, deep sown with wheat. The established morphological and chemical characteristics of soil correspond to the identifier Pellic Vertisol (Mollic, Bathyhumic, Hypereutric, Loamic, Bathypetrocalcic) from the World Reference Base (IUSS Working Group WRB, 2015).

Field study and laboratory assays

The field study complies with FAO principles (2006) and BDS ISO 8400-205 (2019) standard for soil description and study. Soil samples were collected at different depths corresponding to genetic horizons of each soil on 2-th of June 2018. For the purposes of this study additional samples from topsoil (0-20 cm) of vulnerable Smolnitsa were taken from five points positioned in parallel line with the Thermal Power Plant (TPP). The specially designed monitoring network was georeferenced with a density of 50x50 m. Samples from crossing points located at 50 m, 100 m, 150 m, 300 m and 350 m distance from profile 2 (also located in crossing point of monitoring network) were collected (Figure 1).

- Chemical and physical soil properties were analysed through the standard protocols.
  - Pre-treatment of samples for physico-chemical analysis – BDS ISO 11464:2012.
  - Determination of pH in H_2O – BDS ISO 10390:2010.
  - Particle size distribution – method described in ISO 11277:2009.
- The content of soil nutrients known for their significance to soil microflora were also determined.
  - Extractable humus fractions – by Kononova-Belchikova method (Filcheva & Tsadilas, 2002) in three extracts at soil: solution ratio 1:20: a) total organic carbon (TOC) – by acidified dichromate solution (0.4 N $K_2Cr_2O_7$ and concentrated H_2SO_4 in a ratio 1:1, soil oxidation at 120°C for 45 min. in the presence of Ag_2SO_4 followed by titration with 0.2 N Mohr's salt); b) humic acids (HA) – in a mixed solution of 0,1 M $Na_4P_2O_7$ and 0,1 M NaOH; c) free or linked to sesquioxides humic and fulvic acids representing the potentially mobile humic and fulvic acids (HA+FA) – extracted with 0.1 M NaOH. Optical hallmarks (E4/E6) were determined in humic acid fraction as a ratio of its optical density at λ 465 and 665 nm.
  - Main available mineral nitrogen forms (NH_4+NO_3) – by Bremner & Keeney (1965) procedure and available phosphorus (P) and potassium (K) contents using the method of Ivanov (1984).
- Microbiological analyses included determination of:
  - Microbial biomass carbon (Cmic) content – samples were treated with glucose, incubated for 4 hours at 22°C and produced CO_2 was trapped by NaOH. The microbial biomass carbon content (mg Cmic/100 g soil) was calculated according to the equation proposed by Anderson & Domsch (1978).
  - Population density of main microbial groups involved in organic residue mineralization and nutrient cycling in soils – assessed by plating ten-fold serial dilutions on selective agar media (Grudeva et al., 2006). Microbial density of heterotrophic, oligotrophic and oligonitrophilic bacteria, actinomycetes, fungi and cellulolytic microorganisms were presented as a number of colony forming units per gram of dry soil (CFU/g).

Data analyses

Data were subjected to ANOVA analysis and mean comparison was made by LSD test at $p < 0.05$ (values followed by the same letters in the columns of the tables are not significantly different). Pearson's correlation coefficients were used to assess relationships between microbial, chemical and physical soil parameters.

Coefficient of variation is used to evaluate the degree of spatial homogeneity of studied parameters (equation 1):

$$V = \frac{s}{x}.100, \quad (1)$$

where S is standard deviation; x – the mean content of studied parameter in soil.

The V values in the range of 10-12% showed homogeneous distribution of the element, 12-30% – slight heterogeneity and > 30% – strong heterogeneity. If we assume that the slight inhomogeneity is due to natural geochemical and biological processes, then only values above 30% can be interpreted as a result of modern processes of redistribution increasing heterogeneity.

Results and Discussion

Studied soil determinants of microbiological status slightly differed in two soils. In the referent soil (Profile 1) they were:

neutral to slightly alkaline soil reaction (pH 7.5-7.1 down the profile), medium levels of available N (26.5-39.7 mg/kg) and K (21.0-32.2 mg/100 g), medium to low levels of available P (0.3-10.4 mg/100 g), moisture content 8.49-8.23%, clay content between 42.3% and 46.8% and carbonate content between 0.27% and 1.39%. The organic matter despite the low content in this soil (1.72-0.84% down the profile) was of high quality: the Mull type of humus entirely dominated by humic acids (especially below 30 cm depth where FA were not found) with strongly condensed nuclei (E4/E6 varied from 3.46 to 3.74) and high maturity. Humic acids were strongly bound to the mineral matrix by stable calcium humates. The easily mobile humic and fulvic acids were also present in not minor amounts (from 0.11 to 0.06%). Pyrogenic carbon content fluctuated between 0.64 % and 0.38 % down the profile and formed average 29% of SOC.

The vulnerable soil (Profile 2) was also characterized with neutral to slightly alkaline soil reaction (pH 7.0-8.1 down the profile), medium levels of available N (20.7-55.9 mg/kg) and K (11.3-21.5 mg/100 g), but low level of available P (0.5-3.6 mg/100 g). Moisture content varied between 5.57% and 6.71%, carbonates – 0.42-12.52%, and clay content between 35.8% and 43.7%. The organic matter also had almost the same characteristics: low total organic carbon content (1.45-0.25% down the profile), Mull type of humus, domination of mature, strongly condensed (E4/E6 varied from 3.17 to 3.33), stable and hydrophobic humic acids (CHA/CFA 2.37-2.87) and equal amount of easily mobile humic and fulvic acids 0.10-0.06%. The content of pyrogenic carbon was lower (0.55-0.23%) and formed up to 22% on average of SOC.

Soil properties in the first three points (at 50 m, 100 m and 150 m) were similar to those of the surface horizon of profile 2. Samples in the most distanced points were characterized with alkaline reaction, lower TOC and higher moisture, clay and available P and K contents. Data obtained showed a strong spatial heterogeneity in available phosphorus distribution (V = 43%) and extremely strong fluctuation of carbonates' content (V = 121%) in vulnerable soil, which was not registered in their previous study (Nedyalkov & Mladenov, 1979).

Microbial biomass carbon (Cmic) was the highest in plough horizons and decreased by depth in both Smolnitsas (Table 1). Such a decreasing trend was demonstrated in other studies (Taylor et al., 2002). In the top horizon of profile 2, higher Cmic was registered comparing to profile 1. Surprisingly, the lowest value of microbial biomass carbon was registered in the middle part of the profile 2 and this was an indication for biopedoturbation. In most of the monitoring points Cmic amount was similar to that of profile 2 ploughing horizon and predominantly exceeded the value of profile 1. Cmic for all surface horizons in vulnerable soil averages 28.62 mg C/100 g and could be compared to the values found in Chernozems (Frunze, 2013).

Table 1. Content and statistical differences between microbial biomass carbon in studied Vertisols

Pellic Vertisol horizons	Horizon depth, cm	Cmic, mg C/100 g
Pellic Vertisol 1 (Profile 1)		
A_p	0-30	17.43 a
A″	30-50	10.77 b
A‴	50-75	7.83 c
A_k	75-100	5.17 d
Pellic Vertisol 2 (Profile 2)		
A_p	0-30	30.22 a
A″	30-75	7.83 b
A″	75-105	1.31 d
AC_k	105-130	8.37 b
C_{1k}	130-155	4.63 c
Pellic Vertisol top horizons and monitoring points		
A_p	0-30 (Profile 1)	17.43 e
A_p	0-30 (Profile 2)	30.22 cd
A_p – I point	Monitoring points near Profile 2 (0- 20 cm)	45.15 a
A_p – II point		29.16 d
A_p – III point		32.36 b
A_p – IV point		31.82 bc
A_p – V point		14.23 f

Microbial population density followed the common for native soils distrubution along the profile – a downward decreasing numbers (Fierer, 2003). Perfanova & Lyubenova (2017) reported descending microbial numbers along the profile of Pellic Endocalcic Vertisol from North Bulgaria. Taylor et al. (2002) also found that bacteria and fungi declined with the depth in a clay soil and that this was positively correlated with the decrease in soil organic matter. Still, the exceptions to this trend could be seen in case of specific soil properties or anthropogenic influence. In profile 1, the density of microbial groups (except for oligotrophic bacteria) sharply decreased in depth by 1-3 orders of magnitude (Table 2) while in profile 2 the distribution curve is smoother and decrease – gradual with the exception, again, of oligotrophic bacteria. Oligotrophic bacteria were one of the prevailing microbial groups in studied Smolnitsas especially in the deepest horizons wherein they dominate alone. Nikolova (2012) found high amount of oligotrophs in calcareous Chernozem from Russe district (Bulgaria) and this suggests that calcareous soil-parent rocks could determine the observed similarity. Still, the minimums of oligotrophic population were not regis-

tered in last horizons of both soils and this specific behaviour could be related to biopedoturbation. The process of argillipedoturbation (clay illuviation) which could cause biological matter movement was not registered in these soils, but strong positive interlinks between silt fraction, TOC and basic nutrients were observed. Since the calculated correlations (Table 3) show a relationship between oligotrophs and organic carbon simultaneously with liable organic carbon fraction (HA+FA), it could be assumed that this kind of biopedoturbation (oligotroph-pedoturbation) presumably had aroused as a result of floral and faunal successions in the past. Predation is another factor that could control microbes' abundance and hydromorphic stage during the earlier genesis of Smolnitsas could enable predators spreading (such as Amoebozoa). Kurm et al. (2019) established that predation reduced the abundance of bacterial taxa in soils and it could be as essential as nutrients availability and physiological or behavioural features of microbiome.

As a whole, typical features of both Smolnitsas were: dominance of heterotrophic, oligotrophic and oligonitrophilic bacteria and low density of slow-growing cellulolytic microorganisms and fungi.

The comparative analysis of microbial density in topsoil samples showed that heterotrophic bacteria were plenteous in profile 1 (Table 2). The amount decreased in profile 2 and further dropped in distanced points (I -V). The similar trend was noticed for actinomycetes and fungi – lower density in profile 2. Cellulolytic microorganisms contrariwise were considerably higher in distanced points comparing to both profiles and more abundant in soil located close to the mining region (profile 2). In fact the density of all microbial groups in ploughing horizon of vulnerable soil strongly varies. The highest variation was calculated for oligonitrophilic bacteria (V=72%), followed by heterotrophic bacteria (V = 53%), oligotrophs (V = 50%), cellulolytic microorganisms (V = 45%), fungi (V = 42%) and actinomycetes (V = 33%). The lack of previous data does not allow explicit conclusions to be made, but it can be assumed that oligonitrophilic bacteria are the most sensitive to various anthropogenic impacts such as fertilization, bad agricultural practices, industrial pollution, etc. Therefore, the oligonitrophilic bacteria could be used as bioindicator of soil anthropogenization including pyrogenic carbon accumulation.

Usually, microbial groups and taxa have different abundance in the soil. Factors most influencing soil bacterial communities are pH, soil organic carbon, moisture, nitrogen and phosphorus availability, temperature, vegetation type, and agricultural practices (Fierer, 2017). Labile organic carbon content

Table 2. Population density of different microbial groups and statistical differences in Pellic Vertisols

Pellic Vertisol horizons and depths, cm		Hetero- trophic bacteria	Oligo- trophic bacteria	Oligo- nitroph. bacteria	Actino- mycetes	Fungi	Cellulo- lytic microorg.
		CFU/g . 10^4					
Pellic Vertisol 1 (Profile 1)							
$A_{pк}$	0-30	72.00 a	65.60 a	18.07 a	10.73 a	0.633 a	0.094 a
$A''_{к}$	30-50	3.53 b	34.80 b	0.57 b	0.59 b	0.032 b	0.019 b
$A'''_{к}$	50-75	1.11 b	6.60 d	0.16 b	0.03 b	0.003 b	0.002 b
A''''_{k}	75-100	1.73 b	20.27 c	0.05 b	0.01 b	0.000 b	0.002 b
Pellic Vertisol 2 (Profile 2)							
A_p	0-30	34.80 a	72.0 a	29.07 a	4.53 a	0.273 a	0.179 a
A″	30-75	17.67 b	76.8 a	6.77 b	1.17 b	0.025 b	0.082 b
$A'''_{к}$	75-105	10.47 c	52.0 c	0.48 c	0.23 c	0.003 b	0.013 c
AC_k	105-130	6.20 d	34.4 d	0.44 c	0.09 c	0.001 b	0.005 c
C_k	130-155	6.47 d	66.1 ab	0.10 c	0.03 c	0.000 b	0.003 c
Pellic Vertisol top horizons and monitoring points near Profile 2 (0-20 cm)							
A_{pk}	0-30 (Profile 1)	72.00 a	65.60 b	18.07 b	10.73 a	0.633 a	0.094 f
A_p	0-30 (Profile 2)	34.80 b	72.0 ab	29.07 a	4.53 b	0.273 b	0.179 def
A_p	Monito- ring points near Profile 2	14.9 c	48.27 c	19.33 b	4.53 b	0.213 bc	0.380 bc
A_p		16.9 c	67.20 b	8.0 cd	4.13 bc	0.040 d	0.267 cd
A_p		16.2 c	79.47 a	10.0 c	4.80 b	0.247 b	0.460 ab
A_p		6.5 d	18.53 d	5.33 cde	2.40 cd	0.190 bc	0.520 a
A_p		7.7 d	15.90 d	3.33 e	1.67 d	0.130 cd	0.127 ef

Table 3. Correlation coefficients (r) between studied microbial and soil parameters

Parameters	Heterotr. bact.	Oligotroph. bact.	Oligonitr. bact.	Act.	Fungi	Cellulolytic micr.
Pellic Vertisol 1 – profile 1						
Heterotroph. bact.	1.000					
Oligotrophic bact.	0.903	1.000				
Oligonitrophilic b.	1.000	0.899	1.000			
Actinomycetes	1.000	0.909	1.000	1.000		
Fungi	1.000	0.907	1.000	1.000	1.000	
Cellulolytic micr.	0.989	0.947	0.988	0.992	0.991	1.000
Horizon depth	-0.788	-0.838	-0.789	-0.802	-0.801	-0.862
pH	0.594	0.833	0.584	0.597	0.593	0.642
Available N	0.831	0.521	0.838	0.826	0.828	0.767
Available P	0.960	0.961	0.960	0.967	0.966	0.992
Available K	0.999	0.890	0.999	0.997	0.997	0.981
TOC	0.929	0.907	0.930	0.937	0.937	0.966
HA	-0.178	-0.056	-0.172	-0.156	-0.146	-0.065
Liable HA+FA	0.978	0.872	0.980	0.982	0.986	0.980
PyC	0.972	0.826	0.975	0.973	0.982	0.962
Hydrosc. moisture	-0.510	-0.774	-0.499	-0.512	-0.472	-0.561
Sand	-0.002	0.364	-0.015	0.002	-0.003	0.063
Silt	0.824	0.805	0.827	0.836	0.835	0.876
Clay	-0.680	-0.927	-0.674	-0.693	-0.689	-0.771
Carbonates	-0.453	-0.460	-0.460	-0.471	-0.485	-0.536
C mic	0.910	0.909	0.911	0.920	0.918	0.956
Pellic Vertisol 2 – profile 2						
Heterotr. bact.	1.000					
Oligotrophic bact.	0.650	1.000				
Oligonitrophilic b.	0.975	0.525	1.000			
Actinomycetes	0.981	0.538	0.999	1.000		
Fungi	0.936	0.439	0.986	0.983	1.000	
Cellulolytic micr.	0.982	0.574	0.977	0.980	0.931	1.000
Horizon depth	-0.903	-0.498	-0.816	-0.833	-0.755	-0.893
pH	-0.877	-0.579	-0.778	-0.795	-0.698	-0.883
Available N	-0.283	-0.759	-0.274	-0.272	-0.281	-0.262
Available P	0.876	0.369	0.922	0.920	0.961	0.833
Available K	0.953	0.422	0.917	0.926	0.877	0.950
TOC	0.879	0.481	0.784	0.803	0.724	0.864
HA	0.708	0.469	0.565	0.592	0.496	0.678
Liable HA+FA	0.834	0.702	0.709	0.729	0.622	0.833
PyC	0.645	-0.133	0.636	0.647	0.665	0.584
Hydrosc. moisture	0.281	-0.330	0.207	0.229	0.209	0.222
Sand	0.348	0.817	0.234	0.242	0.159	0.245
Silt	-0.254	-0.567	-0.035	-0.067	0.083	-0.182
Clay	-0.233	-0.550	-0.241	-0.230	-0.231	-0.162
Carbonates	-0.382	0.406	-0.336	-0.351	-0.331	-0.355
Cmic	0.908	0.362	0.970	0.962	0.974	0.916

Legend: N, P, K – available forms of nitrogen, phosphorus and potassium; TOC – total organic carbon; HA – humic acids; HA+FA – humic + fulvic acids; PyC – pyrogenic carbon

Table 4. Correlation coefficients (r) between microbial parameters and basic determinants in surface horizons of studied soils

Soil properties	Cmic	Heterotr. bact.	Oligotr. bact.	Oligonitr. bact.	Actin.	Fungi	Cellulol. mic.
pH	-0.521	0.055	-0.594	-0.616	0.014	0.150	0.033
N	-0.041	0.626	0.066	0.188	0.748	0.679	-0.280
P	-0.552	-0.076	-0.795	-0.616	-0.141	0.142	0.039
K	-0.445	-0.350	-0.926	-0.731	-0.416	-0.155	0.081
TOC	0.216	0.634	0.838	0.473	0.796	0.478	-0.172
PyC	-0.571	0.132	0.389	-0.844	0.214	-0.115	-0.276
Carbonates	-0.400	-0.507	-0.929	-0.679	-0.625	-0.331	0.105
Moisture	-0.494	0.909	0.201	0.213	0.902	0.903	-0.522
Clay	-0.638	0.215	-0.556	-0.378	0.082	0.343	-0.129
Cmic		-0.370	0.215	0.307	-0.190	-0.299	0.700

also often correlated with microbial properties of soils (Taylor et al., 2002; Carpenter-Boggs et al., 2003; Fierer, 2003). In our study, the most of microbial groups had strong positive interlinks with TOC, available phosphorus, potassium, easily mobile humic and fulvic acids, and available nitrogen especially in profile 1 (Table 3). All exceptions are observed in profile 2 – for oligotrophs with respect to available P and K and for fungi regarding liable carbon pool (HA + FA) in addition to lack of correlation with available nitrogen. These data generally coincided with other reports showing the primary importance of carbon, nitrogen and phosphorus for bacterial growth in soils (Alden et al., 2001; Demoling et al., 2007).

All microbial groups created strong synergetic linkages and actively utilize nutrients supplied by SOC and PyC in profile 1 while in vulnerable soil this pattern was less pronounced especially for oligotrophs (Table 3). Data suggested that additional inputs of PyC in soils surrounding coalfield (profile 2) could decouple SOM and PyC, supress microbiome synergy and dependency on PyC.

There are much fewer correlations between soil ingredients and microbiome traits in surface horizons – mainly between soil hydroscopic moisture and three microbiological groups (heterotrophs, actinomycetes and fungi), as well as between TOC and oligotrophs and actinomycetes. These data showed that in surface horizons, exogenous nutrient sources are as important to the microbiome as endogenous soil resources.

Conclusion

Due to the inherent three dimensional variations of soil properties, the different crops and cultivation manner, microbial parameters (microbial biomass carbon and population density) of studied Pellic Vertisols predominately varied in depth but followed the typical for native soils trend of decreasing values down the profiles. In both soils, heterotrophic, oligotrophic and oligonitrophilic bacteria were the most numerous groups but in the deepest horizons only oligotrophs dominated. The oligotroph-pedoturbation observed in $A'''_{к}$ and AC_{k} horizons as an atypical population decline is a specific feature of studied local Smolnitsas.

Due to the high spatial variation of oligonitrophils' density this group could be used as bioindicator of soil anthropogenization including pyrogenic carbon (PyC) accumulation. Data suggests that additional inputs of PyC in soils surrounding coalfield could decouple SOM and PyC, supress microbiome synergy and dependency on PyC.

Only some nutrients (organic carbon, phosphorus, potassium and easily mobile humic and fulvic acids) determined the microbial growth and density in studied soils while soil moisture, depth, carbonates and clay contents were not significant factors. Available nitrogen positively influenced microbiome status only in control soil with exception of oligotrophs for which soil pH was determinative. PH is not a microbial determinant in natural soils beyond the mentioned exception.

The spatial variability of studied parameters in surface horizons revealed that exogenous nutrient sources are as important to the microbiome as endogenous resources.

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