Relationships between Numbers of Microbial Communities in Polish Agricultural Soils and Properties of these Soils, paying Special Attention to Xerophilic/Xerotolerant Fungi

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Abstract

Our study was conducted with seven typical Polish soils very different in their physical and chemical features and fertility (a Phaeozem, an Eutric Fluvisol, three Eutric Cambisols, and two Dystric Cambisols). Samples of the soils were taken from a plot experiment established in Puławy at the end of the 19th century and cultivated only by hand tillage. Correlation analyses of the results obtained suggest that soil microorganisms differing in their water requirements were associated with different soil environments: bacteria with small particles and small pores usually filled with water, nonxerophilic fungi with medium particles and medium pores which are usually the region of near the air-water interface, and xerotolerant/xerophilic fungi (especially penicillia) predominantly with big particles and big pores usually filled with air. Moreover, CFU of xerotolerant/xerophilic fungi and particularly formulated indices of xerotolerance of the soil fungal communities (in contrast to CFU of bacteria), were negatively correlated with water content, SOM content, soil pH, and an index of soil quality.

Keywords: soil texture, pore size distribution, xerophilic fungi, nonxerophilic fungi, Penicillium

Introduction

Due to its variability, soil quality is the most important factor influencing crop yields on the flat areas of Poland. Differences in soil quality are caused mainly by variability in soil texture [1, 2]. Kern [3] found on the basis of analyses of 48,000 Polish soil profiles that with decreasing percentage of clay and increasing percentage of sand fraction, the soil carbonate content also decreases. Moreover, in Poland, a land with a humid climate where precipitation exceeds evaporation, the upper horizons of the majority of soils, especially sandy ones, are devoid of carbonates [3]. In these soils, the texture and humus content (exactly the content of bases sorbed by mineral and organic colloids) determine the soil buffering capacity [4]. Due to low absorption capacity and high water permeability, many of the sandy soils have become acidified. These soils, of glacial origin, show low fertility and productivity [3-5]. The same variables, the texture and SOM content, which is also dependent on texture [6-9] are also the main determinants of pore space and pore size distribution in soil, and in consequence the air-water relationships in the soil environment [10-12]. Therefore in general, soil moisture, field water capacity, the amount of water available to plants and the field water con-
sorption by plants, as well as soil pH, increase in direction from the lighter soils to heavier ones [3-5, 13-15].

The quality of soils is connected not only with their physical and chemical properties, but also with their microbiological features. The water availability and pH are also important factors affecting the numbers, activities, and ecological relationships among microorganisms. In the soils with properties favourable for plant production, e.g. heavier texture, higher SOM content and higher pH, intensive development of bacteria is observed [16-21]. On the other hand, the growth of fungi, except water moulds, are favoured by drier soil conditions than the growth of bacteria [22, 23]. Therefore, the lighter soils provide habitats suiting fungal requirements better than heavier ones [18, 24]. Furthermore, in the acidified soils the growth of fungal flora was promoted [16, 19] due to the tolerance of many soil fungi to high hydrogen ion concentration, as opposed to most soil bacteria [25].

From the studies cited above, low bacterial number and high fungal number in soils can reflect the features of coarse-textured soils, e.g. the low level of water retention and the low pH. In these studies, the numbers of fungi were determined on media containing water easily available to the microorganisms, mainly on Martin’s medium [26]. It seems that the additional determination of the numbers of xerotolerant/xerophilic fungi on an agar medium containing very little available water could better reflect the features of the coarse-textured soils.

The aim of this work was to study the relationships between features of different Polish soils and numbers of different groups of soil microorganisms with a focus on xerotolerant/xerophilic fungi.

Material and Methods

Soils, Plants and Sampling Procedure

The experimental site was located in Puławy, in the eastern part of Poland (51° 24' N, 21° 57' E). The field experiment used in this study was established in 1881. It consists of seven plots (14 m²), each 1 m deep with concrete walls, which were filled with profiles of seven soils:

#1. a Phaeozem – sandy loam;
#2. a Eutric Fluvisol - loam;
#3. a Eutric Cambisol developed from loess – loam;
#4. a Eutric Cambisol – sandy loam;
#5. a Eutric Cambisol – loamy sand;
#6. and #7. Dystric Cambisols – loamy sand.

The soils represent common soil types occurring in Poland [27]. The soils of the plots have always been (since 1881) cultivated by hand tillage in the same manner. The same plant species were always grown in all plots at the same time, and the soils received the same or very similar fertilization. Since 1979 the plots have been planted mostly with cereals as the main crop with mineral fertilizers, e.g. according to Podolska [27] and Sulek [28], and some plants for green manure as the second crop, mainly mustard, sometimes phacelia or leguminous plants. In 1984 the plots were fertilized with compost (80 t ha⁻¹) under potatoes.

Some physico-chemical properties of these soils are given in Table 1. Table 1 also contains values of the point index of soil quality – a parameter presenting the general fertility and productivity of the soils [27, 28], calculated by Witek et al. [2], on the basis of results of more than 5,900 experiments with cereals during 1970-75 [1]. The values of this point index of soil quality concerning the plot soils are significantly correlated with the grain yields of various crops growing in these soils, e.g. winter wheat (r=0.89 at P<0.01) obtained in Podolska’s studies [27]; winter wheat (r=0.97 at P<0.01), triticale (r=0.88 at P<0.01) and buckwheat (r=0.84 at P<0.05) in Martyniuk’s studies [29]; and with the grain yields of spring wheat (r=0.79 at P<0.05) in Sulek’s studies [28].

Soils under spring wheat were sampled from intercrops on June 20, 2007. Ten soil cores (2.5 cm in diameter and 25 cm in depth) were collected from each soil and mixed thoroughly together to make one sample, and passed through a 2 mm sieve. For drawing of the soil moisture characteristic curves, undisturbed soil cores of 100 cm³ volume (4.9 cm in diameter and 5.5 cm in depth) were sampled in three replicates after removing the top 10 cm layer of soil.

Physical and Chemical Analyses

The soil water content and the bulk density were determined after oven drying at 105°C [30] in three replicates. Total porosity was calculated from the bulk density of the soils and 2.65 g cm⁻³ particle density. Soil pH was measured in three replicates with a glass electrode in a suspension made by mixing 10 g of soil and 25 cm³ of distilled water. The soil organic matter (SOM) content was determined in three replicates by the modified Tiurin’s method, in which soil organic carbon was oxidized to CO₂ by a mixture of potassium dichromate and sulphuric acid and excess of dichromate was back titrated with a solution of Mohr’s Salt [31].

Soil texture classes were determined by the hydrometer method, modified by Casagrande and Prószyński, based on measurements of the density of soil suspensions during progressive sedimentation, supplemented with the sieve method to fractionate sand [32]. In this study the particle sizes were divided into the following classes: in Table 1 as sand, silt, and clay according to the FAO/USDA classification system (sand, 2.0-0.05 mm; silt, 0.05-0.002 mm; clay, <0.002 mm) [33], and as <0.002, 0.002-0.006, 0.006-0.02, 0.02-0.05, 0.05-0.1, 0.1-0.25, 0.25-0.5, and 0.5-2.0 mm (Figs. 1 and 3). The mean size of the soil particle (MSPart) was calculated according to the equation:

\[
MSPart = \left(\%_{0.2-1}\text{mm}\right) \times 1.5 + \%_{0.1-0.5}\text{mm} \times 0.75 + \%_{0.05-0.25}\text{mm} \times 0.375 + \%_{0.25-0.5}\text{mm} \times 0.175 + \%_{0.01-0.05}\text{mm} \times 0.075 + \%_{<0.01}\text{mm} \times 0.005 + \%_{0.01-0.02}\text{mm} \times 0.013 + \%_{0.02-0.006}\text{mm} \times 0.004 + \%_{0.006-0.02}\text{mm} \times 0.001 \right)
\]

where % is the percentage of the individual classes of soil particles.
Table 1. Characteristics of the sampled soils: point index of soil quality and selected physico-chemical properties of the soils.

<table>
<thead>
<tr>
<th>Soils in accordance with FAO/UNESCO (1994) taxonomy</th>
<th>Index of soil quality</th>
<th>Content of soil particle size classes [%]</th>
<th>Mean size of particle [mm]</th>
<th>Bulk density [g cm⁻³]</th>
<th>Total porosity [% v/w]</th>
<th>Volume of pore size classes [cm³ kg⁻¹]</th>
<th>Mean size of pore [μm]</th>
<th>Water content [%] (pF / PND in μm*)</th>
<th>pH (H₂O)</th>
<th>SOM content [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Phaeozem</td>
<td>100</td>
<td>Sand 50</td>
<td>Silt 50</td>
<td>Clay 46</td>
<td>4</td>
<td>0.16</td>
<td>1.18 a</td>
<td>55.5</td>
<td>136</td>
<td>142</td>
</tr>
<tr>
<td>2. Eutric Fluvisol</td>
<td>92</td>
<td>Sand 48</td>
<td>Silt 48</td>
<td>Clay 34</td>
<td>18</td>
<td>0.15</td>
<td>1.31 b</td>
<td>50.6</td>
<td>168</td>
<td>41</td>
</tr>
<tr>
<td>3. Eutric Cambisol</td>
<td>83</td>
<td>Sand 45</td>
<td>Silt 45</td>
<td>Clay 48</td>
<td>7</td>
<td>0.12</td>
<td>1.30 b</td>
<td>50.9</td>
<td>105</td>
<td>122</td>
</tr>
<tr>
<td>4. Eutric Cambisol</td>
<td>70</td>
<td>Sand 55</td>
<td>Silt 55</td>
<td>Clay 38</td>
<td>7</td>
<td>0.12</td>
<td>1.37 bc</td>
<td>48.3</td>
<td>98</td>
<td>107</td>
</tr>
<tr>
<td>5. Eutric Cambisol</td>
<td>42</td>
<td>Sand 71</td>
<td>Silt 26</td>
<td>Clay 26</td>
<td>3</td>
<td>0.22</td>
<td>1.34 b</td>
<td>49.4</td>
<td>70</td>
<td>92</td>
</tr>
<tr>
<td>6. Dystric Cambisol</td>
<td>30</td>
<td>Sand 78</td>
<td>Silt 18</td>
<td>Clay 18</td>
<td>4</td>
<td>0.26</td>
<td>1.37 bc</td>
<td>48.3</td>
<td>49</td>
<td>81</td>
</tr>
<tr>
<td>7. Dystric Cambisol</td>
<td>18</td>
<td>Sand 83</td>
<td>Silt 14</td>
<td>Clay 14</td>
<td>3</td>
<td>0.33</td>
<td>1.45 c</td>
<td>45.3</td>
<td>28</td>
<td>57</td>
</tr>
</tbody>
</table>

* – in a range <30μm;
** pF values correspondent to moisture and PND – the effective pore neck diameter at the time of sampling;
a, b, c, d – the values in separate columns marked with different letters are statistically different at P≤0.05.
Pore size distributions were derived from the soil moisture characteristic curves measured from undisturbed soil cores. The cores, saturated with water, were drained to obtain matric potentials of -10, -20, -40 and -80 hPa \((pF=1.0, 1.3, 1.6, \text{and} 1.9, \text{respectively})\) on the sand table (Eijkelkamp, The Netherlands) and -250, -500, -1,000, -2,000, -4,000, -8,000, and -15,000 hPa \((pF = 2.4, 2.7, 3.0, 3.3, 3.6, 3.9, \text{and} 4.2)\) on the ceramic plates (Soil Moisture Inc., USA). At all \(pF\) values presented above, the soil water content was determined. The effective pore neck diameters \((y - \text{expressed in \(\mu m\)})\) were estimated from these \(pF\) values \((x)\) according to the regression equation: \(\log y = 3.477 - x\) \((R^2=1)\) developed from data presented by Czyż [34], and Skawina et al. [35] showing various \(pF\) values and the corresponding effective pore neck diameters. The volume of different pore classes in the range of 0.2-300 \(\mu m\) were obtained from the retention curves after subtraction of the soil water content at a higher \(pF\) (a smaller effective pore neck diameter) from the water content at a lower \(pF\) (a larger effective pore neck diameter). For example: in the case of Eutric Fluvisol #2, water content at \(pF\) 3.0 (which corresponds to 3 \(\mu m\) effective pore neck diameter) was determined as 168 cm\(^3\) kg\(^{-1}\), and at \(pF\) 2.7 (6 \(\mu m\) effective pore neck diameter) as 189 cm\(^3\) kg\(^{-1}\). In this case the volume of pores with neck diameter 3–6 \(\mu m\) equals 189 – 168 = 21 cm\(^3\) kg\(^{-1}\).

In this study pore sizes were divided into the following classes: <3, 3-30 and 30-300 \(\mu m\) (Table 1) and <0.5, 0.5-3, 3-6, 6-10, 10-17, 17-30, 30-50, 50-100, 100-300 \(\mu m\) (Figs. 2 and 4). The mean size of the soil pore \((MSPore)\) was calculated according to the equation:

\[
MSPore = \frac{V_{[<0.5 \mu m]} \cdot 0.25 + V_{[0.5-3 \mu m]} \cdot 1.75 + V_{[3-6 \mu m]} \cdot 4.5 + V_{[6-10 \mu m]} \cdot 8 + V_{[10-17 \mu m]} \cdot 13.5 + V_{[17-30 \mu m]} \cdot 23.5 + V_{[30-50 \mu m]} \cdot 40 + V_{[50-100 \mu m]} \cdot 75 + V_{[100-300 \mu m]} \cdot 200}{V_{[<300 \mu m]}}
\]

...where \(V\) is the volume of the individual pore size classes.

![Fig. 1. Particle-size distribution of the sampled soils.](image)

![Fig. 2. Pore-size distribution of the sampled soils.](image)
Microbiological Analyses

The soil samples were analyzed in four ways:

(1) the total numbers of bacterial CFU (colony forming units) after incubation at 25°C for seven days on an agar medium containing: 200 cm³ of extract of a fertile alluvial soil (a suspension of 1 kg of the soil and 1 dm³ of tap water was left for 24 h, mixed again before and after autoclaving, filtered hot and then the obtained soil extract was autoclaved again); K₂HPO₄, 0.5 g; agar, 10 g and tap water, 800 cm³;

(2) the total numbers of fungal CFU after incubation at 25°C for five days on Martin’s medium [26];

(3) the total CFU numbers of xerotolerant/xerophilic fungi after incubation at 25°C for 5-6 days on DG18 medium, developed for enumeration of moderately xerophilic molds in food analysis [36], and after incubation at 20°C for 5-7 days on DSMG – an excellent medium for detection of Penicillium verrucosum in cereals and soil [37], which enables growth of many other xerophilic fungi; and

(4) the CFU numbers of fungi belonging to Penicillium genus on Martin’s, DG18 and DSMG media.

DG18 contains anhydrous glycerol (200 g cm⁻³) and DSMG anhydrous glycerol (220 g cm⁻³) and sucrose (150 g cm⁻³) as the agents reducing water availability [36, 37]. The Penicillium colonies were identified by a microscopic analysis. The CFU numbers of all microbial groups were determined in four replicates.

Formulation of Xerotolerance Indices

On the basis of the assumption that xerotolerance of the fungal communities increased in the following order: (A) fungi other than Penicillium (FOTP) on Martin’s medium < (B) FOTP on DG18 < (C) FOTP on DSMG < (D) Penicillium, these communities were rated on a 1–4 scale. Xerotolerance index of fungal communities #1 (XIFC 1) was formulated as a sum of products of cardinal numbers (CFU numbers of different fungal communities – A, B, C, and D) and ordinal ones (correspondent scale values – 1, 2, 3, and 4) divided by a sum of numbers of these fungal communities, according to the equation:

\[ \text{XIFC}_1 = \frac{(A * 1 + B * 2 + C * 3 + D * 4)}{(A+B+C+D)} \]

Indices, similar to XIFC 1, are used for evaluating severity of plant diseases or degree of infestation by pathogenic fungi [38-40].

Two other indices (#2 and #3) are based only on cardinal numbers - a quotient of a difference between CFU numbers of xerotolerant/xerophilic (PM = Penicillium on Martin’s medium or C = FOTP on DSMG) and nonxerotolerant/xerophilic fungi (A = FOTP on Martin’s medium) and a sum of them, according to the equations:

\[ \text{XIFC}_2 = 1 + \left| \frac{(PM - A)}{(PM + A)} \right|; \]
\[ \text{XIFC}_3 = 1 + \left| \frac{(C - A)}{(C + A)} \right| \]

A technical value “1” is added to transform the obtained negative index values to be positive. These equations can be presented in simpler forms:

\[ \text{XIFC}_2 = 2 PM / (PM + A) \]
\[ \text{XIFC}_3 = 2C / (C + A), \]

because

\[ 1 = (PM + A) / (PM + A) \text{ or } (C + A) / (C + A). \]

Statistical Analyses

All data were screened for normality and homogeneity of variance. Most of them were found to be normally distributed, excluding data of SOM content, CFU of bacteria, total fungi and penicillia on Martin’s medium. These data were normally distributed after transformation to logarithmic forms. The values of percentage of penicillia were transformed for statistical evaluation according to the equation \( y = \arcsin \sqrt{x} \). Then all data were subjected to one-way analysis of variance (at \( P=0.05 \)) and the means were separated with Tukey’s test with a level of significance of \( P=0.05 \). Tukey’s test did not differentiate data concerning CFU numbers of FOTP on Martin’s medium. In this case Fisher’s LSD test was applied (at \( P=0.05 \)). For estimation of the relationships between physico-chemical and biological features of the soils, the simple Pearson’s correlation analysis was used. Together with correlation coefficients (r), level of probability (P) and number of observations (n) are presented. For evaluation of the relationships between the XIFCs and MSPore, the regression analysis was applied. Together with regression equations, determination coefficients (R²) are presented.

Results

Differences in Soil Properties

The soils varied much in their fertility and physico-chemical features (Table 1, Figs. 1 and 2). The three least fertile soils (the lowest index of soil quality) were characterized by the highest MSPart and MSPore, because of the high percentage of the sand fraction, high volume of pores with a neck diameter of 30-300 μm and low volume of <10 μm pores in these soils (Table 1, Figs. 1 and 2). Furthermore, these soils had low pH and very low water content (Table 1). The most fertile soil, No. 1 was characterized by high pH, silt content, volume of pores with a neck diameter of <6 μm, and especially by the highest content of SOM and water, as well as by the highest total porosity and volume of 6-17 μm pores and the lowest bulk density (Table 1, Figs. 1 and 2). The highest pH, clay content and volume of <3μm pores, as well as the lowest MSPore, were the main features of the alluvial soil (No. 2). However, two Eutric Cambisols (loessial soil No. 3 and soil No. 4) had the lowest MSPart, high content of the silt fraction and high volume of pores <30 μm, but the loessial soil, contrary to soil No. 4 (and also to soils Nos. 1 and 2) contained a fairly high volume of pores with a neck diameter of 30-300 μm (Table 1, Figs. 1 and 2).
Except Eutric Fluvisol No. 2 (pF = 3.6), pF values of the examined soils at the time of sampling were similar and ranged from 2.6 to 3.0 (Table 1). This means that in these soils the effective neck diameters of the largest pores filled with water ranged from 3 to 8 μm (in soil No. 2 ca. 1 μm).

Microbial Community Structure

Bacterial CFU numbers (Table 2) are ranked in the same order as the fertility of the soils as given by the index of soil quality (Table 1).

The fungal CFU numbers on Martin’s and DG18 media were lowest in the case of soil Nos. 1, 2, and 6, and highest in the case of soil Nos. 5, 7, and 4. But when the fungal CFU numbers on Martin’s medium were assumed as “total” CFU numbers of soil fungi (100%), the percentage of fungi growing on DG18 were highest in the case of the two lightest soils (Nos. 6 and 7) (Table 2). Similarly, the three lightest soils (Nos. 5, 6, and 7) distinctly differed from other soils in the percentages of xerotolerant/xerophytic fungi growing on DYSG (Table 2). Even more distinct differences between the soils examined were noticeable in the case of CFU numbers and percentages of Penicillium on all used agar media. Table 2 shows that these values were greatest in the case of the three lightest soils (5, 6 and 7) and smallest in soils 1 and 2. Also, the ranking order of CFU numbers and (especially) the percentages of Penicillium were opposite the ranking orders of the index of soil quality and bacterial CFU numbers (Tables 1 and 2).

The CFU numbers of fungi other than Penicillium (FOTP) on Martin’s medium were highest in the case of Eutric Cambisols (soils 3, 4, and 5) and lowest in the Dystric Cambisols (soils 6 and 7) (Table 3). In comparison with Martin’s medium, the CFU numbers of FOTP, determined on both media with the lower availability of water, decreased substantially, especially in the case of DYSG. These decreases were smallest in the case of the three lightest soils (5, 6, and 7) (Table 3).

The values of XIFC 1 and XIFC 2 (Table 3) increased in the opposite order to the values of the index of soil quality (Table 1) and the bacterial CFU numbers (Table 2) from the best soil (Phaeozem No. 1) to the worst one (Dystric Cambisol No. 7) with an exception – the values concerning loessial Eutric Cambisol No. 3 were higher than those of Eutric Cambisol No. 4, although soil No. 3 was more fertile than soil No. 4. The values of XIFC 3 varied less than those of the other two indices. In this case only the three lightest soils differed from the other soils.

Relationships between Soil Microbial Parameters and Physico-Chemical Soil Features

The bacterial CFU numbers are positively correlated with the index of soil quality, soil pH, volume of pores <3 μm and the content of silt (especially with the content of silt fraction <0.02 mm), water and SOM, and negatively correlated with the sand content, the volume of pores 30-300 μm, MSPart and MSPore (Table 4, Figs. 3 and 4).
The observed weak correlation between the bacterial number and the volume of pores 6-10 μm (Fig. 4) was caused by high volume of this class of pores in soil 1 (which is probably connected with a high content of SOM in this soil) containing the highest number of bacterial CFU. After exclusion of the data for this soil, the bacterial CFU number was only correlated with volume of pores <3 μm (results not shown).

The CFU numbers of fungi recorded on Martin’s medium were not significantly correlated (even at P<0.1) with any of the soil parameters determined (Table 4, Figs. 3 and 4).

Table 4 shows that only a few weak correlations (at P<0.1), which were opposite to those of bacteria, were found in the case of the fungal CFU numbers on DG18. The CFU numbers of all fungi on DG18 were also not significantly positively correlated with content of any texture fraction, but the highest positive correlation coefficients were found in the case of the fungal numbers on Martin’s medium that changed the most and those of fungi on DYG changed the least (Table 4, Figs. 3 and 4). Moreover, Figs. 3 and 4 show that the maxima of correlation coefficient lines concerning fungal CFU numbers on Martin’s medium became shifted to the smaller texture fractions (from 0.1-0.25 mm to 0.02-0.1 mm) and smaller pore classes (from 17-30 μm to 3-6 μm) and the correlation became significant at P<0.05 and P<0.1, respectively. The subtraction of Penicillium CFU numbers also made all correlations concerning fungal numbers on DG18 become insignificant, and correlations of CFU numbers on DYG became insignificant (Table 4, Fig. 3) or less significant (Fig. 4).

Table 4 shows that the correlation coefficients gradually changed from very positive to very negative, and from very negative to very positive in transition from hydrophilic bacteria through different fungal groups with gradually increasing xerotolerance (FOTP on Martin’s medium < FOTP on DG18 < FOTP on DYG < Penicillium). Even stronger correlations (than those concerning Penicillium) were found in most cases between the determined soil features and the xerotolerance indices of the fungal communities (XIFCs) (Table 4, Figs. 3 and 4). All XIFCs are strongly negatively correlated with the index of soil quality, as well as with the soil water content (Table 4), and strongly positively correlated with sand content, MSPart, the content of particles 0.25-2.0 mm (r=0.91, 0.90, and 0.98 at P<0.01 in the case of XIFC 1, XIFC 2, and XIFC 3, respectively) and especially with MSPore (Table 4 and Fig. 5) and the volume of pores 30-300 μm (r=0.96, 0.95, and 0.95 at P<0.01 for XIFC 1, XIFC 2, and XIFC 3, respectively). Moreover, the values of correlation coefficients (significant at P<0.01 or

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**Table 3. CFU numbers of Penicillium and other fungi different from Penicillium in examined soils, as well as xerotolerance indices (XIFCs) of the soil fungal communities.**

| Soils               | Fungal CFU numbers [x 10^3 g⁻¹] on different media after subtracting of Penicillium CFU numbers | Highest measured CFU number of Penicillium on the three media [x 10^3 g⁻¹] (Table 2, columns 9-11) | Xerotolerance indices of fungal communities: 1. (A^*1+B^*2+C^*3+D^*4)/(A+B+C+D)  
2. 1+ [(PM-A) / (PM+ Α)] *  
3. 1+ [(C-A) / (C+ A)] |
<table>
<thead>
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<tbody>
<tr>
<td>Phaeozem</td>
<td>Martin’s (A) 117.2 ab* 29.3 a* 24.3 a* 2.0 1.49 0.00 0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eutric Fluvisol</td>
<td>DG18 (B) 94.4 ab 49.0 ab 21.7 a 3.3 1.61 0.03 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eutric Cambisol</td>
<td>DYG (C) 131.1 ab 52.7 ab 30.0 a 27.3 1.81 0.23 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eutric Cambisol</td>
<td>DYSG (D) 168.1 b 60.7 ab 29.0 a 20.0 1.64 0.15 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eutric Cambisol</td>
<td>154.2 b 70.3 b 58.3 c 63.3 2.09 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystric Cambisol</td>
<td>57.8 a 30.3 a 27.0 a 25.0 2.14 0.51 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystric Cambisol</td>
<td>66.9 a 54.7 ab 44.3 b 93.3 2.63 1.16 0.80</td>
<td></td>
<td></td>
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</tbody>
</table>

* – the means in separate columns marked with different letters are statistically different at P≤0.05; 
# – “PM” is CFU numbers of penicillia on Martin’s medium (see Table 2).

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* After subtraction of Penicillium CFU numbers from the total fungal CFU numbers on all media, all correlation coefficients distinctly changed in direction to those of hydrophilic bacteria. Correlation coefficients concerning fungi on Martin’s medium changed the most and those of fungi on DYG changed the least (Table 4, Figs. 3 and 4). Moreover, Figs. 3 and 4 show that the maxima of correlation coefficient lines concerning CFU fungal numbers on Martin’s medium became shifted to the smaller texture fractions (from 0.1-0.25 mm to 0.02-0.1 mm) and smaller pore classes (from 17-30 μm to 3-6 μm) and the correlation became significant at P<0.05 and P<0.1, respectively. The subtraction of Penicillium CFU numbers also made all correlations concerning fungal numbers on DG18 become insignificant, and correlations of CFU numbers on DYG became insignificant (Table 4, Fig. 3) or less significant (Fig. 4). The observed weak correlation between the bacterial number and the volume of pores 6-10 μm (Fig. 4) was caused by high volume of this class of pores in soil 1 (which is probably connected with a high content of SOM in this soil) containing the highest number of bacterial CFU.
Table 4. Correlation coefficients between bacterial or fungal (all fungi, fungi other than *Penicillium*, and *Penicillium* on different agar media) CFU numbers as well as xerotolerance index of the fungal communities and different soil features.

<table>
<thead>
<tr>
<th></th>
<th>Index of soil quality</th>
<th>Sand content</th>
<th>Silt content</th>
<th>Clay content</th>
<th>Mean size of particle</th>
<th>Mean size of pore</th>
<th>Bulk density</th>
<th>Moisture content</th>
<th>pH (H₂O)</th>
<th>SOM content</th>
<th>Bacterial CFU number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria on soil extract medium</td>
<td>0.97 ***</td>
<td>-0.89 ***</td>
<td>0.83 **</td>
<td>0.53</td>
<td>-0.74 *</td>
<td>-0.84 **</td>
<td>-0.88 **</td>
<td>0.94 ***</td>
<td>0.97 ***</td>
<td>0.80 **</td>
<td>[0.75 *]</td>
</tr>
<tr>
<td>Fungi on Martin’s medium</td>
<td>-0.23</td>
<td>0.04</td>
<td>0.00</td>
<td>-0.39</td>
<td>-0.01</td>
<td>0.18</td>
<td>0.26</td>
<td>-0.28</td>
<td>-0.41</td>
<td>-0.11</td>
<td>[0.34]</td>
</tr>
<tr>
<td>Fungi on DG18 medium</td>
<td>-0.67 *</td>
<td>0.55</td>
<td>-0.48</td>
<td>-0.39</td>
<td>0.46</td>
<td>0.65</td>
<td>0.70 *</td>
<td>-0.74 *</td>
<td>-0.74 *</td>
<td>-0.59</td>
<td>[-0.12]</td>
</tr>
<tr>
<td>Fungi on DYSG medium</td>
<td>-0.84 **</td>
<td>0.78 **</td>
<td>-0.68 *</td>
<td>-0.59</td>
<td>0.73 *</td>
<td>0.86 **</td>
<td>0.68 *</td>
<td>-0.86 **</td>
<td>-0.82 **</td>
<td>-0.53</td>
<td>[-0.43]</td>
</tr>
<tr>
<td>Fungi on Martin’s medium without <em>Penicillium</em></td>
<td>0.42</td>
<td>-0.50</td>
<td>0.60</td>
<td>-0.02</td>
<td>-0.68 *</td>
<td>-0.54</td>
<td>-0.28</td>
<td>0.40</td>
<td>0.14</td>
<td>0.30</td>
<td>[0.77 *]</td>
</tr>
<tr>
<td>Fungi on DG18 medium without <em>Penicillium</em></td>
<td>-0.23</td>
<td>0.08</td>
<td>-0.10</td>
<td>0.02</td>
<td>-0.05</td>
<td>0.13</td>
<td>0.46</td>
<td>-0.35</td>
<td>-0.34</td>
<td>-0.41</td>
<td>[0.57]</td>
</tr>
<tr>
<td>Fungi on DYSG medium without <em>Penicillium</em></td>
<td>-0.62</td>
<td>0.58</td>
<td>-0.47</td>
<td>-0.54</td>
<td>0.50</td>
<td>0.63</td>
<td>0.43</td>
<td>-0.64</td>
<td>-0.65</td>
<td>-0.32</td>
<td>[0.08]</td>
</tr>
<tr>
<td><em>Penicillium</em> on Martin’s</td>
<td>-0.82 **</td>
<td>0.79 **</td>
<td>-0.72 *</td>
<td>-0.52</td>
<td>0.80 **</td>
<td>0.89 ***</td>
<td>0.71 *</td>
<td>-0.88 **</td>
<td>-0.75 *</td>
<td>-0.51</td>
<td>[0.50]</td>
</tr>
<tr>
<td>Xerotolerance index #1</td>
<td>-0.94 ***</td>
<td>0.89 ***</td>
<td>-0.85 **</td>
<td>-0.50</td>
<td>0.92 ***</td>
<td>0.99 ***</td>
<td>0.80 **</td>
<td>-0.95 ***</td>
<td>-0.83 **</td>
<td>-0.69</td>
<td>[0.82 **]</td>
</tr>
<tr>
<td>Xerotolerance index #2</td>
<td>-0.92 ***</td>
<td>0.89 ***</td>
<td>-0.83 **</td>
<td>-0.54</td>
<td>0.91 ***</td>
<td>0.98 ***</td>
<td>0.78 **</td>
<td>-0.95 ***</td>
<td>-0.82 **</td>
<td>-0.61</td>
<td>[0.76 *]</td>
</tr>
<tr>
<td>Xerotolerance index #3</td>
<td>-0.91 ***</td>
<td>0.93 ***</td>
<td>-0.90 ***</td>
<td>-0.47</td>
<td>0.98 ***</td>
<td>0.98 ***</td>
<td>0.67 *</td>
<td>-0.90 ***</td>
<td>-0.73 *</td>
<td>-0.57</td>
<td>[0.84 **]</td>
</tr>
</tbody>
</table>

***,**,* significant at *P*≤0.001, *P*≤0.05 and *P*≤0.1, respectively (*n*=7).
The values in square brackets are obtained after exclusion of the Phaeozem data (*n*=6).
<0.05) between all XIFCs and the volume of pores 30-50 μm, 50-100 μm, and 100-300 μm gradually increased in transition from the smallest pores (r=0.90, 0.87, and 0.87 for XIFC 1, XIFC 2, and XIFC 3, respectively) through medium pores (r=0.94, 0.92, and 0.91), to the largest pores (r=0.98, 0.98, and 0.98). Correlation of XIFCs with silt content, bulk density and soil pH, although significant at P<0.05 or <0.01, were distinctly weaker (Table 4).

Discussion

The plots used in this study, established at the end of the 19th century, are special. The soils of the plots, very different in their physico-chemical features, can be compared under the same weather conditions. The structure of the soils is not destroyed by compaction because there is no wheel traffic and they have been always cultivated by hand tillage.

Fig. 3. Correlations between the CFU numbers of different groups of soil microorganisms (as well as XIFC 1 values) and the content of various particle-size fractions. Significance limits of the correlation coefficients: 0.87, 0.75, 0.67 at P<0.01, 0.05, and 0.1, respectively.

Fig. 4. Correlations between the CFU numbers of different groups of soil microorganisms (as well as XIFC 1 values) and the volume of various pore-size classes. Significance limits of the correlation coefficients: 0.87, 0.75, 0.67 at P<0.01, 0.05, and 0.1, respectively.
Furthermore, the same plants are always grown in all plots at the same time, and they receive the same or very similar fertilization. Owing to the exclusion of influence of these factors, the soil quality in these plots, including the size and distribution of soil pores as well as the numbers and distribution of different groups of soil microorganisms in the soil structure, are mainly dependent on the soil texture and SOM content as well as soil pH.

The soil bacteria, restricted to water films, were mostly associated with the silt fraction <0.02 mm and pores with a neck diameter <3 µm, whereas fungi, not restricted to water films due to the formation of hyphae, were connected with the particle size fraction >0.02 mm and pores >3 µm (Figs. 3 and 4). These results are consistent with the literature data and confirm the knowledge that fungi and bacteria occupy two separate soil microenvironments. Kandeler et al. [41] reported that the total bacterial phospholipid fatty acids (PLFAs) increased with diminishing soil particle size, whereas fungal PLFAs decreased. Also Chiu et al. [42] reported that the larger-sized fractions contained more fungal ergosterol than the smaller ones. Hattori [43] suggested that the living space within soil aggregates can be divided into two categories: one is the inner part that consists of smaller pores and the other is the outer part that consists of larger pores. The majority of bacteria were found in the inner part, but most of the fungi were located in the outer part. The critical size of pores dividing them into the two parts was estimated to be between 2.5 and 6 µm in the diameter of the pore neck. Similarly, Strong et al. [44] found that microbial biomass determined after fumigation with chloroform was correlated with pores <3 µm, and ergosterol concentrations were most positively correlated with pores 15-60 µm.

As expected, the bacterial CFU number was positively related to soil features beneficial from a viewpoint of their fertility (positive correlations with the index of soil quality, pH, the volume of pores with neck diameters 0.5-3 µm and <0.5 µm, the content of silt, SOM and water and negative correlations with sand content, bulk density, MSPart, MSPore and the volume of pores 30-300 µm), whereas the total number of fungi, presented as the fungal CFU number on Martin’s medium, containing easily available water, did not show significant correlations with any parameters determined. This means for us, that this group of fungi is a mixture of both nonxerophilic and xerophilic organisms. However, fungal CFU numbers on the media with a reduced availability of water, especially on DYSG, indicated opposite relationships with the soil parameters determined to those of bacteria (Table 4, Figs. 3 and 4). This means that these groups of fungi (particularly determined on DYSG) consist mainly of xerophilic organisms.

We decided to determine the CFU numbers of Penicillium and Aspergillus genera because they are known to be very xerotolerant and even xerophilic [45]. In our opinion, their CFU numbers could better reflect the dryness of the coarse-textured soils than those of total xerotolerant/xerophilic fungi. Kouyeas [22] reported that Penicillium spp. and Aspergillus spp. appeared to be the most drought-tolerant soil fungi, being able to grow at a relative humidity as low as 86 or even 84 percent. They appeared to be suppressed in soil at high water content and were rarely observed in wet soil. These fungi appeared to become active only as soil moisture stress exceeded the 1-atmosphere level (pF 3). They were dominating at water content corresponding to soil moisture stress levels of 15 to 20 atmospheres (pF 4.2-4.3) [22]. Therefore, fungal flora of pure sands of Grande Erg dunes was represented mainly by Penicillium spp. [46], and about 70% of fungi in the outer part of the soil aggregates, and only 5% in the inner part (described above) belonged to the xerophilic genera Aspergillus and Penicillium [43].

![Fig. 5. Relationships between the mean size of soil pores and values of the xerotolerance indices of the fungal communities. (A – XIFC 1, B – XIFC 2, and C – XIFC 3).](image-url)
In our paper only CFU of penicillia are presented, because no colony of Aspergillus was found after inoculation of the agar media with the soil suspensions. It is consistent with the data presented by Domshch et al. [47] that Aspergillus occurs mainly in warmer regions, and Penicillium species predominate in soils of temperate regions.

We assumed that the numbers of penicillia should also be a very good biological indicator of soil acidity, because, among soil fungi, penicillia are especially tolerant to low pH. Król et al. [48] reported that in highly acidic soils (pH 4 and lower) the fungal communities almost exclusively consisted of the genus Penicillium. Penicillia were also more predominant in untreated soils (pH 4.9-5.5) than in those treated with mineral fertilizers (pH 6.0-6.8) [49]. Similarly, on the basis of results presented by Anderson and Domshch [50], significant negative correlation (at \( P<0.1 \)) could be found between soil pH and the percentage of penicillia in the communities of soil fungi.

The CFU number of penicillia in the fungal communities on all used agar media proved to be very well related to the studied features of coarse-textured soils that are disadvantageous to soil fertility. The values of CFU number of penicillia were significantly negatively correlated with soil index quality, soil pH and the silt and water contents, and positively correlated with sand content (Table 4). Very high positive correlations with the particle size fraction 0.25-2 mm and the volume of pore class with neck diameters of 30-300 μm suggest that CFU of these xerophilic microorganisms were located (probably mainly in the form of conidia) in a soil microenvironment usually dry, having only thin water films [44], on the opposite side from bacteria, which were predominantly placed in a soil microenvironment usually wet – the particle size fraction <0.02 mm and pores <3 μm (Figs. 3 and 4). These results are consistent with data of Elmholt and Labouriau [51], who reported that organic farming soils with low clay contents had significantly (at \( P<0.01 \)) more Penicillium spp., determined on the DG18, than organic farming soils with a high clay content.

After subtraction of CFU numbers of penicillia from total CFU numbers of fungal communities on Martin’s, DG18 and DYSG media, three communities of fungi other than Penicillium (FOTP) with gradually increasing level of xerotolerance were obtained. Correlation coefficients (Figs. 3 and 4) suggest that FOTP determined on Martin’s medium were mainly hydrophilic. They were presumably associated with the medium particle size fraction (0.02-0.1 mm) and medium pores (3-6 μm) – which belonged to the soil microenvironment, often containing water and responsible for the retention of water easily available to plants [52], but they were rather not connected with the coarse sand fractions (0.25-2 mm) and big pores (30-300 μm). It should be mentioned that these data correspond to the effective neck diameters of the highest pores filled with water at the time of sampling (1-8 μm) derived from the matric potential values (pF 2.6-3.6) (Table 1). This is the region near the air-water interface, supplying the soil microorganism both in water and oxygen. Strong et al. [45] found that at matric potential of -75 kPa (pF 2.9), pores 4-8 μm were the soil region of fastest decomposition of added plant material.

FOTP determined on DG18 were probably an equal mixture of hydrophilic and xerophilic microorganisms, because their CFU numbers did not correlate with any particle size fraction and any pore class (Figs. 3 and 4). However, FOTP determined on DYSG consists from xerophilic organisms in the greater degree than nonxerophilic ones because its CFU were rather associated with pores 30-300 μm and sand fractions (0.1-2 mm) (Figs. 3 and 4).

On the basis of CFU numbers of four fungal communities with gradually increasing level of xerotolerance (FOTP on Martin’s medium < FOTP on DG18 < FOTP on DYSG < penicillia), we formulated mathematical indices (XIFC 1, XIFC 2, and XIFC 3) presenting in one number the level of xerotolerance of fungal communities in examined soils. All these indices are better related to soil features determined than CFU numbers of xerotolerant/xerophilic fungi and penicillia. The correlation presented in Table 4 and Figs. 3 and 4 between the values of these indexes and values of various parameters, both describing the fertility and physical, chemical and biological properties of the soils, are much stronger in most cases than those of CFU numbers (Table 4 and Figs. 3 and 4) of total xerotolerant/xerophilic fungi or penicillia.

The values of XIFCs were strongly correlated with MSPore (Table 4 and Fig. 5), the volume of pores 30-300 μm (especially of pores 100-300 μm), the soil water content as well as with MSPart (Table 4), which suggests that these indices can reflect the air-water relationships in the soil environment.

On the basis of XIFC 1 and 2, MSPore and volume of pores 30-300 μm, the examined soils can be separated into four groups:

1. Phaeozem No. 1, Eutric Fluvisol No. 2 and Eutric Cambisol No. 4;
2. the loesial Eutric Cambisol No. 3;
3. Eutric Cambisol No. 5 and Dystric Cambisol No. 6; and
4. Dystric Cambisol No. 7.

It should be mentioned that loesial Eutric Cambisols No. 3 has high water content similar to Eutric Fluvisol No. 2, but it has higher values of XIFCs and MSPore, because besides its high volume of small (<3 μm) and medium (3-30 μm) pores, it contains a fairly high volume of big pores (30-300 μm) (Table 1 and Fig. 2). It is consistent with data presented by Konecka-Betley et al. [53], who reported that loesial Eutric Cambisols are characterized by a fairly high content of macropores.

Conclusions

1. The CFU numbers of soil bacteria, contrary to the CFU numbers of xerophilic fungi, were positively related to the soil water, silt fraction <0.02 mm and SOM content, the volume of pores <3 μm, pH and the index of quality of the soils;
2. The CFU numbers of xerophilic fungi (especially the CFU numbers of penicillia), contrary to the soil bacteria, well reflected the soil features facilitating soil to be dry as the content of sand ≥ 0.25 mm and the volume of pores 30-300 μm;

3. The CFU numbers of nonxerophilic fungi were mainly related to the soil environment intermediate to those of bacteria and xerophilic fungi – medium soil pores (3-6 μm) and soil particles 0.02-0.1 mm;

4. The indices of xerotolerance of the soil fungal communities, presenting the relationships between hydrophilic, xerotolerant and xerophilic fungi, were evidently better related to soil dryness than the CFU numbers of total xerotolerant/xerophilic fungi or penicillia.

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