The Ratio of Fungi and Bacteria in the Biomass of Different Types of Soil Determined by Selective Inhibition

N. D. Ananyeva\textsuperscript{a,1}, E. A. Susyan\textsuperscript{a}, O. V. Chernova\textsuperscript{b}, I. Yu. Chernov\textsuperscript{c}, and O. L. Makarova\textsuperscript{b}

\textsuperscript{a} Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, ul. Institutskaya 2, Pushchino, Moscow oblast, 142290 Russia
\textsuperscript{b} Severtsev Institute of Ecological and Evolutionary Problems, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 119071 Russia
\textsuperscript{c} Moscow State University, Vorobyevy gory 1, Moscow, 103009 Russia

Received March 6, 2006

Abstract—Tundra, chernozem (virgin and arable), soddy-podzolic (coniferous forest, meadow, and arable), and grey forest (larch forest) soils were used to separate the contributions of fungi and bacteria to substrate-induced respiration (SIR) with the help of antibiotics. For soils with a high content of organic matter (tundra and chernozem: 12 and 8\%, respectively), the procedure of selective inhibition of SIR has been optimized. This procedure consists in application of high concentrations of streptomycin (50–120 mg/g of soil) and cycloheximide (50–80 mg/g of soil) and decreasing the weight of the analyzed soil sample. Soils under study have shown the predominant contribution of fungi (63–82\%) to the total SIR. The fungal–bacterial ratio in the soils of natural ecosystems (0–5 cm, without litter) was 4.3, 2.2, 1.5, and 1.5 for tundra soil, virgin chernozem, coniferous (soddy-podzolic soil), and larch (grey forest soil) forests, respectively. The lower layers of soddy-podzolic (5–10 cm) and grey forest (48–58 cm) soils showed a decrease in the fungal and increase in the bacterial component in the total SIR.

DOI: 10.1134/S0026261706060130

Key words: soil, antibiotics, fungi, bacteria.

Assessment of the contribution of microscopic fungi and bacteria to microbial biomass and the change of their ratio in different types of soil, including arable ones, is the key problem of soil microbiology associated with the study of the functioning of terrestrial ecosystems [1–3]. Direct microscopic methods successfully differentiate fungi and bacteria in soil but do not reveal their activities. The method of selective inhibition based on the separation of fungal and bacterial substrate-induced respiration by antibiotics evaluates, to a certain extent, their ratio in microbial biomass [4]. The complexity of this method is in the strict observation of experimental conditions (the synergetic effect of bactericides and fungicides must not exceed 5\%) for reliable calculation of the fungal–bacterial ratio in each type of soil [4–7].

It has been shown that fungi dominate in soils for the most part [1, 4, 8], but there are reports on bacterial dominance as well [9, 10]. The fungal–bacterial ratio is used as a parameter of the microbial community structure depending on, e.g., humidity gradient [11], distance from pollution source [12], agricultural practices [13], and decomposition of plant debris [14]. The ratio of fungi and bacteria in soil has been shown to correlate with environmental factors such as pH [3], combination of pH and plant substrate [15], and the content of organic carbon in soil [2]. However, there are very few data on the structure of microbial communities in different types of soil, including those with contrasting properties (C\textsubscript{org}, pH, vegetation).

The goal of our study was to assess the contribution of fungi and bacteria to substrate-induced respiration (SIR) in different types of soil by the method of selective inhibition. The objectives of the research were (1) to optimize the procedure of separation of the contributions of fungi and bacteria to SIR in soils with high content of organic matter; (2) to determine the structure of microbial communities in different types of soil and at different land use practices; and (3) to determine the fungal–bacterial ratio in different soil layers.

MATERIALS AND METHODS

Soils (tundra, soddy-podzolic, grey forest, chernozem) of different ecosystems (forest, meadow, virgin, arable) were the object of the present research. The samples were taken from the upper humus horizon (0–5 cm), from no less than five points. The samples...
The rate of SIR was expressed in gas chromatography. The time of gas sampling was with glucose was incubated for 3–5 h at metabolically sealed and the time was recorded. The soil concentration of 10 mg/g soil); then the vial was hermetically sealed and the time was recorded. The soil was also added to the soil without cycloheximide.

were averaged, sifted through a 2-mm sieve, and stored in a refrigerator at 8–10°C for no more than three weeks before the beginning of experiments. The samples were wet, with the water content of no less than 20% of soil weight. Additional samples were taken from the 5- to 10-cm and 48- to 58-cm layers of forest soddy-podzolic (spruce) and grey forest (larch) soil, respectively. The physicochemical characteristics of the soils under study are given in Table 1.

**Table 1.** Some properties of the soils under study (SIR, substrate-induced respiration)

<table>
<thead>
<tr>
<th>Type of soil</th>
<th>Sampling region (site)</th>
<th>Ecosystem</th>
<th>Dominant vegetation</th>
<th>C_{org}, % (depth, cm)</th>
<th>pH_{water}</th>
<th>C : N in soil</th>
<th>SIR, µg C-CO_2 g^{-1} h^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tundra</td>
<td>Nenets Autonomous Area (Dolgii Island)</td>
<td>tundra</td>
<td>forbs–mossy</td>
<td>12.28 (0–5)</td>
<td>4.20</td>
<td>6.2</td>
<td>62.08 ± 6.55</td>
</tr>
<tr>
<td>soddy-podzolic</td>
<td>Tver region (Savelovo)</td>
<td>forest</td>
<td>spruce</td>
<td>2.21 (0–5)</td>
<td>4.50</td>
<td>7.3</td>
<td>11.53 ± 0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>meadow</td>
<td>grass</td>
<td>1.59 (5–10)</td>
<td>4.35</td>
<td>7.2</td>
<td>6.08 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>arable</td>
<td>rye</td>
<td>0.72 (0–5)</td>
<td>5.35</td>
<td>13.8</td>
<td>7.10 ± 1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>forest</td>
<td>larch</td>
<td>1.39 (0–5)</td>
<td>6.10</td>
<td>9.9</td>
<td>5.49 ± 1.00</td>
</tr>
<tr>
<td>Grey forest</td>
<td>Moscow region (Pushchina)</td>
<td>forest</td>
<td>larch</td>
<td>2.42 (0–5)</td>
<td>5.95</td>
<td>3.0</td>
<td>6.31 ± 0.22</td>
</tr>
<tr>
<td>Leached chernozem</td>
<td>Penza region (Popercheneoe)</td>
<td>virgin</td>
<td>meadow steppe</td>
<td>0.28 (44–58)</td>
<td>5.90</td>
<td>n/d*</td>
<td>1.86 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>arable</td>
<td>beet</td>
<td>8.04 (0–5)</td>
<td>5.85</td>
<td>11.6</td>
<td>26.00 ± 0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.38 (0–5)</td>
<td>6.25</td>
<td>12.2</td>
<td>10.41 ± 0.80</td>
</tr>
</tbody>
</table>

* n/d, not determined.

All respirometric measurements were made in soil samples after preliminary incubation at 22°C and humidity about 55% of WHC for five days.

**Coefficient of antibiotic activity overlap**, or inhibitor additivity ratio (IAR), has been calculated from the equation IAR = [(A – B) + (A – C)] / (A – D), where A is the respiration (CO₂ production) of soil with glucose; B is the respiration of soil with glucose and fungicide; C is the respiration of soil with glucose and bactericide; D is the respiration of soil with glucose, bactericide and fungicide [2]. If IAR = 1, there is no overlapping antibiotic effect on non-target microorganisms or antagonistic effect of one antibiotic on the other (lower effectiveness of streptomycin and cycloheximide at their combined introduction). At IAR > 1, the overlapping antibiotic effect is induced, which points to the low reliability of determining the fungal–bacterial ratios; IAR < 1 indicates the presence of an antagonistic effect.

**The ratio of fungal (F) and bacterial (B) contributions to soil SIR** has been determined according to the formulas: F = (A – B)/(A – D) × 100%; B = (A – C)/(A – D) × 100% (designations as above), provided that A – [(A – B) + (A – C)] = D ± 5% [5].

All measurements were made in five replicates. Standard deviation for SIR inhibition and the contributions of fungi and bacteria to the total biomass were calculated as an error of the function (quotient) of random variables by the formula: 

\[
s_y = \frac{1}{z^2} \sqrt{\left(\frac{sx}{z^2}\right)^2 + \left(\frac{s_z}{z^2}\right)^2},
\]

where 

- \( s_y \) was the standard deviation for the quotient; 
- \( x \) was the numerator value; 
- \( z \) was the denominator value; 
- \( s_x \) and \( s_z \) were the standard deviations for \( x \) and \( z \) [16].