ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 6, pp. 702–707. © Pleiades Publishing, Inc., 2006.
Original Russian Text © N.D. Ananyeva, E.A. Susyan, O.V. Chernova, I.Yu. Chernov, O.L. Makarova, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 6, pp. 807–813.

EXPERIMENTAL ARTICLES

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

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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 synergic 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_{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 soils 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

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

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

* n/d, not determined.

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.

Substrate-induced respiration (SIR) of soil was assessed by the rate of initial maximal respiration of microorganisms after glucose introduction into soil. A weighed soil sample (1 g) was placed into a vial (15 ml) and 0.2 ml of glucose solution was added (to the final concentration of 10 mg/g soil); then the vial was hermetically sealed and the time was recorded. The soil with glucose was incubated for 3–5 h at 22°C; then an air sample was taken from the vial and analyzed on a gas chromatographer. The time of gas sampling was also recorded. The rate of SIR was expressed in μg C-CO_2 g^{-1} of soil h^{-1}.

Antibiotics. Preliminary experiments were performed to determine the concentration of antibiotic (streptomycin and cycloheximide were introduced separately) and the time of its contact with soil before glucose addition, which provided the greatest inhibition of SIR of soil microorganisms. Streptomycin sulfate (aqueous solution) and cycloheximide (powder) were introduced into soil separately and in combination; SIR was measured after glucose introduction. Cycloheximide in different concentrations and streptomycin were added 4 and 0.5 h before glucose addition, respectively. In the case of combined introduction of antibiotics, cycloheximide was incubated with soil for 4 h, then streptomycin was added, and glucose was introduced after 0.5 h. The soil sample containing glucose only was used as a control. Soil humidity in all variants of the experiment was 60–65% of total water holding capacity (WHC). For better distribution of cycloheximide in soil, an inert material (talcum) was used in the mass ratio of cycloheximide : talcum = 1 : 2. Talcum was also added to the soil without cycloheximide.

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 (CO_2 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: $s_y = \frac{\sqrt{(xs_z)^2 + (zs_x)^2}}{z^2}$, where s_y was the standard deviation for the quotient; x was the numerator value; z was the denominator value; and s_x and s_z were the standard deviations for x and z [16].

Table 2. Maximal inhibition of SIR in virgin chernozem soil under the effect of antibiotics (IAR = 1.05)

Introduced into soil, mg/g	SIR inhibition (average ± standard deviation)	
	μg C-CO_2 g^{-1} soil h^{-1}	% of control
Glucose control	34.91 ± 0.17	0
Streptomycin (50)	23.60 ± 1.68	32 ± 5
Cycloheximide (60)	18.41 ± 1.24	47 ± 4
Streptomycin (50) + cycloheximide (50)	10.63 ± 0.74	70 ± 2

Table 3. Inhibition of SIR by antibiotics in arable chernozem

Introduced into soil, mg/g		SIR inhibition*	
		μg C-CO_2 g^{-1} soil h^{-1}	% of control
Glucose control		13.33 ± 0.27	0
Streptomycin, 20		8.72 ± 0.56	35 ±4
Cycloheximide, 40		6.01 ± 0.43	55 ± 3
Streptomycin + cycloheximide (IAR)	10 + 40	4.88 ± 0.82	63 ± 6 (1.22)
	20 + 40	4.78 ± 0.34	64 ± 3 (1.40)
	10 + 30	2.57 ± 0.13	81 ± 1 (1.11)

* Average ± standard deviation.

Table 4. Inhibition of SIR by antibiotics in tundra soil (weighed sample = 0.25 g)

Introduced into soil, mg/g		SIR inhibition*	
		μg C-CO_2 g^{-1} soil h^{-1}	% of control
Glucose control		72.94 ± 5.58	0
Streptomycin, 80		67.03 ± 1.46	8 ± 2
Streptomycin, 120		63.75 ± 5.25	13 ± 3
Cycloheximide, 40		38.87 ± 1.86	47 ± 3
Cycloheximide, 80		33.41 ± 2.31	54 ± 3
Streptomycin + cycloheximide(IAR)	80 + 40	33.31 ± 1.03	54 ± 1 (1.01)
	120 + 40	30.03 ± 1.30	59 ± 2 (1.01)
	80 + 80	24.21 ± 0.60	67 ± 1 (0.93)
	120 + 80	24.58 ± 0.48	66 ± 1 (1.01)

* Average ± standard deviation.

RESULTS

Tundra and chernozem soils contain a lot of organic carbon (12 and 8%, respectively), while grey forest and soddy-podzolic soils have about 2% (Table 1). Soils with the high content of organic carbon presumably require higher antibiotic concentrations to inhibit the fungal and bacterial SIR. The maximal inhibition of SIR in virgin chernozem soil was revealed at 50 mg/g soil for streptomycin and at 60 mg/g for cycloheximide (Table 2). In arable chernozem soil, in contrast to virgin soil, SIR was most inhibited at low antibiotic concentrations: 20 and 40 mg/g for streptomycin and cycloheximide, respectively (Table 3). It was also revealed that the antibiotic concentrations providing maximal inhibition of chernozem SIR were lower in combination than in the case of individual introduction (Table 2, 3). In arable chernozem soil, SIR inhibition by antibiotics was 63–81% of the control; however, the IAR value was relatively satisfactory (for calculation of the fungal–bacterial ratio) in the variant with 10 + 30 mg of bactericide and fungicide per 1 g of soil (Table 3).

Preliminary experiments with tundra soil showed that high antibiotic concentrations provided no more than 40% of SIR inhibition. For more intense SIR inhibition in tundra soil, the weight of the analyzed samples was reduced fourfold, to 0.25 g. The maximal SIR inhibition in this weighed soil sample was 13% for streptomycin (120 mg/g) and 54% for cycloheximide (80 mg/g) (Table 4). At the same time, the combined introduction of antibiotics caused SIR inhibition of 54 to 67%, while the difference of IAR value from 1 was within 5%, except for the variant with streptomycin + cycloheximide in concentrations of 80 + 80 mg/g soil (IAR = 0.93).

Thus, high antibiotic concentrations in combination with lower analyzed sample weight should be used for soils with high content of organic matter (tundra, chernozem).

In soddy-podzolic soil with low content of organic matter, the maximal SIR inhibition was observed at lower antibiotic concentrations as compared with tundra and chernozem soils (Table 5). Streptomycin in a concentration of 30 mg/g of soil inhibited SIR by 32, 21, and 9% of the control in forest, meadow, and arable soddy-podzolic soils, respectively. Cycloheximide had the greatest inhibiting effect in concentrations of 20 (arable soil) and 30 (forest, meadow) mg/g of soil. Combined introduction of antibiotics resulted in SIR suppression in forest soil by 74% and in meadow and arable soils by 63% and 45%, respectively.

Thus, combined introduction of antibiotics into analyzed soils with different contents of organic matter resulted in SIR inhibition by 45 to 81% of the control, and the minimal inhibition (45%) was observed in arable soddy-podzolic soil.

The fungal and bacterial contributions to the total SIR (or biomass) of analyzed soils were calculated on the basis of satisfactory IAR values (1 ± 5%) (the figure). The maximal contribution of fungi was in the tundra soil (F : B = 4.32). The F : B ratios in virgin and arable chernozem soils were 2.09 and 1.58, respectively. For soddy-podzolic soil, the minimal fungal contribution to the total SIR (63%) was in spruce forest and the maximal one was in arable soil (80%). The F : B values

Table 5. Inhibition of SIR (% ± standard deviation) by antibiotics in soddy-podzolic soil of different ecosystems

Introduced into soil, mg/g		Ecosystem		
		forest	meadow	arable
Streptomycin	10	29 ± 3	5 ± 4	4 ± 2
	20	35 ± 4	19 ± 3	5 ± 3
	30	32 ± 3	21 ± 2	9 ± 2
Cycloheximide	10	30 ±8	34 ± 7	28 ± 2
	15	31 ± 3	43 ± 2	35 ± 3
	20	n/d*	39 ± 1	39 ± 2
	30	35 ± 3	44 ± 4	n/d
Streptomycin + cycloheximide (IAR)	10 + 10	54 ± 2 (1.10)	41 ± 3 (0.96)	38 ± 2 (0.85)
	20 + 15	74 ± 1 (1.06)	49 ± 2 (1.27)	35 ± 4 (1.10)
	30 + 20	44 ± 3 (1.31)	59 ± 4 (1.00)	44 ± 2 (0.99)
	30 + 30	n/d	63 ± 2 (1.03)	45 ± 3 (1.05)

* n/d, not determined.

Table 6. The maximal inhibition of SIR and the fungal–bacterial biomass ratio in different layers of soddy-podzolic and grey forest soils

Soil vegetation	Depth of sampling, cm	Maximal inhibition of SIR, %			Contribution to biomass, %	
		streptomycin	cycloheximide	streptomycin + cycloheximide	fungi	bacteria
		mg antibiotic/g soil				
Grey forest larch	0–5	31 ± 4 (10)	47 ± 1 (40)	73 ± 1 (5 + 40)	64 ± 2	42 ± 2
	48–58	46 ± 0 (10)	51 ± 1 (20)	87 ± 1 (5 + 20)	59 ± 2	52 ± 2
Soddy-podzolic spruce	0–5	32 ± 2 (30)	47 ± 1 (40)	74 ± 2 (20 + 15)	63 ± 3	43 ± 4
	5–10	24 ± 1 (30)	46 ± 1 (40)	66 ± 1 (20 + 15)	70 ± 1	37 ± 1

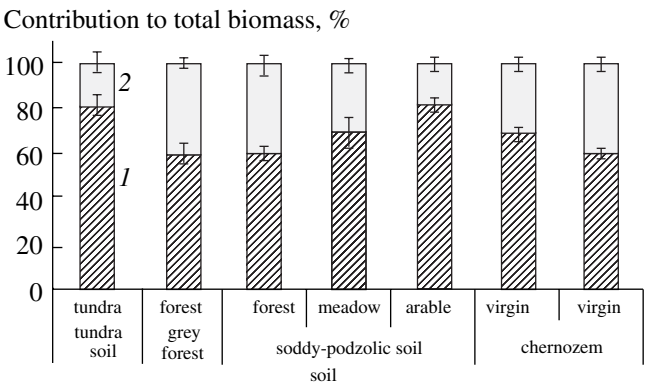
in soddy-podzolic soil were 1.47, 2.12, and 4.0 for coniferous forest, meadow, and arable soil, respectively. It should be mentioned that the F : B ratio in grey forest soil of larch forest was 1.52, and this value was close to that for the soddy-podzolic soil of spruce forest.

The fungal and bacterial contributions to SIR (biomass) of different soil layers were assessed. In the 0- to 5-cm layer of the grey forest soil of the larch forest, the maximal SIR inhibition by cycloheximide was achieved at 40 mg/g of soil, while in the 48- to 58-cm layer it was at a two times lower concentration and more intensive SIR inhibition (Table 6). The maximal inhibition of bacterial respiration in both layers of grey forest soil was observed at the same streptomycin concentrations (10 mg/g). The content of fungi in the lower horizon of grey forest soil was less than in the upper one. The maximal SIR inhibition in soddy-podzolic soil was achieved at equal antibiotic concentrations, which was evidence of the approximately equal contributions of fungi to the microbial biomass of the upper layers under study.

Thus, in the microbial communities of spruce (soddy-podzolic) and larch (grey forest) forest soils, the fungal biomass constituted about 60%; more than one third of the microbial substrate respiration of these soils was bacterial. The microbial communities of deep soil horizons showed a tendency towards a decrease of the fungal component and an increase of the bacterial one.

DISCUSSION

The specificities of the procedure of separation of fungal and bacterial respiration in soils with a high content of organic matter (tundra, chernozem) consisted in the application of high antibiotic concentrations for SIR inhibition and in decreasing the weight of the analyzed samples. Other works reported that sufficient SIR inhibition in arable soils was provided by antibiotic concentrations of 0.5–3 mg/g [4], while carbon-enriched beech forest soils reqired higher concentrations, up to 16 mg/g [17]. The authors noted the probable deactivation of antibiotics in soils enriched in organic matter due to their chemical absorption by



The fungal : bacterial ratios in the biomass of different types of soil (determined by SIR method): *1*, fungi; *2*, bacteria.

humus substances and utilization by autochthonous microorganisms as a substrate [6].

It is no less evident that soil acidity also plays a role in the selection of antibiotic concentrations providing SIR inhibition. Cycloheximide is a neutral antibiotic which binds weakly to soil and remains active in it, while streptomycin is an alkaline antibiotic which can be absorbed and inactivated by soil [5]. The tundra soil under study was not only characterized by a high content of organic carbon, but also had a low pH value (4.2; Table 1). Therefore, the maximal SIR inhibition in tundra soil required high streptomycin concentrations (80–120 mg/g).

Another approach to the optimal separation of fungal and bacterial contributions to the total SIR of soil with high organic carbon content was to decrease the weight of the analyzed samples. This procedure was also used in the work of Bailey et al. [2], where the fungal and bacterial biomass ratio was determined in soils (desert, prairie, forest, arable) with different organic matter contents (the weight of the analyzed samples was 1 to 5 g). It should be noted that reduction of the weight of the tundra soil sample results in a decrease in the sample volume (low specific weight) and thereby in better distribution of antibiotics in soil.

In our experiments, SIR inhibition by the two antibiotics in analyzed soils reached 45–87%. Minimal SIR inhibition (45%) was registered in arable soddy-podzolic soil; maximal inhibition (87%) was found in the illuvial horizon (48–58 cm) of grey forest soil. Different authors evaluate SIR inhibition at combined introduction of antibiotics as 40–70% [8, 14]. For instance, it was shown that the maximal SIR inhibition in different layers of forest litter was 37–64%, while in the humus layer it was only 27% [18]. In the meadow, arable, and forest soils (0–10 cm) of England, SIR inhibition by antibiotics was 47–53% [5]. The study of soils under arboreal and herbaceous vegetation in various climatic conditions showed the maximal suppression of respiration by antibiotics of 29–59% [2].

The structure of a microbial community (fungi : bacteria) is the most important characteristic of soil functioning, which is associated with carbon storage in terrestrial ecosystems. In the soils under study, the fungal contribution to the total SIR was 63–82% and the bacterial component was minor (18–37%). We have recorded the fungal biomass as highest in tundra soil (81%), lower in chernozem (68–71%), and lowest in soddy-podzolic soil under spruce forest (63%).

The fungal–bacterial ratio in the analyzed soils was 1.5–4.3. This value was the highest in tundra soil. Other authors also mentioned that the fungal–bacterial ratio in the tundra soil of Taimyr was rather high: 8.2–11.3 and 3.6–14.9 for the layers of 0–5 and 5–10 cm, respectively [10]. For the soddy-podzolic soils investigated, the fungal–bacterial ratio was higher in arable soil (4.0) than in the forest and in the meadow (1.5 and 2.1, respectively). This fact can probably be explained by the difference in the physicochemical properties of soddy-podzolic soils in different ecosystems. Previously it has been mentioned that the fungal–bacterial ratio increases together with the increase of C : N in soil [19]. For the three analyzed ecosystems of soddy-podzolic soil, this condition is true (Figure, Table 1). In addition, the acidic soil of pine forest (pH_{KCl} 2.6) was shown to contain more bacteria (F : B = 0.3) as compared with arable soils (pH_{KCl} 3.8–5.8) [9]. In [3], the study of soils of the broad-leaved forests (0–10 cm) of Germany with different pH values (3.0–7.2) showed a significant decrease in the fungal–bacterial ratio upon increasing pH. In these soils, the F : B ratio was 9 at pH 3 and 2 at pH 7. The combination of such factors as pH and plant substrate also proved to influence the fungal–bacterial ratio [15]. It was mentioned that the quantity and physiological diversity of bacteria increased on coniferous substrate as compared with leaf waste [20]. In [2], Bailey et al. reported the fungal–bacterial ratio of 1.1 in a pine forest and 13.5 in a prairie. The fungal–bacterial ratio in traditionally arable soils was low: 0.5–0.6 [9], while in pasture soil it was 1.0 [8]. In addition, it was hypothesized that soils where fungi are dominant provide better maintenance of the carbon pool [2].

Thus, the result of our studies was the optimization of the procedure for separating the fungal and bacterial contributions to microbial biomass (the method of selective inhibition) in different soils, including those with high content of organic matter. Inhibition of substrate-induced respiration (SIR) by antibiotics at their combined introduction was 45–87% of the total SIR in soils under study, which significantly exceeded this value reported in the works of other authors. The soils showed the predominant contribution of fungi (63–82%) to the total SIR.

The work was supported by the Russian Foundation for Basic Research (project no. 04-04-48715) and the Scientific School Program no. 3096.2006.4 “Biological Sources, Reservoirs, and Sink of Greenhouse Gases in Soils and Terrestrial Ecosystems.”

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