

Change in Microbiological Conditions of Lawn Grass Root Zones as a Result of Fertilization with Sewage Sludge

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Abstract

Sewage sludge, thanks to its content of nutrients, can be used as a fertilizer for lawns. The aim of this study was to determine the relationship between the applied dose of sewage sludge and variety of lawn grasses and changes in the quality and quantity of microorganisms in the root zone of grass. The community of fungi isolated from the rhizoplane was significantly dominated by fungi belonging to the *Fusarium* genus, including *F. oxysporum*. The share of these fungi in the isolated community increased with the dose of sewage sludge. It was also found that the share of some saprotrophic fungi significantly increased after application of the sludge at a dose of 60 t•ha⁻¹ and decreased after its application at the highest dose of 150 t•ha⁻¹. The lowest number of fungi of the *Fusarium* genus was observed in the roots of perennial rye-grass (30.3% of all isolates), while their highest number occurred in creeping red fescue (44.3% of all isolates). Based on all the results, such as the level of dominance, the number of potential pathogens and bacteria and fungi saprotrophic observed that the dose of 60 t•ha⁻¹ is the most favorable option, while the use of a dose of 150 t•ha⁻¹ may increase the share of pathogenic fungi in the community fungi, without causing beneficial changes in the number of microorganisms inhabiting rhizosphere terms lawns where no fertilizer was applied.

Keywords: sewage sludge, fertilization, lawn grass, fungal community, root zone microorganisms

Introduction

One of the main problems faced by municipal sewage treatment plants is excessive accumulation of waste produced during the sewage treatment process, i.e., sewage sludge. Sludge utilization using landfills is

an uneconomical solution. Alternative and more cost-effective ways to use sewage sludge include: composting, direct use as a fertilizer, remediation of degraded areas, and as a source of energy [1-5]. Before application, the sludge should be properly prepared by a process of dehydration and stabilization [6-8].

Sewage sludge is rich in nutrients and elements responsible for the normal development of plants, and its

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proper application results in improved chemical parameters of the soil, an increase in pH value, and the content of humic substances, the formation of soil aggregates and improving their stability and sorption capacity. Due to its positive effects, this substrate can successfully compete with other types of organic fertilizers, as well as with mineral fertilizers [2, 9-10]. However, the possibility of using sewage sludge for fertilization is regulated by a decree by the Ministry of the Environment of 1 August 2002 (Dz. U. 2002/134/1140) on municipal sewage sludge.

A disadvantage of this type of fertilizer, formed on the basis of sewage sludge, is their variable chemical composition and the presence of toxic substances, including increased doses of heavy metals that may accumulate in the soil. The high content of organic matter and the introduction of pre-treatment and quality studies, as well as the adaptation of the sludge dose to the crop, can effectively minimize the impact of toxic substances, which is of particular importance in the cultivation of crops intended for consumption [10-12].

The rhizosphere is very important for the functioning of plants. As a zone of direct interaction between the soil and the roots, it is mainly responsible for the circulation of elements in nature and the formation of diverse specific groups of microorganisms [13]. In the rhizosphere, the microbial community can be formed by approximately 10^4 species, and their count may reach 10^8 - 10^9 per g of wet weight of soil. The positive impact of plant-friendly microorganisms may result in the facilitation of nutrient uptake by roots, protection against colonization by pathogenic organisms, induction of defence mechanisms in the host plant, and easier distinction of harmful microorganisms from beneficial ones. An important aspect is the ability of the affected root zone to stimulate beneficial groups of microorganisms displacing plant pathogens, which has an impact on the health and yield of plants [14]. Important groups of microorganisms present in the rhizosphere that have a positive effect on plants comprise rhizobacteria, including the genera of *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bradyrhizobium*, and *Rhizobium* [14-16], as well as saprotrophic filamentous fungi [17] and yeast-like fungi [18].

Lawns have become an integral component of the urban environment due to their aspects related to aesthetics, recreation, scenery, and their positive impact on health owing to the detoxification of air and soil [19-20]. Proper fertilization improves the density of plants in the lawn, and their colour and ability to regenerate. The application of organic fertilizers in the spring or summer protects the sod and soil from drying and facilitates cultivation [19], as

well as reduces the need for mineral fertilization [21-22]. Grasses are potential hosts for many fungal and bacterial pathogens that can disrupt the functioning of lawns and grasslands [23]. An area covered with grass and infested by pathogens becomes a focus of infection, threatening adjacent ecosystems. One of the most important factors that determine the usefulness of grass species and varieties for lawns is their resistance to pathogens. The influence of fertilization with sewage sludge on the healthy condition of lawns is not sufficiently described in literature; therefore, research in this field has been undertaken.

The aim of this study was to analyse the change in the count of selected types of rhizosphere microorganisms, and the structure of fungal communities present in the rhizoplane of three grass species under diversified fertilization with sewage sludge.

Material and Methods

Our study was conducted in the years 2003-06. The studied material consisted of three species of lawn grass: perennial rye-grass var. Trubadur (A), common meadow-grass var. Alicja (B), and creeping red fescue var. Areta (C). The scope of the study included a two-factor experiment in a randomized split-plot design on incomplete soil (aerosol) formed from loose sand on loamy dust, conducted in quadruplicate in the area of the Research and Education Centre of the Department of Horticulture at the University of Warmia and Mazury in Olsztyn (N:53°45'36", E: 20°27'08"). The experimental plots had dimensions of 1 m • 1 m. It was assumed that the use of the plots would be moderately intense; they would be irrigated during water deficiency periods and mowed 10-15 times during the growing season, corresponding to extensive use of the lawn grass. The characteristic properties of the soil are given Table 1.

Prior to sowing the grass, the soil was fertilized with a dose of 60 or 150 tons of sewage sludge per 1 ha. The sewage sludge was mixed with a 10 cm layer of soil. The higher dose of sewage sludge used in the study was the maximum dose that allows for mixing with the soil surface. The control group included plots that were not fertilized with sewage sludge. The characteristics of sewage sludge are given in Table 2.

Samples of roots were collected from the 3-6 July each year of the study. The microbiological analysis of the rhizosphere used the dilution method. From each experimental setting, 10 g of soil adhering to grass roots was placed in Erlenmeyer flasks containing 90 ml of sterile water

Table 1. Physicochemical parameters of soil*.

Moisture (% vol.)	Organic matter (% of d.m.)	pH (in KCl)	Total N (% of d.m.)	Total content of macronutrients (g·kg ⁻¹ d.m.)				
				P	K	Mg	Ca	Na
	2.80	6.20	0.10	0.19	0.09	0.03	0.71	0.01

*Vol. – volume; d.m. – dry mater

Table 2. Primary parameters of sewage sludge used in this study.

Dry mass (%)	Organic matter (% of d. m.)	pH (in KCl)	Total content of macronutrients (% of d. m.)						
			N	P	K	Ca	Mg	Na	
18.25	54.56	7.7	4.44	1.76	1.00	4.42	0.72	0.18	
			Total concentration of heavy metals (mg·kg ⁻¹ d. m.)*						
			Zn	Cu	Pb	Ni	Cr	Cd	Hg
			2290 (3,500)	344 (1,200)	37.6 (1,000)	27.3 (200)	47.7 (1,000)	0.27 (25)	1.70 (10)

*Value in parentheses describes the maximum allowable concentration of heavy metals contained in sewage sludge used for land reclamation for non-agricultural purposes by the decree of the Ministry of the Environment of 1 August 2002 (Dz. U. 2002/134/1140) on municipal sewage sludge.

each. The samples were shaken using an Elpin plus 385S type bench laboratory shaker for 30 min. Subsequently, by consecutive diluting 1 cm³ of the studied samples in test tubes containing 9 cm³ of sterile saline distilled water (0.85% NaCl), a series of dilutions was obtained and 0.1 ml of sample was transferred into the appropriate medium. In the quantitative microbiological analysis of the rhizosphere, filamentous and yeast-like fungi were isolated on a Martin's Rose Bengal *Agar* medium, while an agar medium without nitrogen and a King's B medium were used to determine the count of bacteria of *Azotobacter* and *Pseudomonas* genera, respectively. Due to technical problems, the total count of filamentous and yeast-like fungi isolated from the rhizosphere was not determined in 2004, which was the second year of the study. The results of the quantitative study of the microorganisms isolated from the rhizosphere were statistically analysed with Statistica 10 software (ANOVA) using Tukey's test.

Mycological analysis of the rhizoplane involved placing six fragments of thoroughly rinsed roots on a Petri dish with a potato dextrose agar (PDA) medium. Growing hyphae of the mycelium were transferred into agar slants and incubated at 25°C and 18 root samples from each experimental setting were applied. Microscopic analysis of the fungi was performed using generally available guidelines [24-30].

Microbiological analyses of the rhizosphere were performed in triplicate. The structure of the communities colonizing the rhizoplane was described using the species richness index, Simpson's dominance index, Simpson's diversity index, and Simpson's evenness index. The determination of taxonomic dominance hierarchy was performed using a five-point percentage scale applied in population-based studies of fungi [31-32].

Results and Discussion

During the four years of the experiment, a total number of 1,215 colonies, which represented 64 taxa, were isolated (Table 3). During the study we noted a decrease in the number of taxa of fungi. The population obtained during the four years was analysed as a whole. In terms of

the specific aspect, the presence of eudominant (>50%) in the three communities was not observed.

Among all the taxa present in the study, 18 groups occurred in each community. Analysis of 64 fungal species typically observed in the studies demonstrated only a dominance of *Fusarium oxysporum* (25.9%). Sub-dominants included: *Rhizopus stolonifer* (12.3%), *Penicillium* spp. (7.7%) and *Thanatephorus cucumeris* (anamorph = *Rhizoctonia solani* Kühn) (7.5%). The dominance of *F. oxysporum* and sub-dominance of the three above-mentioned species was found for all the analysed grass species and sewage sludge doses. According to the applied dominance scale, both the analysis of the total population collected in the study and the analyses in terms of the grass species and fertilization doses did not show the presence of a eudominant taxon.

Analysis of the population of fungi present on the roots showed significant differences between varieties of grasses. In the community present in the rhizoplane of perennial rye-grass var. Trubadur consisting of 447 colonies belonging to 39 taxa, *F. oxysporum* constituted 21.7% of the population, while *Rhizopus stolonifer*, *Penicillium* spp., and *Thanatephorus cucumeris* constituted 12.1%, 9.8%, and 5.4% of the population, respectively. Other sub-dominant individuals were *Phialophora* spp. (11.2%) and *Mortierella alpina* (6.5%). Moreover, in this community, 10 rare taxa and 13 occasional taxa occurred. The community of fungi present in the rhizoplane of common meadow-grass var. Alicja consisted of a population of 374 colonies belonging to 38 species. *F. oxysporum*, *Rhizopus stolonifer*, *Penicillium* spp., and *Thanatephorus cucumeris* constituted 28.9%, 10.2%, 6.4%, and 9.6% of the population, respectively. Another sub-dominant, *Pyrenochaeta* spp. (16.0%), was present in this community. The number of rare and casual species equalled 11 and 21, respectively. Three-hundred and ninety-four colonies belonging to 42 species were obtained from the rhizoplane of creeping red fescue var. Areta. *F. oxysporum* constituted 27.9% of the population, while the sub-dominants comprised *Rhizopus stolonifer* 14.7%, *Penicillium* spp. 6.3%, *Thanatephorus cucumeris* 7.9%, and *Mucor hiemalis* 5.1%. Moreover, 16 rare taxa and 21 occasional taxa occurred in this community.

Table 3. Structure of the fungal community isolated from the rhizoplane in terms of grass type and sewage sludge dose (the total results for the years 2003-06).

Strain of fungi	Species of grass*			Dose of sludge [t*ha ⁻¹]			Sum
	A	B	C	0	60	150	
<i>Acremonium charticola</i> (Lindau) W. Gams	0	2	0	2	0	0	2
<i>Acremonium furcatum</i> (F. et V. Moreau) ex W. Gams	1	0	0	0	0	1	1
<i>Acremonium persicinum</i> (Nicot) W. Gams	4	0	0	4	0	0	4
<i>Acremonium rutilum</i> W. Gams	0	0	1	0	1	0	1
<i>Alternaria alternata</i> (Fries) Keissler	1	0	2	1	1	1	3
<i>Arthrinium phaeospermum</i> (Corda) M.B. Ellis	0	1	0	1	0	0	1
<i>Arthrinium</i> spp.	0	0	5	5	0	0	5
<i>Aspergillus fumigatus</i> Fresenius	1	0	0	0	0	1	1
<i>Aspergillus niger</i> vanTiegh	0	1	1	1	0	1	2
<i>Aureobasidium pullulans</i> (de Bary) Arnaud.	8	1	9	12	4	2	18
<i>Cephalotrichum purpureofuscum</i> (Schwein.) S. Hughes	10	1	0	10	1	0	11
<i>Chaetomium</i> spp.	4	0	1	0	0	5	5
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	4	2	5	4	3	4	11
<i>Cladosporium herbarum</i> Link ex Fr.	0	3	0	3	0	0	3
<i>Clonostachys rosea</i> (Link) Schroers, Samuels, Seifert et W. Gams	0	1	2	0	2	1	3
<i>Epicoccum nigrum</i> Link	1	2	0	0	3	0	3
<i>Fusarium chlamydosporum</i> Wollenw. et Reinking	3	0	1	1	1	2	4
<i>Fusarium culmorum</i> W.G. Smith	0	0	6	0	4	2	6
<i>Fusarium incarnatum</i> (Desm.) Sacc.	1	0	0	0	1	0	1
<i>Fusarium oxysporum</i> Schldtl.	97	108	110	93	101	121	315
<i>Gibberella acuminata</i> Wollenw	0	2	0	0	2	0	2
<i>Gibberella avenacea</i> R.J. Cook	2	7	9	3	4	11	18
<i>Gibberella intricans</i> Wollenw.	11	4	9	8	9	7	24
<i>Gibberella zeae</i> (Schwein.) Petch,	0	0	1	0	1	0	1
<i>Haematonectria haematococca</i> (Berk. et Broome) Samuels et Rossman	1	1	4	2	3	1	6
<i>Hormiactis candida</i> V. Höhnelt	3	0	0	3	0	0	3
<i>Humicola fuscoatra</i> Traaen	1	0	0	0	1	0	1
<i>Humicola grisea</i> Taaen	0	8	1	0	8	1	9
<i>Microdochium bolleyi</i> (Sprague) de Hoog et Herm.-Nijh	10	2	2	3	9	2	14
<i>Monodictys castaneae</i> (Wallr.) Hugnes	0	0	1	1	0	0	1
<i>Monographella nivalis</i> (Schaffnit) E. Müll.	0	6	9	5	0	10	15
<i>Mortierella alpina</i> Peyronel	29	9	18	21	29	6	56
<i>Mortierella capitata</i> Marchal	3	0	0	3	0	0	3
<i>Mortierella globulifera</i> O. Rostr.	1	0	0	0	1	0	1
<i>Mortierella jenkini</i> (Naumov) Smith	0	1	4	0	5	0	5
<i>Mortierella simplex</i> van Tieghem et Le Monnier	0	1	1	1	0	1	2
<i>Mortierella verrucosa</i> Linn.	0	0	1	0	0	1	1

<i>Mortierella verticillata</i> Linn.	1	0	0	1	0	0	1
<i>Mortierella zonata</i> Linnem. ex W. Gams	6	7	5	5	9	4	18
<i>Mucor circinelloides</i> van Tieghem	5	0	2	0	4	3	7
<i>Mucor hiemalis</i> Wehmer	3	3	20	7	5	14	26
<i>Myrothecium verrucaria</i> (Alb. et Schwein.) Ditmar	3	2	6	2	3	6	11
<i>Penicillium</i> spp.	44	24	25	34	35	24	93
<i>Phialophora</i> spp.	50	0	0	9	0	41	50
<i>Phoma eupyrena</i> Sacc.	0	0	2	0	0	2	2
<i>Phoma herbarum</i> Westend	17	7	12	19	15	2	36
<i>Phoma humicola</i> Gilman et Abbott	0	0	2	2	0	0	2
<i>Pseudonectria buxi</i> (DC.) Seifert, Gräfenhan et Schroers	4	7	0	0	10	1	11
<i>Pyrenochaeta</i> spp.	0	60	0	60	0	0	60
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	54	38	58	66	37	47	150
<i>Sarocladium strictum</i> (W. Gams) Summerb.	2	1	4	4	1	2	7
<i>Scopulariopsis brumptii</i> Salvanet-Duval	0	3	2	2	1	2	5
<i>Scopulariopsis chartarum</i> (G. Smith) Morton et G. Smith	2	1	0	1	0	2	3
<i>Sphaerostilbella aureonitens</i> (Tul. et C. Tul.) Seifert, Samuels & W. Gams	0	0	1	1	0	0	1
<i>Thanatephorus cucumeris</i> (A.B. Frank) Donk	24	36	31	25	21	45	91
<i>Trichoderma aureoviride</i> Rifai	8	4	3	8	7	0	15
<i>Trichoderma deliquescens</i> (Sopp) Jaklitsch	0	2	0	0	2	0	2
<i>Trichoderma hamatum</i> (Bon.) Bain.	13	0	10	8	2	13	23
<i>Trichoderma harzianum</i> Rifai	0	7	1	2	6	0	8
<i>Trichoderma koningii</i> Oud.	0	1	0	0	1	0	1
<i>Trichoderma viride</i> Persoon ex S.F. Gray	1	2	1	1	2	1	4
<i>Trichothecium roseum</i> (Persoon ex Fries) Link	1	0	0	0	0	1	1
<i>Umbelopsis isabellina</i> (Oudem.) W. Gams	0	6	4	5	4	1	10
<i>Wardomyces ovalis</i> W. Gams	13	0	2	0	2	13	15
Sum	447	374	394	449	361	405	1,215

* A – perennial rye-grass, B – common meadow-grass, C – creeping red fescue

Scale of domination	
20-50%	dominant
5-20%	subdominant
1-5%	rare individuals
<1%	casual individuals
0%	not observed

The number of species present in the respective studies was relatively similar. However, only 18 species were the same in each of the communities. Species richness (S) of fungal communities analysed in terms of grass variety reached similar values. The highest richness (S = 42) occurred in the rhizoplane of creeping red fescue var. Areta.

Simpson's dominance index (λ) revealed that none of the three communities occurring in the grass rhizoplane was distinctly dominated by any of the fungal species (Table 4), which indicates the lack of a eudominant species (>50% of the population). The only form exhibiting dominance was *F. oxysporum* present in each population,

Table 4. Structural indicators for the communities of fungal species.

Indicator	Formula	Species of grass*			Dose of sludge (t•ha ⁻¹)		
		A	B	C	0	60	150
Species richness (S)	Number of taxa	39	38	42	42	41	39
Simpson's dominance index (λ)	$\lambda = \sum p_i^2$	0.10	0.14	0.12	0.10	0.12	0.13
Simpson's diversity index (C)	$C = 1/\sum p_i^2$	0.90	0.86	0.88	0.90	0.88	0.87
Simpson's diversity index (D)	$D = 1/(\sum p_i^2)$	10.27	7.31	8.34	10.09	8.69	7.41
Simpson's evenness index (E)	$E = D/S$	0.22	0.16	0.20	0.21	0.18	0.18

p_i^2 – probability of sequentially selecting two individuals of the same species

* A – perennial rye-grass, B – common meadow-grass, C – creeping red fescue

and in addition, each of the communities comprised three sub-dominants (Table 3). Diversity index (C) showed that each of the fungal populations was characterized by a high level of species diversity (C = 0.90-0.86).

As for the aspect of sewage sludge fertilization, the distribution of the individual taxa share was similar for each dose. The community of fungi isolated from the

rhizoplane of grasses not fertilized with sewage sludge, comprising 449 species, was dominated by *F. oxysporum* (20.7%). The sub-dominants included four species: *Rhizopus stolonifer* (14.7%), *Pyrenochaeta* spp. (13.4%), *Penicillium* spp. (7.6%), and *Thanatephorus cucumeris* (5.6%). Moreover, in this community, 13 rare taxa and 24 occasional taxa were recorded. In the community isolated

Table 5. Structure of communities of the most numerous significant group of fungi isolated from the rhizoplane in terms of grass type and sewage sludge dose (total results for 2003-06).

Strain of fungi	Strain of grass*			Dose of sludge			Sum
				(t•ha ⁻¹)			
	A	B	C	0	60	150	
<i>Acremonium</i> spp. + <i>Sarocladium strictum</i>	7	3	5	10	2	3	15
<i>Aureobasidium</i> spp. + <i>Microdochium bolleyi</i>	18	3	11	15	13	4	32
<i>Fusarium</i> spp. + <i>Gibberella</i> spp. + <i>Haematonectria haematococca</i> + <i>Monoglyphella nivalis</i>	115	128	149	112	126	154	385
<i>Gliocladium</i> spp.	3	5	9	3	7	7	17
<i>Mortierella</i> spp. + <i>Umbelopsis isabellina</i>	40	24	33	36	48	13	97
<i>Penicillium</i> spp.	44	24	25	34	35	24	93
<i>Phialophora</i> spp.	50	0	0	9	0	41	50
<i>Pyrenochaeta</i> spp.	0	60	0	60	0	0	60
<i>Thanatephorus</i> spp.	24	36	31	25	21	45	91
<i>Rhizopus</i> spp.	53	38	56	63	37	47	147
<i>Scopulariopsis</i> spp.	2	4	2	3	1	4	8
<i>Trichoderma</i> spp.	23	14	15	19	18	15	52
Sum	379	339	336	389	308	357	1,047

* A – perennial rye-grass, B – common meadow-grass, C – creeping red fescue

Scale of domination	
20-50%	dominant
5-20%	subdominant
1-5%	rare individuals
<1%	casual individuals
0%	not observed

from the rhizoplane of grasses fertilized with sewage sludge at a dose of 60 t•ha⁻¹, including 361 colonies, no presence of a eudominant taxon was found. The dominant form was *F. oxysporum* (28.9%). The group of sub-dominants consisted of the following species: *Rhizopus stolonifer* (10.2%), *Penicillium* spp. (9.7%), *Mortierella alpina* (8.0%), and *Thanatephorus cucumeris* (5.8%). The remaining colonies formed 15 rare taxa and 21 occasional taxa. In the community of 405 colonies, isolated from the grasses fertilized with sewage sludge at a dose of 150 t•ha⁻¹, the dominant form was *F. oxysporum* (29.9%). The sub-dominant group included: *Rhizopus stolonifer* (11.6%), *T. cucumeris* (11.1%), *Phialophora* spp. (10.1%), and *Penicillium* spp. (5.9%). The community comprised nine rare taxa and 25 occasional taxa.

Analysis of ecological indicators of fungal communities occurring in the rhizoplane of plants treated with different doses of sewage sludge revealed similar relationships, as in the case of the analysis of fungal communities occurring in the rhizoplane of different grass varieties. Species richness (S) equalled 42 for the community present in unfertilized plants, while for the 60 t•ha⁻¹ and 150 t•ha⁻¹ doses it equalled 41 and 39, respectively. Simpson's dominance index (λ) did not indicate the dominance of any taxon. However, the diversity index (C) demonstrated that each fungal population was characterized by a high level of species diversity (0.84-0.76), which was also confirmed by the evenness index (D). Similar to the case of the analysis of the fungal community according to species, Simpson's evenness index (E) had low values due to the presence of numerous individual casual taxa.

Due to the high proportion of rare and casual taxa in the isolated community, an analysis of the dominance of the major fungal species was performed (Table 5). The highest count and share in the analysed community was reached by fungi of the *Fusarium* genus (average share 36.8%). In the case of varieties, their count and share were significantly higher in creeping red fescue (share 44.4%), while in the case of other sewage sludge fertilization variants, the count increased with the dose and, at a dose of 150 t•ha⁻¹, was 37% higher in comparison to the population originating from unfertilized plants.

The distribution of species richness for the analysis of the specific factor and fertilization dose was similar. In pe-

rennial rye-grass, the species richness was low, while in common meadow-grass and creeping red fescue, the share of the dominant was more pronounced ($\lambda > 20$). This result was confirmed by both evenness indices, which were significantly higher for perennial rye-grass, and low evenness indices, which reached only E = 10 for common meadow-grass and red fescue. In the case of fertilizer doses, the dominance index increased with the dose, but the difference between 60 and 150 t•ha⁻¹ variants was small ($\lambda = 1$). The diversity and evenness indices were similar for 60 and 150 t•ha⁻¹ doses, and significantly lower in comparison to the unfertilized variant (Table 6).

During the study of fungal communities, it was observed that the number of pathogenic fungi isolated from the rhizosphere of grasses increased with doses of sewage sludge. The ratio of phytopathogens to saprophytes was similar in both doses of fertilization and was approximately 0.42, while in unfertilized grass it was 0.36. Depending on the species of grasses, most pathogenic fungi were isolated from the rhizosphere of creeping red fescue, and the ratio of phytopathogens to saprophytes was 0.44 and increased by 0.14 and 0.06 in comparison with the population of fungi isolated from rye-grass and common meadow-grass, respectively (Table 5).

In the analysed population, we found the presence of saprotrophically developing fungi such as *Aureobasidium*, *Mortierella*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichoderma*. Due to antagonistic action against plant pathogens, a high proportion of these species in the community is a limiting factor for the infection of plants with phytopathogens [33].

Previous studies suggest that in the autumn and winter period, the fungal species *Limonomyces roseipellis*, *Laetisaria fuciformis*, and *Monographella nivalis* occur with high intensity on grasses with a high proportion of perennial rye-grass. By contrast, the infection of red fescue can be caused, in addition to typical autumn and winter period pathogens, by *Rhizoctonia*, *Fusarium*, *Bipolaris*, and *Drechslera*. Common meadow-grass is susceptible to infection by *Puccinia* spp., *Drechslera poae*, and *Blumeria graminis* fungi [34-36]. However, during the studies of sports ground turf, it was observed that *Fusarium* and *Rhizoctonia* fungal species also adversely affect the healthy condition of the grass in the summer.

Table 6. Structural indicators for the communities of frequently occurring significant groups of fungi.

Indicator	Formula	Species of grass*			Dose of sludge (t•ha ⁻¹)		
		A	B	C	0	60	150
Species richness (S)	Number of taxa	11	11	10	12	10	11
Simpson's dominance index (λ)	$\lambda = \sum p_i^2$	0.16	0.21	0.25	0.16	0.23	0.24
Simpson's diversity index – fraction (C)	$C = 1 - \sum p_i^2$	0.84	0.79	0.75	0.84	0.77	0.76
Simpson's diversity index (D)	$D = 1 / (\sum p_i^2)$	6.24	4.80	4.00	6.41	4.43	4.18
Simpson's evenness index (E)	$E = D/S$	0.13	0.10	0.10	0.14	0.09	0.10

p_i^2 – probability of sequentially selecting two individuals of the same species

* A – perennial rye-grass, B – common meadow-grass, C – creeping red fescue

Table 7. Count of bacteria of the *Azotobacter* genus isolated from the rhizosphere of grasses ($\log_{10}\cdot\text{g}^{-1}$ soil).

Strain of grass		Perennial rye-grass			Common meadow-grass			Creeping red fescue			Mean dose of sewage sludge		
Dose of sewage sludge ($\text{t}\cdot\text{ha}^{-1}$)		0	60	150	0	60	150	0	60	150	0	60	150
Year	I	5.53c	5.64b-c	5.88ab	5.56c	5.67bc	5.59c	5.64b-c	5.73a-c	6.00a	5.58	5.68	5.82
	II	5.58c	5.55c	5.52c	5.57c	5.55c	5.49c	5.58c	5.54c	5.61b-c	5.58	5.55	5.54
	III	4.11e-h	3.72j	4.33de	4.15e-g	4.17e-g	4.13e-g	4.13e-g	3.93g-j	4.02f-i	4.13	3.94	4.16
	IV	3.76ij	4.28ef	4.55d	4.18e-g	4.15e-g	3.93g-j	3.85h-j	5.74a-c	4.21ef	3.93	4.72	4.23
Mean		4.75	4.80	5.07	4.87	4.89	4.79	4.80	5.24	4.96	4.80Y	4.97X	4.94X
		4.87B			4.85B			5.00A					

Results with the same letter (year • dose: small letters; mean for types of grasses: A or B; mean for doses: X or Y) are not significantly different; LSD ($p < 0.05$)

The authors emphasized that perennial rye-grass (*Lolium perenne* L.) and common meadow-grass (*Poa pratensis* L.) are more resistant to some factors determining the quality of grasses, such as low mowing and intensive trampling [37].

In other studies, based on the example of perennial rye-grass, it was shown that one of the factors affecting the healthy condition of grass was the course of weather conditions after winter, resulting from lack of snow cover and long-lasting temperatures of approximately 0°C . Mould was observed in the period after winter snow, and its intensity was higher every year of observation. Powdery mildew dominated among the pathogens affecting grass health during the blooming period, while rusts dominated in the autumn. But the presence of pathogens was not the only cause of grass dying out [38]. In contrast, the degree of grass decay with the years passing is affected by grass variety, but grass susceptibility to infections is higher in humid weather and at high temperatures, which favours the development of fungi of the *Fusarium*, *Rhizoctonia*, and *Bipolaris* genera [36]. The intensification of occurrence of snow mould was also dependent on the type of mineral

fertilizers, which was observed in the studies of Jankowski et al. [39]. However, apart from long-lasting snow cover, another important factor that intensifies grass infestation with pathogens is an unfavourable balance of fertilization. An increase in the occurrence of snow mould (*M. nivalis*) and rhizoctoniosis (*Rhizoctonia* spp.) can be caused by over-fertilization with nitrogen [36].

Bacteria of the *Azotobacter* genus occurred most often in red fescue, while in terms of sludge fertilization a comparable count of *Azotobacter* spp. was isolated from plants fertilized with doses of 60 and $150 \text{ t}\cdot\text{ha}^{-1}$, which was significantly higher in comparison to unfertilized plants. Among grass species, the least variable bacterial count occurred in common meadow-grass. Analysis of individual years did not show any significant differences for any of the studied years. In the first and last year of the study, an increase in bacterial count with the sludge dose in perennial rye-grass occurred, while in 2005 a significantly lower result was obtained for the dose of $60 \text{ t}\cdot\text{ha}^{-1}$. Analysis of the second year did not demonstrate any significant changes in the *Azotobacter* spp. count for any of the doses. A similar dependency was observed in red fescue.

Table 8. Count of bacteria of the *Pseudomonas* genus isolated from the rhizosphere of the grasses ($\log_{10}\cdot\text{g}^{-1}$ soil).

Strain of grass		Perennial rye-grass			Common meadow-grass			Creeping red fescue			Mean dose of sewage sludge		
Dose of sewage sludge ($\text{t}\cdot\text{ha}^{-1}$)		0	60	150	0	60	150	0	60	150	0	60	150
Year	I	8.45bc	8.63a-c	8.81a	8.39c	8.58a-c	8.63a-c	8.43c	8.52a-c	8.73ab	8.42	8.58	8.72
	II	4.51n	4.53mn	5.01jk	5.21h-j	4.84kl	4.84kl	4.83k-m	5.21h-j	5.22h-j	4.85	4.86	5.02
	III	4.52n	4.01o	4.48n	3.53p	4.56l-n	4.37n	4.53mn	5.06i-k	4.59l-n	4.19	4.54	4.48
	IV	5.18h-j	5.63ef	6.28d	5.43f-h	5.79e	5.66ef	5.60e-g	5.32g-i	6.18d	5.40	5.58	6.04
Mean		5.67	5.70	6.15	5.64	5.94	5.88	5.85	6.03	6.18	5.72Z	5.89Y	6.07X
		5.84B			5.82B			6.02A					

Results with the same letter (year • dose: small letters; mean for types of grasses: A or B; mean for doses: X, Y or Z) are not significantly different; LSD ($p < 0.05$)

Table 9. Count of filamentous fungi isolated from the rhizosphere of the grasses ($\log_{10} \cdot \text{g}^{-1}$ soil).

Strain of grass		Perennial rye-grass			Common meadow-grass			Creeping red fescue			Mean dose of sewage sludge		
Dose of sewage sludge ($\text{t} \cdot \text{ha}^{-1}$)		0	60	150	0	60	150	0	60	150	0	60	150
Year	I	4.49b	4.53ab	4.75a	4.51ab	4.11c	4.62ab	4.52ab	4.40b	3.03ij	4.51	4.38	4.52
	III	2.52k	2.52k	3.12hi	2.52k	2.83j	3.37e-g	2.83j	3.03ij	3.22g-i	2.65	2.82	3.25
	IV	2.48k	3.85d	3.60de	2.48k	3.31f-h	3.78d	2.48k	3.30f-h	3.48ef	2.48	3.56	3.64
Mean		3.16	3.63	3.82	3.17	3.42	3.92	3.28	3.58	3.24	3.21Z	3.59Y	3.80X
		3.54A			3.50A			3.37B					

Results with the same letter (year • dose: small letters; mean for the types of grasses: A or B; mean for doses: X, Y or Z) are not significantly different; LSD ($p < 0.05$)

However, in 2006 the highest count of microorganisms occurred at a dose of $60 \text{ t} \cdot \text{ha}^{-1}$, reaching the same result as in the first year of the study, and the increase in the bacterial count was two orders of magnitude higher than in 2005 (Table 7).

Changes in the bacterial count of the *Pseudomonas* genus showed a partially similar tendency, as in the case of *Azotobacter*. An increase in the bacterial count was reported in the last year of the study. *Pseudomonas* spp. were the most frequent in red fescue, and the increasing sludge dose had a stimulating effect on their development. In the case of perennial rye-grass, the bacteria count significantly increased in each of the studied years (with the exception of 2005, in which a significant result among the doses of 60 and $150 \text{ t} \cdot \text{ha}^{-1}$ was found only for $60 \text{ t} \cdot \text{ha}^{-1}$). The count of bacteria isolated from common meadow-grass fertilized with different sludge doses showed various tendencies, depending on the year of studies. Nevertheless, based on the analysis of the average value from all years for the 60 and $150 \text{ t} \cdot \text{ha}^{-1}$ doses, an increased bacterial count was observed, especially for the $60 \text{ t} \cdot \text{ha}^{-1}$ dose. Red fescue showed a tendency comparable to perennial rye-grass. In each year (except for 2005) an increase in the *Pseudomonas* spp. count in the plants fertilized with sludge was demonstrated, which occurred most visibly in the first and fourth years of the study (Table 8).

The results of the year-long study of the abundance of filamentous fungi present in grass rhizosphere showed a different trend than the number of bacteria. Analysis of averaged results indicated that the rhizospheres of perennial rye-grass and common meadow-grass were the richest in filamentous fungi, while the amount of microorganisms significantly increased with the sewage sludge dose. Fungal count in perennial rye-grass significantly increased for the dose of $150 \text{ t} \cdot \text{ha}^{-1}$ in the two first years of the study, and for the doses of 60 and $150 \text{ t} \cdot \text{ha}^{-1}$ in 2006, reaching a similar level. Fungal count isolated from common meadow-grass in the first year of the study significantly decreased for the dose of $60 \text{ t} \cdot \text{ha}^{-1}$ in comparison to the control, and insignificantly increased for the dose of $150 \text{ t} \cdot \text{ha}^{-1}$. In the first year of the study, the population size of filamentous fungi in the rhizosphere of red fescue decreased insignificantly for the dose of $60 \text{ t} \cdot \text{ha}^{-1}$ and significantly for the dose of $150 \text{ t} \cdot \text{ha}^{-1}$. In 2005 and 2006 the fungal count increased with dose, and only in 2005, for the dose of $60 \text{ t} \cdot \text{ha}^{-1}$, did it not demonstrate any significance as compared to the control (Table 9).

Diversity in the count of yeast-like fungi was much lower than for filamentous fungi. In terms of grass species, the rhizosphere of perennial rye-grass was the richest in yeast-like fungi, and the lowest count was

Table 10. Count of yeast-like fungi isolated from the rhizosphere of grasses ($\log_{10} \text{CFU} \cdot \text{g}^{-1}$ soil).

Strain of grass		Perennial rye-grass			Common meadow-grass			Creeping red fescue			Mean dose of sewage sludge		
Dose of sewage sludge ($\text{t} \cdot \text{ha}^{-1}$)		0	60	150	0	60	150	0	60	150	0	60	150
Year	I	3.85e-g	4.20d	4.34cd	4.56b	4.46bc	3.70g-i	3.60hi	3.70g-i	3.90ef	4.00	4.12	3.98
	III	3.60hi	3.12k	3.37j	3.00k	3.60hi	3.80e-g	3.75f-h	3.56i	3.70g-i	3.45	3.43	3.62
	IV	4.18d	4.83a	4.74a	3.95e	4.18d	4.18d	3.60hi	4.28d	3.78e-g	3.91	4.43	4.23
Mean		3.87	4.05	4.15	3.84	4.08	3.89	3.65	3.85	3.79	3.79Z	3.99X	3.95Y
		4.03A			3.94B			3.76C					

Results with the same letter (year • dose: small letters; mean for types of grasses: A, B or C; mean for doses: X, Y or Z) are not significantly different; LSD ($p < 0.05$)

represented by red fescue. With respect to sludge fertilization, the dose of $60 \text{ t}\cdot\text{ha}^{-1}$ stimulated yeast-like cells in the best manner, while a lower value was reached for the dose of $150 \text{ t}\cdot\text{ha}^{-1}$, and the lowest value was observed for the control. In 2003 and 2006 we observed a significant increase in fungal count in the rhizosphere of perennial ryegrass as compared to the control, and the differences in the number of cells between the doses were small. The highest value was reached by the control in 2005, while the dose of $60 \text{ t}\cdot\text{ha}^{-1}$ was characterized by the lowest fungal count. In 2003, in the case of common meadow-grass, we found a significant reduction in the value for the dose of $150 \text{ t}\cdot\text{ha}^{-1}$, while in 2005 we observed a noticeable increase in yeast cell count with a sludge dose. In the last year of the study the fungal count did not change significantly, although the value for both doses was slightly higher as compared to the control. In the rhizosphere of red fescue, similarly to the two above-mentioned grass species, the amount of yeast-like fungi demonstrated a variable tendency depending on the studied year. In the first year of the study, despite the increase in cell count, a significant result was observed only for the dose of $150 \text{ t}\cdot\text{ha}^{-1}$. In 2005 the cell count decreased for the dose of $60 \text{ t}\cdot\text{ha}^{-1}$ and did not change significantly as compared to the control for the dose of $150 \text{ t}\cdot\text{ha}^{-1}$. In 2006 all the results differed significantly. The highest fungal count was observed for the dose of $60 \text{ t}\cdot\text{ha}^{-1}$, and for the highest dose and control it was much lower (Table 10).

Soil bacteria and bacteria living on the surface of plant roots may have antagonistic properties against pathogenic fungi. The group of microorganisms with a high potential for reducing plant diseases includes *Pseudomonas* spp. bacteria, which has been confirmed by numerous scientific studies [40-41]. Analysis of the microbial count of the grass rhizosphere (Tables 8-9) demonstrates that the *Pseudomonas* spp. bacteria count increased with the dose of sewage sludge, while the filamentous fungi count did not significantly change. This may indicate that the limiting factor for fungal development was the count of antagonistic bacteria, which increased under the influence of fertilization. As indicated by Garbeva et al. [42], among the investigated populations of *Pseudomonas* spp., bacteria isolated from the rhizosphere of grasses and corn were characterized by the strongest antagonistic properties against pathogens. The author also points out two important factors, which are soil and plant species that determine the population structure of rhizosphere bacteria of the *Pseudomonas* and *Bacillus* genera.

Diazotrophic (non-symbiotic) bacteria, producing nitrogenase, belong to the bacteria responsible for the fixation of molecular nitrogen. The studies of wheat root zone [43] showed that *Azotobacter* spp. colonizes the rhizosphere ($2\cdot 10^5$ CFU) and soil ($1.5\cdot 10^5$ CFU) most frequently, while it is less frequent in the direct vicinity of plant roots ($6.2\cdot 10^4$ CFU). The amount and activity of these bacteria in the soil are mainly dependent on the type of soil, the type of agrotechnical treatments, and the use of chemicals in agriculture [44-46]. Bacteria of the *Azotobacter* genus can fix significant amounts of nitrogen in

the soil environment (about $5\text{-}17 \mu\text{g N}\cdot\text{g}^{-1}$). As has been shown, this process is more efficient in clayey soil than in sandy soils [44]. The data presented by Mujiyati and Supriyadi [46] prove that fertilization influences the count of nitrogen-fixing bacteria. The population growth for *Azotobacter* spp. and *Azospirillum* spp., originating from the rhizosphere of a chili pepper plantation, equalled 43% and 16% sequentially for mineral fertilization, and 29% and 68% for manure. The use of fertilization also increased nitrogen content in the soil by 41% in the case of mineral fertilizer, and by 36% in the case of manure. Moreover, the sorption capacity and the amount of organic carbon and organic matter increased. As shown in the studies [47], the count of *Azotobacter chroococum* varies depending on the isolation site. The author also points out that the important factors determining the occurrence of *A. chroococum* are pH and the contents of N and C, as well as of heavy metals and other xenobiotics. Additionally, the studies demonstrated that *A. chroococum* bacteria form communities that vary within a species regardless of the environment from which they were isolated, but their count in downtown lawns and other ecosystems present within the area of Poland differs.

The high microbial activity and normal course of biochemical processes in the soil indicate its good quality and its proper functioning, which has a crucial effect on supplying plants with nutrients by bacteria – especially in soils to which no mineral fertilization is applied. Microbial activity depends on the parameters of the soil environment (e.g., pH value) and tillage system. In quantitative studies of bacteria in the ecological system in soil under winter wheat, the bacterial count of the *Azotobacter* genus equalled $98 \text{ CFU}\cdot\text{g}^{-1}$ on 23 June and $143 \text{ CFU}\cdot\text{g}^{-1}$ on 15 September. In the conventional system, no presence of *Azotobacter* spp. was found [48].

The study on the impact of different grass species and the presence of earthworms (*Aporrectodea caliginosa*) on the count of bacteria of the *Azospirillum* genus demonstrated that the bacteria occurred in the soil in a form not connected to the roots – both in red fescue and in the cultivation of a mixture of different grass species – throughout the growing season. There was no correlation between the amounts of isolated bacteria, changes in diversity of varieties in the grass mixture, and the presence of earthworms [49].

Microbiological analysis of the soil under lawns fertilized with nitrogen at five different doses (from 50 to $200 \text{ t}\cdot\text{ha}^{-1}$) indicated that the doses of fertilizer were not a factor that would determine the bacterial and fungal counts in the soil, but weather conditions and the date of collection of samples turned out to be significant. The highest count of bacteria was recorded in May and June, and the highest count of filamentous fungi in August [50]. On the other hand, the application of sewage sludge at its highest dose N ($170 \text{ t}\cdot\text{ha}^{-1}$) to Virginia mallow (*Sida hermaphrodita* (L.) Rusby) resulted in a significant increase in the count of soil fungi and bacteria. It was also found that the period of the most frequent occurrence of bacteria was in July. The count of filamentous fungi contained in the

soil was strongly linked to weather conditions during the experiment as well [51].

Analysis of fungal communities in the soil under winter wheat cultivation demonstrated that the layer 10-20 cm below the surface of the earth is dominated by *Penicillium* spp. (17%) and *Trichoderma harzianum* (9%) taxa in the ecological system, and by *Alternaria alternata* (11%), *Fusarium culmorum* (7%), and *Penicillium* spp. (7%) taxa in the conventional system. The results indicate a greater share of saprotrophic fungi in the soil cultivated in an ecological system [52].

The use of sewage sludge, especially at a dose of 60 t•ha⁻¹, influenced the growth of yeast-like fungi. The presence of yeast cells in the soil environment is responsible for the decomposition of dead organic matter, has a positive impact on the soil macrofauna, and exhibits antagonism against pathogens [53-54].

Conclusions

The largest community of microorganisms was isolated from the control not fertilized with sewage sludge, and the smallest one after fertilization with a dose of 60 t•ha⁻¹. Despite this, the worst parameters (the highest degree of dominance by one species, the lowest level of diversity among taxa) were obtained for analysis of the rhizoplane population originating from grasses fertilized with sludge at a dose of 150 t•ha⁻¹. Furthermore, in grasses fertilized with the highest dose used in the study, not only a higher occurrence of *F. oxysporum* and other closely related pathogens was observed, as compared to the control, but also a decrease in the share of fungi potentially antagonistic against pathogens was observed. On the other hand, at a dose of 60 t•ha⁻¹, both the share of pathogens and of potentially antagonistic fungi such as *Aureobasidium* spp., *Trichoderma* spp., and other saprotrophs was higher as compared to the control.

Perennial rye-grass had the most favourable population indices and the lowest dominance of the root zone by fungi of the *Fusarium* genus. This species was characterized by a significantly more numerous fungal community. All of the analysed parameters, including a better response of rhizosphere bacteria of the *Azotobacter* genus and yeast-like fungi to the doses of sludge than in other grass species, indicate that this grass is characterized by a low susceptibility to root zone colonization by pathogens, and a potentially better use of the nutrients present in the soil and provided by sewage sludge.

Due to the small number of reports on the effects of fertilization of sewage sludge on the microorganisms occurring in the root zone of lawn grasses, it is proved that the use of low doses of sewage sludge on (60 t•ha⁻¹) improves the microbiological parameters. There is also evidence that potentially pathogenic fungi less frequently colonize the root zone of perennial rye-grass combined with fertilization with sewage sludge. Therefore, the combination of sewage sludge fertilization with the use of perennial rye-grass is the most optimal

variant, contributing to the formation of lawns with low emissions of toxigenic fungi and producing the appropriate communities of microorganisms responsible for the high quality of the grass.

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