COMMUNITIES OF FUNGI AND BACTERIA IN THE RHIZOSPHERE OF POTATO AND THEIR EFFECT ON PHYTOPATHOGENS

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Abstract. The purpose of the studies carried out in the years 1996-1998 was to establish the composition of bacteria and fungi communities in the potato rhizosphere and non-rhizosphere soil. Besides, in the examined samples the studies established the proportion of bacteria and fungi antagonistic towards soilborne pathogens. The microbiological analysis of 1 g of dry weight of soil coming from the rhizosphere of potato revealed from 3.96×10^6 to 7.26×10^6 bacteria colonies and from 51.38×10^3 to 69.96×10^3 fungi colonies. In the case of non-rhizosphere soil of 1 g of dry weight of soil revealed from 3.50×10^6 to 4.75×10^6 bacteria colonies and from 16.16×10^3 to 34.10×10^3 fungi colonies. Moreover, potato cultivation had a positive effect on the increase of numbers of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.). A larger number of the communities of bacteria and fungi, including antagonistic ones, in the root area of potato, indicates considerable biological activity, which contributes to a better phytosanitary condition of the soil.

Key words: potato, rhizopshere, pathogenic fungi, antagonistic microorganisms

I. INTRODUCTION

Rhizopshere is characterized by the largest biological activity. The compounds exudated by roots and the flaking root cells (Funck-Jensen and Hockenhull 1984; Huber and Watson 1970; Rovira 1965; 1969; Schroth and Hildebrand 1964) and the remaining roots (Batalin 1962) are the main stimulators of microorganisms development in the period of vegetation. Root exudates are a rich source of amino-acids, sugars, organic acids, vitamins, metal ions, phenol acids and their derivatives (Funck-Jensen and Hockenhull 1984; Pieta 1988; Sytnik et al. 1977). Among the enumerated compounds, sugars soluble in water and acidic amino-acids stimulate, while phenol acids, aromatic and alkaline amino-acids inhibit the growth of pathogenic fungi (Milczak and Piotrowski 1980; Pieta 1988; Piotrowski and Milczak 1982). Particular attention should be paid to the stimulating effect of root exudates on the development of bacteria and fungi antagonistic towards soilborne pathogenic fungi. Among bacteria, the largest ability of antagonistic effect towards phytopathogens was found in the representatives of Bacillus spp. and Pseudomonas spp. (Defago and Haas 1990; Keel 1992), while in the case of fungi in Trichoderma spp. and Gliocladium spp. (Hwang and Chakravarty 1993; Lorito et al. 1993; Marois et al. 1981; Papavizas 1985). Representatives of these genera of antagonistic bacteria and fungi are distinguished by remarkable ability to inhibit the growth of pathogens and to shorten their life in the soil through competition, antybiosis and parasitism (Ahl et al. 1986; Dowling and O'Gara 1994; Kloepper et al. 1999; Lin et al. 1994; Mukherjee et al. 1995; Pietr and Sobiczewski 1993). Considering the

effect of microorganisms on plants, studies were undertaken to determine the quantitative and qualitative composition of bacteria and fungi populations in the potato rhizosphere and non-rhizosphere soil. Besides, in the examined samples the studies established the proportion of bacteria and fungi antagonistic towards soilborne pathogens.

II. MATERIAL AND METHODS

The studies were carried out on the experimental field belonging to the Department of Plant and Soil Cultivation of the University of Agriculture in Lublin in the years 1996-1998. The field is localized in the Experimental Farm of Czesławice near Nałęczów. The object of the research was the rhizosphere soil of potato of cv. Bronka, and the non-rhizosphere soil. In the present experiment potato was cultivated after soybean.

The experiment was established according to randomised block design with three replications, on grey brown podzolic soil formed from loesses, that belonged to the second, good wheat complex. In each year, the rhizosphere soil was sampled at anthesis of potato. Samples of non-rhizosphere soil were taken from the fallow ground. The manner of sampling the rhizosphere soil, and next conducting the laboratory microbiological analysis corresponded with the methods described by Martyniuk et al. (1991). The rhizosphere soil of potato and the non-rhizosphere soil (from the depth of 5-10 cm) was taken to sterile Petri dishes. Afterwards, in sterile laboratory conditions a soil solution in the dilution ranging from 10^{-1} to 10^{-7} was prepared.

The total number of bacteria in 1g of soil dry weight was determined on "Nutrient agar" from the soil solutions with the dilutions of 10⁻⁵, 10⁻⁶, 10⁻⁷. "Triptic soy agar" and dilutions 10⁻⁴, 10⁻⁵, 10⁻⁶ were used for the bacteria from *Bacillus* spp., while "Pseudomonas agar F" and dilutions of 10⁻², 10⁻³, 10⁻⁴ were used for *Pseudomonas* spp. The total number of fungi in 1 g of soil dry weight was determined on Martin nutrient medium (1950) with the dilutions of 10⁻², 10⁻³, 10⁻⁴.

The results concerning the numbers of bacteria and fungi were statistically analysed and the significance of differences was determined on the basis of Tukey confidence intervals (Oktaba 1987).

In each year, the obtained bacteria isolates (200 isolates of *Bacillus* spp. and 200 isolates of *Pseudomonas* spp.) together with all the isolates of saprophytic fungi of *Gliocladium* spp. and *Trichoderma* spp. as well as 5 isolates from each species from *Penicillium* spp. were used in order to determine their antagonistic effect towards such pathogenic fungi as *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Pythium irregulare* and *Rhizoctonia solani*. To establish the antagonistic effect of the examined bacteria on pathogenic fungi a five-degree scale described by Martyniuk et al. (1991) and the degrees of inhibition of phytopathogen growth provided by Pięta (1999) were used. An estimation of the effect of saprophytic fungi on the studied pathogenic fungi was performed by means of the method of biotic rows (Mańka 1974; Mańka and Mańka 1992), and the individual effect of antagonistic effect was determined on the basis of the scale provided by Mańka and Kowalski (1968).

III. RESULTS

The differences in the numbers of particular populations of microorganisms were found out on the basis of the mycological analysis of the potato rhizosphere and non-rhizosphere soil (Tab.1). During the studies the total number of bacteria in 1 g of dry weight of the rhizosphere soil of potato ranged from 3.96×10^6 to 7.26×10^6 bacteria colonies, while in the case of non-rhizosphere soil it was from 3.50×10^6 to 4.75×10^6 bacteria colonies. In the three years of studies the mean number of bacteria from *Bacillus* spp. in 1 g of dry weight of the potato rhizosphere soil was 1.80×10^6 , while in non-rhizosphere soil it was 1.91×10^6 colonies (Tab.1). In the case of bacteria from *Pseudomonas* spp. on the average three times more colonies were isolated from the potato rhizosphere soil than from non-

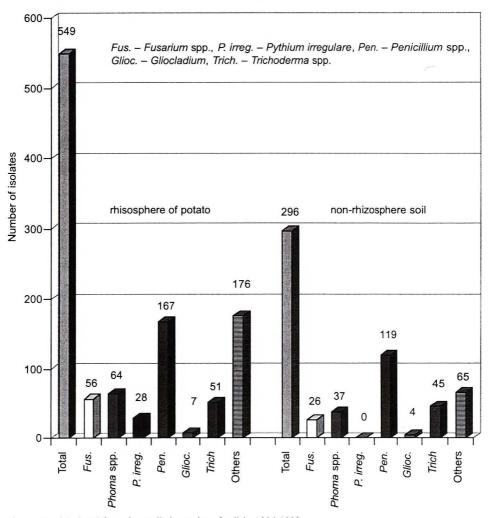


Fig. 1. Fungi isolated from the studied samples of soil in 1996-1998

The number of bacteria and fungi in the plant rhizosphere

Type of soil	Œ)	Total number of bacteria (mln/1g d. m. of soil)	umber teria m. of so	(i	Z o Ē	umber o f <i>Bacill</i> n/1g d.	Number of bacteria of Bacillus genus (mln/1g d. m. of soil)	ia ; il)	Nu of P (m)	Number of bacteria of Pseudomonas genus (mln/1g d. m. of soil)	bacteriz onas gei m. of so	a nus il)	(tho	Total number of fungi (thous./1g d. m. of soil)	Total number of fungi s./1g d. m. of s	(lic
	1996	1997	1998	mean	mean 1996 1997	1997	1998	mean 1996 1997	9661	1997	8661	mean	9661	mean 1996 1997 1998	8661	mean
Rhizosphere of potato Soil of non- rhizosphere	6,69**	3,96° 7,26° 4,47° 3,50°	7,26° 3,50°	5,97ª 4,24ª	3.96° 7.26° 5.97° 3.00° 1,23° 1,19° 1,80° 1,91° 0,96° 2,03° 1,63° 59.90° 51,38° 69.96° 60.13° 4,47° 3,50° 4,24° 2,33° 1,17° 2,25° 1,91° 0,19° 1,03° 0,44° 0,55° 34,10° 27,37° 16,16° 25,88°	1,23ª	1,19ª 2,25b	1,80	1,91 ^b 0,19 ^a	0,96ª	2,03b 0,44ª	1,63 ^b 0,55 ^a	59,90 ^b 34,10 ^a	0,96* 2,03* 1,63* 59,90* 51,38* 69,96* 60,13* 1,03* 0,44* 0,55* 34,10* 27,37* 16,16* 25,88*	69,96 ^b	60,13 ^b 25,88*
* Means in columns differ significantly (P = 0.05), if they are not marked with the same letter	P = 0.05	, if they	are not	marked	with the	same l	etter									

rhizosphere soil. The total number of fungi in 1 g of dry weight of the potato rhizosphere soil ranged from 51.38×10^3 to 69.96×10^3 , while in non-rhizosphere soil it was from 16.16×10^3 to 34.10×10^3 colonies (Tab.1).

The mycological analyses of the rhizosphere potato soil separated 549 isolates, and from non-rhizosphere soil 296 fungi isolates (Fig. 1). Among the obtained fungi the following were included in the pathogenic species: Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua and Pythium irregulare. The last one was not isolated from non-rhizosphere soil, while the others were much more frequently isolated from the rhizosphere potato soil than from non-rhizosphere soil (Fig. 1). A similar relation was observed for such saprophytic fungi as Penicillium spp., Gliocladium spp. and Trichoderma spp. Besides, the species from Acremonium spp., Aspergillus spp., Chaetomium spp., Cladosporium spp., Mucor spp., Rhizopus spp. were obtained in small numbers both from the rhizosphere and non-rhizosphere soil. The number of isolates of those fungi obtained from the three years of mycological analysis of the examined soil samples is shown in the chart as others (Fig. 1).

Within Bacillus spp., Pseudomonas spp., Gliocladium spp., Penicillium spp. and Trichoderma spp. the studies separated the isolates, which were characterized by antagonistic effect towards the tested pathogenic fungi. Totally, 71 Bacillus spp. isolates and 99 Pseudomonas spp. isolates were separated from the rhizosphere potato soil, and correspondingly 18 and 34 isolates were separated from non-rhizosphere soil which were distinguished by their antagonistic effect. Those bacteria genera from the rhizosphere of potato were effective in limiting the growth of Pythium irregulare colonies. On the other hand, those bacteria obtained from non-rhizosphere soil efficiently reduced F. oxysporum (Tabs. 3, 4). Bacteria of Bacillus spp. and Pseudomonas spp. obtained from the rhizosphere of potato showed little effectiveness in limiting the growth of F. oxysporum (+ 215) and F. solani. (+ 247), and their effect was the smallest towards R. solani (+ 28 the total antagonistic effect). The stud-

Table 3

Activity of bacteria Bacillus spp. and Pseudomonas spp. isolated from the rhizosphere of potato towards pathogenic fungi

Genus of bacteria	The number	F. cul	morum	F. oxys	porum	F. so	lani	P. irreg	gulare	R. so	lani	Altogether effect
Genus of bacteria	of isolates	1	2	F. oxysporum 1 2 1 +1 +19 +1 +20 +1 +39 +1 +24 +2 +90 +1 +114 +1 +1 +28 +1 +34 +2 +62 +215	2	1	2	1	2	of antagonistic activity		
Bacillus spp.	19	0	0	+1	+19	+2	+38	+2	+38	0	0	+152
Pseudomonas spp.	20	+1	+20	+1	+20	+1	+20	+3	+60	0	0	+140
Together effect of antagonistic activity			+20		+39		+58		+98		0	+292
Bacillus spp.	24	+1	+24	+1	+24	+2	+48	+3	+72	0	0	+240
Pseudomonas spp.	45	0	0	+2	+90	+1	+45	+4	+180	0	0	+360
Together effect of antagonistic activity			+24		+114		+93		+252		. 0	+600
Bacillus spp.	28	+1	+28	+1	+28	+1	+28	+5	+140	+1	+28	+504
Pseudomonas spp.	34	0	0	+1	+34	+2	+68	+3,5	+119	0	0	+255
Together effect of antagonistic activity			+28		+62		+96		+259		+28	+759
Altogether effect of antagonistic activity	2		+72		+215		+247	- 1	+609		+28	+1,651

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity

Activity of bacteria Bacillus spp. and Pseudomonas spp. isolated from the non-rhizosphere soil towards pathogenic fungi

Genus of bacteria	The number	F. cul	morum	F. oxys	sporum	F. so	lani	P. irre	gulare	R. soi	lani	Altogether effect
Genus of vacteria	of isolates	1	2	1	2	1	2	1	2	1	2	of antagonistic activity
Bacillus spp.	6	+1	+6	+1	+6	+1	+6	+1	+6	0	0	+42
Pseudomonas spp.	11	0	0	+1	+11	+1	+11	0	0	0	0	+33
Together effect of antagonistic activity			+6		+17		+17		+6		0	+75
Bacillus spp.	3	0	0	+2	+6	+2	+6	+1	+3	+1	+3	+30
Pseudomonas spp.	13	0	0	+2	+26	+1	+13	+1	+13	+1	+13	+117
Together effect of antagonistic activity			0		+32		+19		+16		+16	+147
Bacillus spp.	9	+1	+9	+1	+9	0	0	+3	+27	+1	+9	+126
Pseudomonas spp.	10	0	0	+1	+10	0	0	+1	+10	0	0	+40
Together effect of antagonistic activity			+9		+19		0		+37		+9	+166
Altogether effect of antagonistic activity			+15		+68		+36		+59		+25	+388

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity

Activity of saprophitic fungi isolated from the rhizosphere of potato towards pathogenic fungi

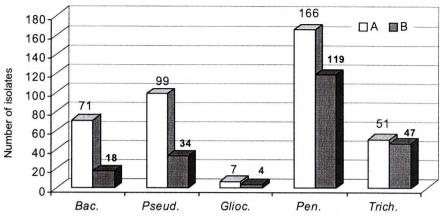
	Mean	F. cul	morum	F. oxy	sporum	F. so	lani	P. irre	gulare	R. so	lani
Fungus species	of isolates in 1996-1998	1	2	1	2	1	2	1	2	1	2
Gliocladium catenulatum	4	+4	+16	+4	+16	+6	+24	+6	+24	+2	+8
Gliocladium roseum	3	+4	+12	+6	+18	+8	+24	+7	+21	+5	+15
Penicillium brevi-compactum	116	+3	+348	+4	+464	+4	+464	+5	+580	-2	-232
Penicillium decumbens	6	+2	+12	+3	+18	+4	+24	+4	+24	+1	+6
Penicillium janthinellum	12	-2	-24	+2	+24	+3	+36	-2	-24	-5	-60
Penicillium meleagrinum	2	+1	+2	+3	+6	+3	+6	-2	-4	-5	-10
Penicillium nigricans	18	+1	+18	+2	+36	+4	+72	-2	-36	-5	-90
Penicillium purpurogenum	1	+2	+2	+3	+3	+3	+3	+3	+3	+2	+2
Penicillium roseo-purpureum	1	+1	+1	+3	+3	+2	+2	+3	+3	+2	+2
Penicillium verrucosum var. cyclopium	2	+7	+14	+7	+14	+5	+10	+2	+4	0	0
Penicillium verrucosum var. verrucosum	8	+3	+24	+4	+32	+4	+32	+2	+16	+1	+8
Torula herbarum	4	+1	+4	0	0	-1	-4	+2	+8	-2	-8
Trichoderma harzianum	20	+8	+160	+8	+160	+8	+160	+8	+160	+8	+160
Trichoderma koningii	27	+8	+216	+8	+216	+8	+216	+8	+216	+8	+216
Trichoderma pseudokoningii	4	+8	+32	+8	+32	+8	+32	+8	+32	+8	+32
Together effect of antagonistic activity	228		+837		+1042		+1101	e l	+1027		+49

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity

Activity of saprophitic fungi isolated from the non-rhizosphere soil towards pathogenic fungi

F	Mean	F. culi	morum	F. oxy	sporum	F. so	lani	P. irre	egulare	R. sc	olani
Fungus species	of isolates in 1996-1998	1	2	1	2	1	2	1	2	1	2
Gliocladium roseum	4	+6	+24	+6	+24	+7	+28	+7	+28	+4	+16
Penicillium chrysogenum	34	+1	+34	+2	+68	+5	+170	+4	+136	-4	-136
Penicillium funiculosum	33	+1	+33	+1	+33	+1	+33	+1	+33	-3	-99
Penicillium nigricans	6	0	0	+1	+6	+2	+12	0	0	-4	-24
Penicillium purpurogenum	12	0	0	+2	+24	+2	+24	+1	+12	-1	-12
Penicillium roseo-purpureum	4	0	0	+1	+4	+3	+12	+1	+4	+1	+4
Penicillium verrucosum var. verrucosum	30	+1	+30	+2	+60	+1	+30	+1	+30	0	0
Torula herbarum	5	0	0	+1	+5	+1	+5	0	0	-3	-15
Trichoderma hamatum	2	+6	+12	+8	+16	+8	+16	+8	+16	+6	+12
Trichoderma harzianum	2	+8	+16	+8	+16	+8	+16	+8	+16	+7	+14
Trichoderma koningii	8	+7	+56	+8	+64	+8	+64	+8	+64	+7	+56
Trichoderma pseudokoningii	23	+7	+161	+8	+184	+8	+184	+8	+184	+7	+161
Trichoderma viride	12	+8	+96	+8	+96	+8	+96	+8	+96	+8	+96
Verticillium tenerum	7	+1	+7	+2	+14	+3	+21	+2	+14	+1	+7
Together effect of antagonistic activity	182		+469		+614		+711		+633		+80

^{1 -} individual effect of antagonistic activity; 2 - total effect of antagonistic activity



Bac. – Bacillus spp., Pseud. – Pseudomonas spp., Glioc. – Gliocladium spp., Pen. – Penicillium spp., Trich. – Trichoderma spp.

Fig. 2. Occurrence of antagonistic microorganisms in the rhizosphere of potato (A) and non-rhizosphere soil (B)

ied bacteria genera from non-rhizosphere soil inhibited the growth of *F. culmorum* in the smallest degree (Tab. 3).

Among the saprophytic fungi isolated from the rhizosphere soil of potato the studies separated 228 isolates of antagonistic effect ($Gliocladium \, spp. -7$, $Penicillium \, spp. -166$, $Trichoderma \, spp. -51$, and $Torula \, herbarum -4$ colonies) (Tab. 4). Antagonistic fungi inhibited the growth of $R. \, solani$ in the smallest degree (+49), while inhibiting $F. \, oxysporum \, (+1042)$ and $P. \, irregulare \, (+1027 - total \, antagonistic \, effect)$ the most.

Non-rhizosphere soil included 182 fungi isolates, which were distinguished by their antagonistic effect, and they belonged to *Penicillium* spp. – 119 and *Trichoderma* spp. – 47 colonies, and the species of *Gliocladium roseum* – 4, *Torula herbarum* – 5 and *Verticillium tenerum* – 7 colonies (Tab. 5). The antagonistic fungi enumerated here inhibited the growth of *R. solani* the least (+ 80), and the growth of *F. solani* the most (+ 711 total antagonistic effect) (Tab. 5).

IV. DISCUSSION

On the basis of the studies it was found out that potato cultivation had a stimulating effect on the development of microorganisms in the soil. The increase of the number of microorganisms, especially in the rhizosphere potato soil, could have occurred under the effect of root exudates of the plant. The fact is explained in numerous publications concerning the role of compounds exudated by the roots of different cultivated plants (Funck-Jensen and Hockenhull 1984; Huber and Watson 1970; Pięta 1999; Rovira 1965; 1969; Schoruvitz and Zeigler 1989; Schroth and Hildebrand 1964). The total number of bacteria and fungi in 1 g of dry weight of the examined soil as compared to the non-rhizosphere soil indicated that the biologicall activity in the rhizosphere potato soil was considerably larger. Besides,

potato created favourable conditions for the development of both antagonistic bacteria Bacillus spp. and Pseudomonas spp. and antagonistic fungi of Gliocladium spp., Penicillium spp. and Trichoderma spp. This is shown by more numerous occurrence of antagonists in the rhizosphere of the analysed plant than in non-rhizosphere soil. According to Keel (1992) and Weller (1988) antagonistic bacteria from Pseudomonas spp. are capable of active colonization of plant roots, owing to which they effectively compete with pathogens for the nutritional elements occurring in root exudates and that is why they become a barrier protecting the roots from infection. An especially important role is also played by metabolites produced by antagonistic Bacillus spp. and Pseudomonas spp., which have a fungistatic or fungitoxic effect on pathogens (Ahl et al. 1986; Dowling and O'Gara 1994; Howell and Stipanovic 1980; 1983; Kloepper et al. 1999; Weller 1988). The effect of antagonistic fungi, especially Gliocladium spp. and Trichoderma spp. towards pathogenic fungi is similar to the effect of antagonistic bacteria (Hwang and Chakravarty 1993; Lin et al. 1994; Mukherjee et al. 1995; Papavizas 1985). In the case of *Penicillium* spp. some authors pay attention to the fact that those fungi produce toxic metabolites, also harmful for plants (Bojarczuk 1974; Smyk 1980).

Microorganisms characterized by antagonistic effect towards pathogenic agents not only limit their growth in the soil but they also reduce soilborne spores (Łacicowa and Pięta 1985a; 1985b; 1989; Papavizas 1985). Hence, one should expect the potato, through the positive effect on the increase of the number of antagonists in the soil, to contribute to the improved phytosanitary condition of the soil.

V. CONCLUSIONS

- 1. The mean number of microorganism population in the rhizosphere soil of potato was larger than in non-rhizosphere soil.
- 2. Potato cultivation had a positive effect on the increase of numbers of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.).
- 3. A larger number of the communities of bacteria and fungi, including antagonistic ones, in the root area of potato indicates considerable biological activity, which contributes to a better phytosanitary condition of the soil.

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ZBIOROWISKA GRZYBÓW I BAKTERII W RYZOSFERZE ZIEMNIAKA I ICH WPŁYW NA FITOPATOGENY

STRESZCZENIE

W latach 1996-1998 przedmiotem badań była gleba ryzosferowa ziemniaka oraz pozaryzosferowa. Na podstawie analizy mikrobiologicznej uzyskane wyniki wykazały, że w 1 g s. m. gleby ryzosferowej ziemniaka średnia liczebność bakterii oraz średnia liczebność grzybów była większa, aniżeli w glebie pozaryzosferowej. Podobna zależność wystąpiła w przypadku bakterii z rodzaju *Pseudomonas*. W przypadku bakterii z rodzaju *Bacillus* o 6% więcej kolonii uzyskano z gleby pozaryzosferowej w porównaniu z glebą ryzosferową ziemniaka.

Na podstawie badań laboratoryjnych stwierdzono, że w obrębie zbiorowisk populacji bakterii i grzybów w glebie ryzosferowej opisanej rośliny było znacznie więcej antagonistycznych *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp., aniżeli w glebie pozaryzosferowej.

Liczebność populacji bakterii i grzybów w strefie przykorzeniowej ziemniaka była większa w porównaniu z glebą pozaryzosferową, co może świadczyć o zwiększonej aktywności biologicznej gleby ryzosferowej.