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Influence of Agricultural Crops and Fertilization on Microbial Activity and Microorganisms in the Rhizosphere

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With 5 tables

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Abstract

In long-term field experiments on loamy sand and sandy loam, legumes (pea and lupine) stimulated microbial activity in the rhizosphere more than cereals (winter rye, winter wheat and spring barley), maize and oil flax. In the rhizosphere of winter wheat and maize, microbial activity and the bacteria species *Pseudomonas*, *Agrobacterium* and *Xanthomonas* were more stimulated by organic manuring than by mineral fertilization. A positive correlation between the stimulated bacteria species and the growth of young plants was found. Various mineral nitrogen applications had no influence on the rhizosphere microflora. The leghemoglobin content of pea nodules – an indicator of nitrogen fixation activity – was reduced by high nitrogen application in crop rotation.

Key words: Agricultural crops — fertilization — microorganisms — rhizosphere

Introduction

Microorganisms influence plant growth through their phytoeffective metabolic activities, especially in the rhizosphere (Curl and Truelove 1986). Analysis of the soil–microorganism–plant interaction is therefore a prerequisite for the effective use of natural resources in sustainable agriculture.

There are many papers describing the influence of differentiated fertilization on soil microflora (microbial activity; groups of microorganisms) and their metabolic capability (cellulose decomposition, soil respiration, nitrification and other enzyme activities) directly in the soil, mostly independent of plant growth (Schinner and Sonnleitner 1996). However, in agriculture the direct interaction between microorganisms and plants plays a special role.

In the present study, the effects of various organic and mineral fertilization measures on plant-specific microbial activity and rhizosphere microorganisms were analysed in long-term field experiments with different crops in sandy loam and loamy sand.

Materials and Methods

The analysis were carried out on winter wheat, winter rye, spring barley, maize, pea, lupine and oil flax in long-term field experiments with differentiated organic and mineral fertilization on loamy sand in Müncheberg (Rogasik et al. 1997) and sandy loam in Dedelow (Smukalski et al. 1990).

An overview of the field experiments is shown in Table 1. The investigation took place mainly at the shooting stage and in part at the stage of the young plant. Bacterial colonization of the soil and rhizosphere (roots with adherent soil) was studied by the plate count technique using glycerine-pepton agar. As described by Hirte (1969a,b), the isolated bacteria were classified into different types using macro- and micromorphological characteristics. Subsequently the number of different types in relation to the total number was calculated. Using the 'Microbial Identification System' (MIS; Microbial ID Inc., Newark, DE) and the method of Miller (1982) (see Sasser and Miller 1984), type-specific isolates were identified on the basis of the analysis of the cellular fatty acid methylester profile. The fungi were cultivated and analysed on biomalt agar. The colonization of plant roots with mycorrhiza was determined following the method of Philipps and Hayman (1970). At the same time, infestation of wheat roots with *Gaeumannomyces graminis* was examined by single plant monitoring.

The analysis of microbial activity was carried out by the DMSO reduction method (Alef 1991). For the analysis of rhizosphere microorganisms a soil: root mixture (3 : 1) was used.

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Table 1: Overview of the field experiments

	Müncheberg	Dedelow
Soil	loamy sand	sandy loam
C_t (%)	0.60–0.75	0.67–0.95
N_t (%)	0.06–0.07	0.08–0.10
P (mg kg^{-1})	53–70	55–86
K (mg kg^{-1})	68–80	69–101
pH	5.6–6.4	6.4–7.0
Start of experiment	1963	1976
Crop rotation	maize–winter rye–oil flax–winter rye–sugar beet–spring barley–pea–winter wheat	grain maize–oil flax–pea–winter wheat
Fertilization	in maize and sugar beet	in maize
Organic:	without or with manure: 4.8 and 12.8 t ha^{-1} DM, respectively	without or with bovine liquid manure: 4 and 8 t ha^{-1} DM, respectively or straw and green manure
Mineral:	0, 70, 120 kg N ha^{-1}	30, 70, 120 kg N ha^{-1}

Because of the close correlation between nitrogen fixing activity and leghemoglobin content of legume nodules, a spectroscopic method for determining the total leghemoglobin content of nodules was used as an indicator of the activity of the nitrogenase complex (Tauschke and Lentzsch 1997).

Statistical analysis was performed by ANOVA and specific effects were tested by the Newman Keuls test with a critical range of 5 %. Data characterized by the same letter were not significantly different.

Results

Influence of crops on microbial activity and bacteria species in the rhizosphere

In field experiments on loamy sand and sandy loam, the analysed crops promoted microbial activity in the rhizosphere independent of the microbial activity in the soil. Legumes (pea and lupine) stimulated activity more strongly than cereals and oil flax (Table 2). With maize, the relative stimulation effect decreased in the later developmental stage. Higher microbial activity in sandy loamy soils compared to loamy sand did not correlate with numbers of certain species of bacteria in the soil (Table 3). In both soils, the *Pseudomonas* spp. were more strongly promoted by the crops than *Agrobacterium* spp., *Bacillus* spp. and *Streptomyces* spp. Numbers decreased in the following order: pea > maize > winter wheat and winter rye. Fungi were not significantly promoted in the rhizosphere (data not shown).

Influence of long-term organic fertilization in crop rotation on microbial processes in the rhizosphere

Compared to mineral fertilization, long-term (18–20 years) straw and green manuring on sandy loam caused higher microbial activity in the rhizosphere of winter wheat, maize and pea (Table 4). There was no difference in the effect on microbial activity when liquid manure was used. In contrast, the bacteria species were stimulated by both organic fertilization measures in the rhizosphere of winter wheat, maize and oil flax, in most cases more strongly than in the soil (Table 4). In the rhizosphere of pea, only *Agrobacterium* spp. were stimulated.

Increased bacteria numbers in the rhizosphere after organic fertilization were correlated with better growth of winter wheat and maize plants until the begin of shooting (Table 4). In pea cultures, the relatively high number of bacteria was only weakly affected and young plant development was not influenced by organic fertilization.

In comparison to mineral fertilization on loamy sand, manuring (4.8 t ha^{-1} DM) also stimulated microbial activity (119 %) and the population of *Pseudomonas* spp. (122 %) and *Agrobacterium* spp. (145 %) in the rhizosphere of winter rye. Higher application of manure (12.8 t ha^{-1} DM in crop rotation compared to 4.8 t ha^{-1} DM) had no additional positive effect.

On loamy sand the leghemoglobin content in pea nodules (an indicator for nitrogen fixation activity) and the nodule number per plant were significantly reduced under the influence of high organic manuring in combination with increased mineral nitrogen fertilization (Table 5). In contrast, on sandy loamy soils organic manuring in crop rotation had no negative effect on nodule development and the leghemoglobin content (Table 5). Independently of organic manuring, higher mineral N fertilization in

Table 2: Microbial activity in the rhizosphere of different crops at two growth stages on loamy sand and sandy loam (field experiments 1994–96, soil without plants = 100)

	Microbial activity (soil = 100)			
	Loamy sand		Sandy loam	
Crop	4–6 Leaf stage	Shooting stage	4–6 Leaf stage	Shooting stage
Soil	100 a (212 a)	100 a (232 a)	100 a (478 b)	100 a (598 b)
Winter rye	184 b	128 b	–	–
Winter wheat	–	130 b	144 b	125 b
Spring barley	–	122 b	–	–
Maize	180 b	100 a	155 b	100 a
Pea	228 c	510 c	167 c	301 c
Lupine	232 c	–	166 c	–
Oil flax	–	121 b	–	–

() ng DMS g^{-1} h^{-1}

Data followed by the same letter are not significantly different ($P < 0.05$).

Table 3: Colonization of different bacteria species in the rhizosphere of different crops (shooting stage) in field experiments (1995) on loamy sand (lS) and sandy loam (sL)

	1×10^{-6} cfu g^{-1} root or soil							
	Pseudomonas spp.		*Agrobacterium* spp.		*Bacillus* spp.		*Streptomyces* spp.	
Crop	IS	sL	IS	sL	IS	sL	IS	sL
Soil	0.5	0.2	0.2	0.1	0.1	0.1	0.2	0.1
Winter rye	11.9[1]	–	2.0	–	1.3	–	0.5	–
Winter wheat	–	19.0[1]	–	2.4	–	1.3	–	0.8
Maize	28.0^{-1}	29.0[1]	2.0	2.4	2.0	0.1	0.5	0.1
Pea	56.0^{-1}	95.0[1]	68.0[1]	40.0[1]	12.0	1.0	5.0	0.1

[1]Significant effect to the soil.

Table 4: Influence of long-term organic fertilization on microbial activity, the bacteria species in the rhizosphere and plant growth (field experiments on sandy loam 1994–96, mineral fertilization = 100)

			Bacteria								Plant growth	
	Microbial activity		*Pseudomonas* spp.		*Agrobacterium* spp.		*Xanthomonas* spp.		*Bacillus* spp.		(shooting stage)	
Crop	St + Gm	Lm	St + Gm	Lm	St + Gm	Lm	St + Gm	Lm	St + Gm	Lm	St + Gm	Lm
Soil	108	102	115	108	133	93	0	100	68	68	–	–
Winter wheat	117*	95	128*	139*	147*	137*	196*	316*	109	98	115*	117*
Maize	127*	98	125*	312*	101	131	350*	140*	188*	225*	110	120*
Pea	125*	–	67*	47*	144*	156*	76	70*	56*	8*	97	94
Oil flax	–	–	119	137*	168*	177	250*	200*	66*	130	–	–

*Significantly different from the mineral fertilization (NPK). St = straw; Gm = green manure; Lm = liquid manure.

Table 5: Influence of different organic and mineral nitrogen fertilization treatments on the leghemoglobin content of pea nodules and nodule development at the shooting stage [field experiments in Müncheberg (lS) and Dedelow (sL) 1996–97]

Soil	Organic fertilization in crop rotation (t ha^{-1} DM) Lm	M	Mineral N (kg N ha^{-1})	Lb (mg/plant)	Nodules/plant
			in crop rotation		
sL	without	–	30	2.95 a	43 a
			120	2.14 b	31 a
	8.0	–	30	3.16 a	38 a
			120	1.75 b	35 a
			to pea		
lS	–	4.8	20	8.52 a	111 a
	–	12.8	40	4.92 b	75 b

Lm = liquid manure; M = manure.

crop rotation inhibited nitrogen fixation activity in both soils.

In both analysed soils, no infestation of cereal roots with *Gaeumannomyces graminis* was observed because the known crop rotation axioms were followed in all fertilization variants.

On sandy loam, the colonization of roots with mycorrhiza was stimulated by the long-term use of straw, green and liquid manure only in winter wheat, by about 20%. No effects could be found in maize and pea. Organic manuring had no unambiguous effect on the saprophytic fungi (*Fusarium* spp., *Trichoderma* spp., *Cephalosporium* spp., *Chaetomium* spp. and *Penicillium* spp.) in the rhizosphere (data not shown).

Influence of different amounts of nitrogen fertilizer on microbial processes in the rhizosphere

Application of high amounts of mineral nitrogen (120 kg N ha^{-1} in crop rotation) was compared to variants without mineral nitrogen on loamy sand and variants with 30 kg ha^{-1} N on sandy loam. Higher nitrogen application caused no stimulation of the microbial activity in the rhizosphere of maize, wheat and rye and no increase in colonization with *Pseudomonas* spp., *Agrobacterium* spp., *Bacillus* spp. and fungi (data not shown). In the rhizosphere of pea the microbial activity and the number of *Pseudomonas* spp. and *Bacillus* spp. were decreased (62 %).

Discussion

In long-term fertilization experiments on loamy sand and sandy loam, the microflora in the rhizosphere were affected differently, and were also affected by the crop and by organic manuring.

To characterize the importance of the analysed rhizosphere bacteria for plant growth, bacteria of different species (*Pseudomonas* spp., *Agrobacterium* spp., *Xanthomonas* spp., *Stenotrophomonas* spp., *Flavobacterium* spp., *Rhizobium* spp., *Arthrobacter* spp., *Azotobacter* spp., *Bacillus* spp. and *Streptomyces* spp.) were isolated from cereals, legumes and winter rape and their effectiveness was tested in vegetation experiments. With isolates of *Pseudomonas* spp., *Agrobacterium* spp., *Stenotrophomonas* spp. and *Rhizobium* spp., a reproducible growth stimulation of legumes, gramineae and crucifers was demonstrated in pot and field experiments (Höflich et al. 1994, 1997, Höflich and Kühn 1996). Although not all isolates of these species had a growth stimulation effect, these bacteria species deserve more attention.

For instance, *Pseudomonas* spp. and *Agrobacterium* spp. were enriched in the rhizosphere of legumes (Table 3) and could migrate to the subsequent crops (Wiehe and Höflich 1995). From these findings it can be assumed that legumes in crop rotation have importance not only for nitrogen fixation, but also for the enrichment of growth stimulating associative bacteria in the soil.

The results show that organic manuring stimulates not only microbial activity in the soil (Bolton et al. 1985, Goyal et al. 1993) but also the colonization of the rhizosphere with bacteria. The better young plant growth seems to be produced by positive combination effects of nutrient release from the organic fertilizers and plant growth stimulation through the enriched phytoeffective bacteria. These combination effects could not be exploited by pure mineral fertilization.

High doses of manure in crop rotation and additional mineral nitrogen fertilization could inhibit the rhizosphere microflora of pea and the nitrogen fixation activity of pea nodules. Doughton et al. (1993) explained the differences in nitrogen fixation caused by dissimilar fertilization and cropping through treatment influences on soil nitrate. The primary impact of mineral nitrogen on N_2 fixation of legumes has been well documented (Tsai et al. 1993, Waterer et al. 1994).

The results of this study show that efficient use of microbial resources in sustainable agriculture demands more analyses of plant-specific rhizosphere processes, with attention to the metabolic capacity and the colonization of microorganisms dependent on management strategies.

Zusammenfassung

Einfluß von landwirtschaftlichen Kulturpflanzen und Düngungsmaßnahmen auf mikrobielle Aktivitäten und Mikroorganismen im Rhizosphärenraum

In langjährigen Feldversuchen auf lehmigem Sand und sandigem Lehm stimulierten Leguminosen (Erbse, Lupine) die mikrobiellen Aktivitäten im Rhizosphärenraum stärker als Getreide (Winterroggen, Winterweizen, Sommergerste), Mais und Öllein.

Organische Düngung förderte im Vergleich zur Mineraldüngung die Besiedlung mit *Pseudomonas* spp., *Agrobacterium* spp. und *Xanthomonas* spp. in der Rhizosphäre von W.-Weizen und Mais. Zwischen stimulierter Rhizosphärenbakterienflora und Jungpflanzenwachstum zeichneten sich positive Beziehungen ab. Unterschiedliche Stickstoffdüngergaben hatten keinen Einfluß auf die Rhizosphärenmikroflora. Der Leghämoglobingehalt – ein Indikator für die N_2-Fixierungsaktivität – war in Erbsenknöllchen bei hoher N-Düngung in der Fruchtfolge reduziert.

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