Nitrogen Balance in a Soil Cropped with Non-Leguminous Plants Inoculated with Nitrogen-Fixing Microorganisms

V. P. SHABAEV,[1] V. Yu. SMOLIN,[1] G. R. BURLUTSKAYA,[2] and M. M. UMAROV[2]

[1]Institute of Soil Science and Photosynthesis, USSR Academy of Sciences, Puschino and [2]Soil Science Department, Moscow State University, Moscow /USSR/

The data available at present on the effect of nitrogen-fixing microorganisms on the yield of non-leguminous plants are contradictory, and the balance experiments taking into account the changes of soil nitrogen content due to the activity of diazotrophs are not abundant.

In pot experiments carried out on a grey forest soil the effect of inoculation of oat /Avena sativa L./ with Azospirillum brasilense sp7 and Azotobacter chroococcum and the inoculation of rape /Brassica napus L./ with Bacillus polymyxa on the yield and nitrogen balance in the soil has been studied. Five oat plants were grown up to complete ripeness in the pot containing 5 kg of air-dried soil to which labelled ^{15}N ammonium sulphate had been applied at a rate of 4 mg N/100 g of soil on a PK background. In the first experiment oat plants were inoculated with suspensions of Azospirillum brasilense sp7, in the second with Azotobacter chroococcum at a rate of 10^6 cells per 1 plant. Ten rape plants were cultivated in the third experiment in a climatic chamber in a pot containing 5 kg of air-dried soil to the end of blooming /45 days/ at 18-20 °C from 8 a. m. till 8 p. m. under illumination of 20 klux, the rest of the time the plants were kept at 15 °C in darkness. $Ca/NO_3/_2$ was applied at a rate of 2 mg N/100 g of soil on a PK background. The rape seeds were inoculated with a suspension of the standard strain Bacillus polymyxa and a mutant strain B. polymyxa, which has an increased nitrogen fixing activity, at a rate of 10^8 cells per 1 plant. In all the experiments the plants were watered with deionized water, maintaining soil moisture at a level of 60% WHC. The activity of C_2H_2/N_2-fixation in the rhizosphere of plants was assayed in dynamics according to the phases of plant development by acetylene technique /UMAROV, 1976/.

After harvesting, the total nitrogen content of the plants and soil was determined by indophenolic method /KUDEYAROV, 1972/ and ^{15}N excess % was assayed by an emission spectrometer NOI-5 /GDR, Stratron/. The total nitrogen content of the soil samples at the end of the experiment was determined in 15 replications for each treatment and the initial one.

Oat inoculation with Azospirillum did not lead to yield increase in the first experiment and had no effect on the uptake of fertilizer and soil nitrogen by the plants /Table 1/. However, after the addition of Azospirillum to the soil a steady increase in the ^{14}N content of the soil was observed at

Table 1

Oat dry weight, nitrogen uptake by plants and their balance in the "soil-plant" system

Experiment	Microorganisms	Fertilizers	Dry weight, g/pot			N uptake by plants, mg/pot		N uptake by total biomass of plants, mg/pot		^{14}N content in soil at the end of the experiment[a]	^{14}N balance[b]
			Grain	Roots	Total biomass	Grain	Total biomass	^{15}N	^{14}N	mg/100 g of soil	
1	Without inoculation	^{15}NPK	13.9	3.6	28.1	241	314	155	159	127,6	+ 0.5
	Azospirillum brasilense sp 7	^{15}NPK	13.6	3.2	26.8	230	310	153	157	132.2	+ 5.1
2	Without inoculation	PK	3.9	0.7	9.8	55	79	-	79	109.6	+ 0.8
	Without inoculation	^{15}NPK	9.9	1.3	20.0	157	242	134	108	110.3	+ 2.1
	Azotobacter chroococcum	^{15}NPK	11.0	2.0	21.9	160	249	140	109	116.1	+ 8.1

a/ Without ^{15}N fertilizer remained in the soil at the end of the experiment

b/ ^{14}N balance = /^{14}N in plants + ^{14}N in soil at the end of the experiment/ - ^{14}N in soil at the beginning of the experiment

Initial nitrogen content of the soil was 127.9 and 110.4 mg/100 g of soil in the first and second experiments respectively. Plants were cropped at addition 4.8.8 mg of ^{15}NPK per 100 g of soil

the end of the experiment as compared with its initial amount which resulted in a positive ^{14}N balance in the "soil-plant" system.

Under the effect of Azotobacter application in the second experiment the grain weight and total biomass of oat plants tended to increase whereas a solid 150% increase of root weight was observed /Table 1/. Azotobacter had practically no effect on the nitrogen uptake by grain and total biomass of plants. As a result of the treatment with Azotobacter and the application of Azospirillum in at the end of the first experiment a solid increase of ^{14}N content of the soil and a positive balance were found.

The data obtained by the balance method are in good agreement with the data on nitrogen-fixing activity determined by the acetylene technique /Table 2/. The nitrogenase activity in the rhizosphere of the oat plants increased during their development and reached maximum values in the blooming and milky stages. Under the effect of the application of Azospirillum, nitrogen-fixing activity in the oat rhizosphere was 4 times higher on the average during all stages of the ontogenesis as compared with the treatment without adding microorganisms. In a number of experiments after the inoculation of non-legumes with Azospirillum an insignificant yield gain or its absence under simultaneous significant enhancement of nitrogen fixation was observed /BARBER et al., 1976, 1979; O'HARA et al., 1981; SMITH et al., 1984/.

Table 2
Acetylene reduction in the rhizosphere of oat

Microorganisms	N, mg/pot/hour x 10^{-3} a/			
	Heading	Blooming	Milky stage	Average during vegetation period
Without inoculation	0.3 ± 0.2	3.3 + 0.9	2.9 ± 0.9	2.4 ± 0.4
Azospirillum brasilense sp 7	3.8 ± 0.7	20.3 ± 9.9	5.2 ± 0.4	9.8 ± 3.7

a/Values are means for 3 replications

Table 3
Weight of rape green mass, nitrogen uptake by plants and acetylene reduction activity in the rhizosphere of rape

Microorganisms	Dry matter, g/pot	Nitrogen uptake by plants mg/pot	Acetylene reduction activity in the rhizosphere of rape, N mg/pot/hour x 10^{-3}	
			beginning of flower budding	flower budding--the beginning of blooming
Without inoculation	14.7	133	3.9	1.18
Bacillus polymyxa, wild type	15.5	151	6.3	2.38
Bacillus polymyxa, mutant strain	14.8	145	1.2	1.49

Acetylene reduction activity was not determined at the end of blooming

In our experiments the application of relatively small amounts of mineral nitrogen /4 mg N/100 g of soil/ stimulated associative nitrogen fixation during oat cultivation /Tables 1, 2 and 3/. The inoculation of non-leguminous plants with Azospirillum and Azotobacter with simultaneous application of low rates of mineral nitrogen are reported to have a positive effect on associative nitrogen fixation and yield /SMITH et al., 1984; MARTINEZ-TOLEDO et al., 1988/.

Since during our experiments the plants were irrigated with water containing no nitrogen, and no ammonia was observed in the surrounding atmosphere, excess ^{14}N found in the soil after the vegetation period and its positive balance at the application of nitrogen-fixing microorganisms can be accounted for only by the enhancement of nitrogen fixation. This is proved by the fact that in the treatments without microorganisms no reliable changes in the nitrogen content of the soil and its positive balance are found after the vegetation period. In our experiments the amounts of nitrogen accumulated in the soil by nitrogen-fixing bacteria can be compared with the rate of applied nitrogen fertilizers. At the end of the experiments an increase of the nitrogen content of the soil and substrate /sand/ available for growing plants was found due to the activity of heterotrophic nitrogen-fixing bacteria after rice cultivation /SINGH and SINGH, 1987; SIROTA, 1982/.

Probably the nitrogen fixed by these diazotrophs is not available to plants during the vegetation period due to its localization in the soil in microbial biomass /AZAM et al., 1986/ or in the humous substances /KALININSKAYA et al., 1979/.

Rape inoculation both with standard and mutant strains of B. polymyxa did not result in the greater yield of the above-ground mass of rape, however, at the same time the nitrogen uptake by plants increased slightly /Table 3/. The experiment showed that at the beginning the inoculated plants grew better than the non-inoculated ones, i.e. the former started to bloom 10 days earlier than the latter ones. But after harvesting, at the end of blooming the difference between them practically disappeared. Determination of nitrogen-fixing activity in the rape rhizosphere also proved that at the beginning of the flower budding stage, when B. polymyxa, especially the mutant strain was applied, the nitrogen-fixing activity was considerably higher as compared with the non-inoculated treatment /Table 3/. At the next stage of plant development the nitrogen-fixing activity in the rhizosphere of inoculated plants was considerably lower than that in the treatment without inoculation.

Thus, the results of balance experiments show that in the absence of Azospirillum and Azotobacter the effect on the oat yield due to the enhancement of nitrogenase activity in the rhizosphere, nitrogen fixed by diazotrophs is accumulated in the soil, thus increasing the amount of nitrogen stored these. Inoculation of rape plants with B. polymyxa stimulates plant development during the first stages of growth, increasing nitrogen fixation in the rhizosphere and slightly increasing nitrogen uptake by the plants but having no favourable effect on the yield of green mass.

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