Polish Journal of Environmental Studies Vol. 11, No. 3 (2002), 193-198

Review

Biological Effects of Mineral Nitrogen Fertilization on Soil Microorganisms

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Received: July 27, 2001
Accepted: October 18, 2001

Abstract

The mineral fertilization of arable land positively affects an increase of the biological productivity of various ecosystems as well as the microbial activity in soil. From the three elements N, P, K used for fertilization - nitrogen is one of the most important factors affecting soil fertility and productivity as well as the growth and development of cultivated plants. The chemical studies show that high N rates of fertilization result in the formation of carcinogenic nitrosamines in soil environments. The use of mineral N resulted in significant changes in microbiocenoses under investigated ecological condition. The decline of some beneficial microorganisms, as well as the occurrence of carcinogenic nitrosamines in soil, were the symptoms of changes in the environment.

Keywords: nitrogen fertilization, nitrosamines, microbial activity, soil agrocenoses

Fertilization in modern agriculture not only maintains, but even gradually increases field crop yield. Modern fertilization systems are not just an isolated element of plant production, but are indispensable link in the system of agrotechnical practice. Efficient fertilization provides plants with nutrients at appropriate proportions and quantities which enable maximum yield increase of crops with high biological and technological quality. Fertilization should also gradually improve soil fertility. It should be mentioned that soil fertility, decisive for proper development of cultivated plants and crop yield, depends on many factors, the most important being nutrient contents, and physical and chemical properties and metabolic activity of many microorganisms implicated in metabolic processes and energy flow [28]. The results of studies conducted by Alexander [1], Myskow [25], Smyk [30]. Myskow et al. [26] and many other authors clearly indicate that microbiological and biochemical processes in soil environment frequently prevail over purely chemical reactions. Although biomass of all microorganisms living in soil constitutes only several percent of organic matter content, they play an important role in the functioning of entire ecosystems because, due to their enormous biochemical and biogeochemical activity, they can exert crucial effects on dynamics of multidirectional microbiological processes [5, 17,18, 31]. Microorganisms, being a part of all natural and man-made ecosystems, compose biocenoses which are significant and essential biochemical elements responsible for the entirety of biogenic element transformation in soil environment, and which exert critical effects on biochemical activity and ecological stability, and biological productivity of many field, forest and grassland ecosystems. They are involved in biochemical transformations of mineral fertilizers,

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particularly NPK fertilizers, synthesis of biologically active substances (amino acids, vitamins, antibiotics, toxins) and nitrogen fixation from the air [19, 20]. They regulate element circulation in soil environment and make them assimilable for plants.

Many years' studies and effects of agrotechnical practice clearly indicate that mineral fertilization, mainly nitrogen supply, has the strongest influence on field crop yield. However, results of investigations carried out by Doran et al. [11], Smyk et al. [32], Jankinson [15], Barabasz [6], Barabsz and Smyk [9] and Gawronska [13] have shown that mineral fertilization also strongly affects a number of microorganisms and qualitative selection of whole communities of soil microorganisms. Although mineral fertilization has been shown to have beneficial effect on crop yield, incorrect agrotechnical measures and improper fertilization practice can lead to seriously disturbed functions of entire agroecosystems and contribute to the formation of different compounds in soil (e.g. nitrosamines, mycotoxins) which are harmful for soil microorganisms and cultivated plants, and then for animals and humans [31, 32]. This detrimental phenomenon, a side-effect of the use of chemistry in agriculture, definitely influences activity of soil microorganisms, and consequently it indirectly affects fertility of arable soils.

Study results of Doran et al. [11], Wqjcik-Wojtkowiak [35], Mallik and Tesfai [21], Mallik et al. [22] and Newbould [27] indicate that the above mentioned agricultural chemicalization, especially caused by the use of high N fertilizer rates applied to arable, grassland and horticultural soils, might not only hazard biological productivity and ecological stability of agroecosystems, but can also threaten with pollution of surface and underground waters by accumulation of nitrates, nitrites and many other organic nitrogen compounds such as amines, nitro and nitroso compounds, including nitrosamines and nitrosamides.

Mineral nitrogen, introduced into soil environments, undergoes various biochemical transformations, in which many microorganisms and plants participate [7, 8]. Their dynamics depend on a number of ecological factors, but serious changes in soil nitrogen transformations can be expected under disadvantageous physicochemical conditions in soil environments, when nitrosamine precursors, such as primary and secondary amines, nitrates, nitrites, nitro and nitroso compounds are formed.

Good N efficiency is particularly important for crop yield and environmental protection and should be discussed in relation to the system comprising soil - water - plant. Nitrogen accumulated in soil, independently of its origin, should be used for production of plant biomass and to maintain high soil fertility. However, under certain circumstances, nitrogen fertilization contributes to mobilization of soil nitrogen. Such a process occurs principally in soils to which a low rate of organic fertilizers was applied, with a low humus content. In this event, decomposition of organic matter in soils generates nitrates which, unless taken up by plants, can be leached by precipitation to deeper soil layers and to ground waters. Mineral N fertilizers are applied to surface layers of soil while organic fertilizers are introduced to the depth of 10-20 cm below soil surface. Both these fertilizer types are the source of mineral N, which increases its concentration in soil solution and saturates soil sorption complex with ammonium ions. An increase in the concentration of soil solution is accompanied by nitrogen leaching, if this element is not absorbed by plants. Hitherto conducted studies estimate that 15-50% fertilizers-derived N is leached to deeper soil layers and ground waters, which contaminate surface waters contributing to facilitation of eutrophication (Table 1). Eutrophication leads to the accelerated phytoplankton and waterside vegetation growth. Many phytoplankton species, including cyanophyte, are harmful for humans and animals since they cause stomach diseases, catarrh or even palsy. In animals, serious poisoning can lead to death due to nervous system paralysis.

Table 1. Nitrogen leaching in relation to the cultivated plants and N fertilizer rate (according to [23]).

Plants	N rate (kg/ha)	N loss (kg/ha)	N content in drainage waters (mg/dm^3)
Meadow vegetation	175	20	11
Cereals	64	43	17
Root plants	128	68	38
Vegetables	270	82	51

The most recent ecotoxicological studies have indicated that out of numerous chemical compounds contaminating natural environment (soil, water), nitrosamines deserve special attention as they are included among the most dangerous ecological poisons for their detrimental biological and ecological effects [4, 24]. The studies of Alexander [2], Barabasz [6], Panchole [29] and Smyk et al. [31, 32] have demonstrated that nitrosamines endanger also all organisms inhabiting ecosystems and biological productivity of agroecosystems, grassland and forest ecosystems as surface water quality (rivers, lakes, ponds), field crops, food products, and health of animals and humans. Moreover, Byrnes [10], Smyk [30] and other authors have reported that nitrosamines isolated from soil environment of various ecosystems exerted strong phytotoxic, mutagenic, teratogenic and carcinogenic effects on micro- and macroorganisms (plants, animals and humans). They are strong inhibitors of DNA and RNA synthesis, block transmission of genetic information connected with amino acid synthesis and formation of certain phytohormones, such as a-NAA responsible for rhizogenesis in higher plants.

Ayanaba et al. [4], Focht and Verstraete [12], Hill [14] and Alexander [3] have shown that nitrosamine precursors are generated with the participation of autotrophic (mainly of the genera *Nitrobacter* and *Nitrosomonas)* and heterotrophic microorganisms, engaged in nitrification and denitryfication processes, and numerous bacteria of the genera *Arthrobacter, Bacillus, Eubacterium, Pseudomonas, Mycobacterium, Nocardia* and *Streptomyces* and soil fungi belonging to the genera *Aspergillus, Candida, Fusarium, Cephalosporium, Penicillium* and

Table 2. Average number of microorganisms in soil environment of grassland ecosystems *Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum* (the data presented as thousands/g of soil were obtained in the period 1975-1995)

Fertilizers rates	*Arrhenatheretum elatioris*			*Gladiolo-Agrostidetum*		
	Bacteria	Actinomycetes	Fungi	Bacteria	Actinomycetes	Fungi
0-Control	640	200	17.4	420	180	12.6
$P_{60}K_{80}$	780	240	16.2	500	200	14.4
N_{60}	1290	260	17.6	610	185	17.2
$N_{60}P_{60}K_{80}$	1340	380	18.5	850	215	22.3
N_{120}	1390	370	25.4	890	230	27.4
$N_{120}P_{60}K_{80}$	1450	440	30.6	1100	220	29.5
N_{180}	1470	460	26.2	1050	260	31.5
$N_{180}P_{60}K_{80}$	1520	520	28.3	1290	250	37.6
N_{240}	1570	610	32.4	1250	290	35.2
$N_{240}P_{60}K_{80}$	1680	740	34.6	1310	320	37.4
N_{360}	1790	380	27.5	1140	310	30.3
$N_{360}P_{60}K_{80}$	1940	420	29.4	1260	360	36.2

Table 3. Influence of many years' mineral NPK fertilization on the changes in number of species belonging to the selected groups of microorganisms in soil environment of grassland ecosystems *Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum* (the data were obtained in the period 1975-1995)

Fertilizers rates	*Arrhenatheretum elatioris*			*Gladiolo-Agrostidetum*		
	Bacteria	Actinomycetes	Fungi	Bacteria	Actinomycetes	Fungi
0-Control	30	22	30	27	25	24
$P_{60}K_{80}$	30	22	30	27	25	24
N_{60}	30	22	30	27	25	24
$N_{60}P_{60}K_{80}$	30	22	30	27	25	24
N_{120}	28	20	29	25	24	20
$N_{120}P_{60}K_{80}$	28	20	30	25	23	20
N_{180}	24	19	28	24	22	20
$N_{180}P_{60}K_{80}$	24	18	27	23	21	17
N_{240}	24	17	24	20	19	16
$N_{240}P_{60}K_{80}$	22	17	28	20	19	18
N_{360}	18	15	29	15	16	20
$N_{360}P_{60}K_{80}$	12	14	32	13	12	21

many others. Dynamics of nitrosoamine precursor generation and formation of different nitrosamines in soil environments depends on many ecological and physicochemical factors, characterizing soil environment, such as pH and type of soil, vegetation cover, amount and kind of nitrogen substrate available for microorganisms, oxygen conditions, precipitation, agrotechnical measures, applied pesticides and N fertilization rate. Higher nitrosoamine concentrations were observed in acid soils with low pH value (pH 4.0-6.0) than in soils characterized by higher pH values (pH 5.0-7.5). In acid soils, nitrosamines are readily produced, and various bacteria and fungi actively participate in this process. Ecotoxicological conditions are worsened by the fact that, as indicated by Kaplan and Kaplan [16] and Tate and Alexander [33, 34], nitrosamines can be deposited in soil for 90-150 days, and their biodegradation is very slow. In fact, some microorganisms can degrade nitrosamines to simple compounds in co-metabolic processes, and then use them as nutrients, however such bacteria are sparse, belonging principally to the genera *Arthrobacter* and *Eubacter.*

An example of negative effect of high rates of NPK

Table 4. Dominating species of microorganisms occurring in the studied environments of grassland ecosystems *Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum* in Jaworki, Pieniny Mountains (the data were obtained in the period 1975-1995).

No	Bacteria	Actinomycetes	Fungi
1	Arthrobacter citreus	Streptomyces alboniger	Absidia glauca
2	Arthrobacter globiformis	Streptomyces albus	Acremonium strictum
3	Arthrobacter terregens	Streptomyces boboli	Alternaria alternata
4	Bacillus brevis	Streptomyces celluloflavus	Alternaria geophila
5	Bacillis cereus	Streptomyces cellulosae	Aspergillus alliaceus
6	Bacillus cereus var. mycoides	Streptomyces flaveolus	Aspergillus chevalierii
7	Bacillus circulans	Streptomyces fradiae	Aspergillus fumigatus
8	Bacillus firmus	Streptomyces globisporus	Aspergillus terreus
9	Bacillus globisporus	Streptomyces globosus	Aspergillus versicolor
10	Bacillus lentus	Streptomyces griseolus	Cladosporium herbarum
11	Bacillus licheniformis	Streptomyces griseus	Cladosporium macrocarpum
12	Bacillus macerans	Streptomyces lavendulae	Fusarium dimerum
13	Bacillus megaterium	Streptomyces longisporus	Fusarium graminearum
14	Bacillus polymyxa	Streptomyces mirabilis	Fusarium moniliforme
15	Bacillus psychrophilus	Streptomyces nigrescens	Fusarium nivale
16	Bacillus pumilis	Streptomyces odorifer	Fusarium oxysporum
17	Bacillus subtilis	Streptomyces rochei	Fusarium sporotrichioides
18	Cellulomonas flavigena	Streptomyces roseolus	Glocladium roseum
19	Clostridium pasteurianum	Streptomyces rutgersensis	Humicola grisea
20	Cytophaga hutchinsonii	Streptomyces tanashiensis	Mortierella alpina
21	Cytophaga krzemieniewska	Streptomyces violaceus-niger	Mortierella hyalina
22	Eubacterium nitritogenes	Streptomyces violatus	Mucor hiemalis
23	Flavobacterium lutescens		Penicillium citrinum
24	Myxococcus macrocarpum		Penicillium implicatum
25	Nitrobacter europaea		Penicillium jenseni
26	Pseudomonas aeruginosa		Penicillium rugulosum
27	Pseudomonas fluorescens		Penicillium tardum
28	Pseudomonas putida		Rhizopus nigricans
29	Rhizobium leguminosarum		Trichoderma viride
30	Sporocytophaga myxococcoides		Verticillium cellulosae
31			Verticillium tenerum
32			Zygorrynchus moelleri

fertilization on permanent grassland is provided by the results of long term (1975-1995) comprehensive agrotechnical, chemical and microbiological studies of montane soils of grassland ecosystems, *Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum* floral communities in Pieniny Mountains at Montane Experimental Station IMUZ in Jaworki near Szczawnica. The data based on field and laboratory investigations gathered for many years indicate that the applied fertilization exerted beneficial effect on an increase in soil microbiological activity and general raise in biological productivity of the grassland ecosystems, however, it led also to conspicuous quantitative and qualitative changes in microbiocenotic composition of soil microflora and species composition of meadow vegetation. Although NPK fertilization caused an increase in green mass and hay yield by about 150-300%, hay feeding value was relatively low due to impoverished biodiversity.

The data averaged over a 20-year period of quantitative studies presented in Table 2 indicate that high NPK fertilization rates significantly elevated the number of microorganisms belonging to the studied groups. Number of bacteria, actinomycetes and fungi increased 2-, 2.5-and 2-fold, respectively, in comparison with control.

Table 5. Effect of many years' mineral NPK fertilization on nitrosamine content in soil environments of grassland ecosystems *Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum* (the data were obtained in the period 1975-1995) (data in µg/ of soil)

Fertilizers rates	*Arrhenatheretum elatioris*			*Gladiolo-Agrostidetum*		
	DMNA	DENA	Others	DMNA	DENA	Others
0-Control	0	0	0	0	0	0
$P_{60}K_{80}$	0	0	0	0	0	0
N_{60}	0	0	0	0	0	0
$N_{60}P_{60}K_{80}$	0	0	0	0	0	0
N_{120}	0.45	0.35	0.20	0.20	0.40	0.10
$N_{120}P_{60}K_{80}$	0.35	0.20	0.10	0.15	0.20	0.10
N_{180}	1.60	1.55	0.75	0.90	0.70	0.80
$N_{180}P_{60}K_{80}$	1.40	1.20	0.65	0.60	0.50	0.55
N_{240}	6.20	5.40	6.80	12.70	8.20	4.75
$N_{240}P_{60}K_{80}$	5.70	4.40	4.50	10.40	6.60	1.90
N_{360}	23.40	25.50	16.40	20.20	17.40	17.25
$N_{360}P_{60}K_{80}$	19.80	22.70	14.20	17.40	16.20	12.40

DMNA = dimethylnitrosamine; DENA = diethylsnitrosamine; Others = dipropylnitrosamine, dibutylnitrosamine, methylethylnitrosamine.

Table 3 shows that fertilization changed a number of species, members of the chosen groups of microorganisms. High N rates reduced number of bacteria and actinomycete species, but the change in the number of fungal species was negligible. It was demonstrated that high mineral N fertilizers rates evoked recession of bacteria of the genera *Arthrobacter* and *Sterptomyces* by 50% on average and complete eradication of bacterial genera *Azotobacter, Rhizobium* and *Bradyrhzobium*. On the other hand, a rise in the number of microorganisms and biomass of the genera *Eubacterium, Pseudomonas* and *Bac.ullus,* and fungi of the genera *Aspergillus, Fusarium, Penicillium, Verticillium* and others was noted (Table 4).

The observed changes in microbiocenotic composition of montane soils of grassland ecosystems should be regarded as harmful from both biological and ecological points of view, since they create disturbances in nitrogen and carbohydrate metabolism in the study ecosystems, which can have negative consequences for their biological productivity.

Chemical studies of soil of montane grassland ecosystems conducted over a many-year period have demonstrated that mineral N fertilization at rates exceeding 120 kg N/ha/year is a direct cause of generation of carcinogenic nitrosamines in soil. Table 5 presents nitrosoamine contents in the studied soils. The highest nitrosamine amounts were observed on plots fertilized at a rate of 360 kg N/ha/year. Nitrosamine level exceeded even 25 µg/g of soil. In should be emphasized that grasslands are overgrown with meadow vegetation, and subject to one-sided constant fertilization and always the same land use. Therefore, there are conditions favoring nitrosamine accumulation in soil environments. A completely different situation occurs on cultivated fields, where crop rotation is applied, and agrotechnical measures, such as plowing, harrowing, sowing or fertilization, are changed, since they depend on the cultivated plant. Thus, on cultivated fields, favorable conditions for nitrosamine accumulation do not develop, although at N rates of 180 kg N/ha/year, nitrosamine contents range 3-5 µg/g of soil.

It should be emphasized that many years' mineral N fertilization of both grasslands and cultivated fields caused a considerable decrease in soil pH value, which dropped as low as pH 4.8-5.2.

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