Microbial transformation of nitrogen in soil after the biochar addition

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Abstract: It is apparent from the several studies that soil amended with biochar (pyrolysed plant-derived material in absence of oxygen) can influence the abundance, diversity and activity of soil organisms. The objective of the present work is to characterize the impact of soil treatment with various amounts of mineral nitrogen fertilizers on specialized group of free-living N_2-fixing bacteria (diazotrophic) bacteria as a potential indicator of the effect of biochar amendment on the soil nitrogen transformation in the planned experiments. The high sensitivity of diazotrophic bacteria from the genus *Azotobacter* to the increasing doses of nitrate fertilizers has been found out in our studies. In terms of model experiment it has been showed that the number of *Azotobacter* was reduced under the influence of nitrogen fertilizers. Moreover, the higher concentration of mineral nitrogen, the more distinct the decreasing trend in the percentage of nitrogen-fixing bacteria has been observed. It was found that soya bean cultivation has led to the increase of the amount of nitrate-nitrogen in the soil, which explains the significant reduction in the percentage of *Azotobacter* genus bacteria. Based on the high value of correlation coefficients between the inversely correlated studied values is to be recommended to use these as an indicator of the effect of above mentioned biochar amendment.

Key-Words: biochar, soil, genus *Azotobacter*, nitrogen fertilizers

Introduction

Biochar, a material obtained from the carbonization of biomass, has many uses as a soil improver and also as a method of carbon sequestration. Therefore, biochar addition has been explored in the last years as a way to mitigate global warming. Different studies have found that biochar can improve both the nutrient- and water-holding properties of soil, and its popularity in recent years also owes to its ability to reduce greenhouse gases by storing carbon in soil, in some cases for many centuries [1,2].

However, responses of plant – soil - microbial complexes to biochar addition in particular in relation to changes in microbial activities changing the form and the availability of soil nitrogen are seldom documented. Nitrogen (N) is an essential nutrient, and research to date shows that biochar has the ability to manipulate the rates of N cycling in soil systems by influencing nitrification rates and adsorption of ammonia and increasing NH_4^+ storage by enhancing cation exchange capacity in soils and it may cause the reduction of gaseous N losses such as N_2O and nitrate leaching [3]. A greater abundance of microorganisms after biochar additions to soil has been relatively well described [4]. But still, it is not satisfactory explained, how biochar application into the soil influences free-living diazotrophs, the transformation mechanisms and fate of N. Finding an answer to some of these questions will be the aim of our future studies.

Though, modern technologies of crops cultivation tend to ensure complete recovery of nitrogen spends [5]. Due to the usage of such fertilizers as mineral and fertilizers of organic origin plants receive a sufficient amount of nutrients. However, fertilizers can not only enhance physiological processes, but also inhibit microbiological processes, including biological nitrogen fixation [6]. It is known, that in the structure of microbial coenosis, *Azotobacter* acts as a sensitive indicator of soil condition changes under the influence of anthropogenic factors and as an indicator of the amount of soil nutrients [7, 8, 9].

The main goal of our previous studies in Ukraine was to determine a quantitative composition of free-living diazotrophs in soils after the application of the nitrate fertilizers and to find possibilities of studying the ways of using *Azotobacter* as a microbial indicator to assess soil state.

Material and Methods

In laboratory conditions, the amount of nitrogen-fixing bacteria in soil samples enriched with nitrate fertilizers has been determined before and after germination of soya bean (*Glycine max* Moench.).

The studies were conducted during 2012 -2013 years in a sod-podzolic soil type. Different fertilizer levels of calcium nitrate ($Ca(NO_3)_2$) and ammonium nitrate (NH_4NO_3) have been added to experimental soil samples. Calcium nitrate in the amount of 30 g, 60 g, 90 g per 100 cm^2 and ammonium nitrate in the amount of 15 g, 30 g, 45 g per 100 cm^2 have been applied into special containers with soil and then soya beans were seeded there. Soil without fertilizers was taken as a control sample. *Azotobacter* genus representatives were determined on nitrogen-free Ashby medium by the method of application and the percentage soil lumps fouling [10]. The concentration of nitrates in soil has been also estimated using ion-selective electrode method with an ion analyzer AI-123 before and after soya bean cultivation [11] and the correlation analysis between the percentage of *Azotobacter* genus representatives and the concentration of nitrate ions in differently fertilized soils before and after soya bean cultivation has been carried out.

Results and Discussion

According to the research results it has been found that the percentage of free-living nitrogen-fixing bacteria has gradually decreased with increasing doses of nitrate fertilizers, with a more clear tendency of *Azotobacter* percentage decrease noted after 60 days of observation (Table 1). Before soya bean planting, the control sample showed a high number of *Azotobacter* – 98.4 ± 1.65%.The greatest inhibition of diazotrophic bacteria has been observed during the application of calcium nitrate in the highest concentration of 90 g/cm^2, at which their number was on the 30th day of the experiment 1.1 times lower (88.4 ± 1.65%), compared to the control sample (98.4±1.65%). Similarly on the 60th day of the experiment was their number 1.3 times lower (76.6 ± 0.3%), compared to the control sample (96.65±3.35%). During the application of ammonium nitrate in the concentration of 45 g/cm^2 has been recorded the following inhibition of nitrogen-fixing microorganisms – it dropped in 1.2 times (81.7 ± 1.65%) on the 30th day of the experiment and in 1.24 times (78.3 ± 1.5%) on the 60th day of the experiment (Table 1).

Table 1 Percentage of the *Azotobacter* genus representatives during nitrate fertilizers application (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrogen-fixing microorganisms	
	30th day of experiment	60th day of experiment
g/cm^2	%	
	Calcium nitrate	
0 (control)	98.4±1.65	96.65±3.35
30	95±1.70	83.4±6.65
60	96.7±3.35	81.7±1.65
90	88.4±1.65	76.6±0.30
	Ammonium nitrate	
0 (control)	98.4±1.65	96.65±3.35
15	93.3±0.50	81.7±1.65
30	93.3±0.50	83.3±1.00
45	81.7±1.65	78.3±1.50

Nitrate fertilizers influence on *Azotobacter* throughout soya bean cultivation has been characterized by further suppression of the microorganisms number (Table 2). Thus, during the application of calcium nitrate in the concentration of 90 g/cm^2 nitrogen-fixing bacteria content decreased in 1.4 times (63.3 ± 3.3%) on the 30th day of the experiment compared to the control sample (91.6 ± 1.65%) and in 3.8 times (21.6 ± 1.65%) on the 60th day of the experiment, compared to the control sample (81,65±1,65%). Ammonium nitrate application throughout soya bean cultivation has also led to the nitrogen-fixing microorganisms inhibition in 1.7 times (53.3 ± 3.3%) on the 30th day of the experiment and in 2.1 times (38.3 ± 2.7%) on the 60th day of the experiment compared to the control samples (Table 2, see Fig.1).

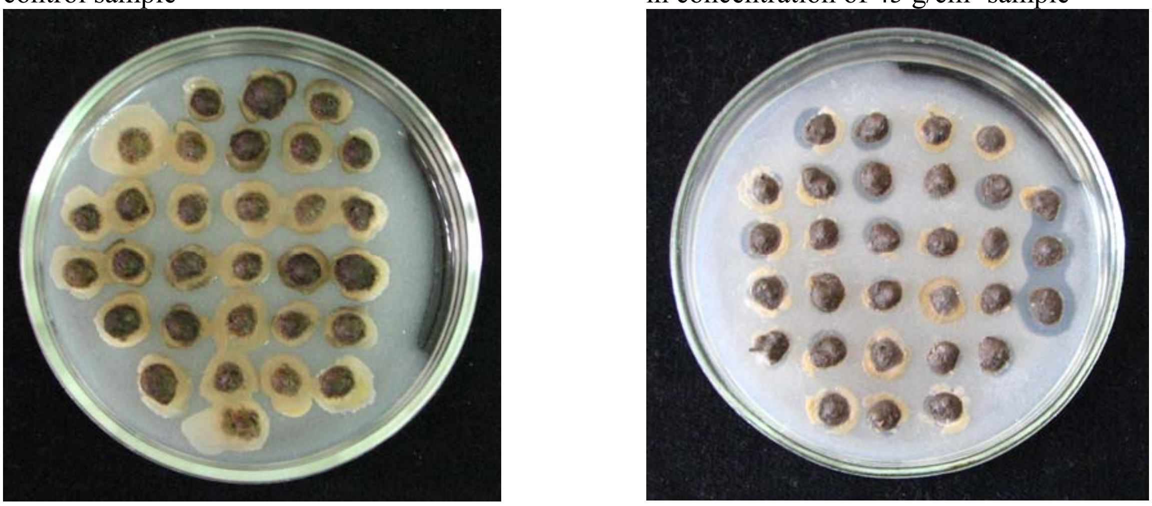
Table 2 Percentage of the *Azotobacter* genus representatives during nitrate fertilizers application and cultivation of soya bean (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrogen-fixing microorganisms	
	30th day of experiment	60th day of experiment
g/cm^2	%	
	Calcium nitrate	
0 (control)	91.6±1.65	81.65±1.65
30	85±1.65	70±6.65
60	76.6±1.70	21.6±1.65
90	63.3±3.30	21.6±1.65
	Ammonium nitrate	
0 (control)	91.6±1.65	81.65±1.65
15	70±0.30	70±3.35
30	66.6±3.35	41.6±8.35
45	53.3±3.30	38.3±2.70

Fig. 1 Representation of the ammonium nitrate fertilizer influence on the *Azotobacter* genus representatives throughout cultivation of soya bean (60th day of experiment)

A) Untreated with nitrate fertilizers control sample

B) Treated with ammonium nitrate fertilizer in concentration of 45 g/cm^2 sample



It has been showed in the works [12, 13, 14] that a long-term usage of mineral fertilizers in high doses inhibits germination of nitrogen-fixing microorganisms. Experimental results testify on whether or not the fertilization with increasing doses of nitrogen, such as ammonium nitrate and calcium nitrate, the percentage of the *Azotobacter* genus representatives reduces. Throughout soya bean cultivation a further nitrogen-fixing bacteria inhibition has been recorded. Using an ion-selective electrode method for the nitrates determination in the soil we have also discovered, that soya bean cultivation leds to an increase of mineral nitrogen amount in the soil, which explains the significant percentage reduction of the investigated bacteria genus (Table 3).

Table 3 Nitrate concentration changes before and after soya bean cultivation in the fertilized soil (x from each variant; n = 3; ± σ)

Fertilizer concentration	Nitrates concentration	
	Before soya bean cultivation	After soya bean cultivation
g/cm^2	mg/kg	
	Calcium nitrate	
0 (control)	151±8	210±15
30	203.4±6	428±7
60	668.1±32	758.2±26
90	712.8±28	1465.4±82
	Ammonium nitrate	
0 (control)	151±8	210±15
15	373.4±18,5	773.5±14,5
30	538.8±28	811.2±19
45	1105±40	1021±8

Our data are consistent with the data of other authors who have observed that the cultivation of legumes in monoculture and legume-cereal stock mixtures is accompanied by the *Azotobacter* number decrease comparing to growing cereals, which in turn is linked with a competition between symbiotic and associative nitrogen-fixing microorganisms, that are specifically supported by the plants in their own rhizosphere suppressing the development of free-living nitrogen-fixing bacteria [15, 16].

Correlation analysis between the percentage of *Azotobacter* in treated soil and the concentration of nitrate ions has been conducted. High and reliable correlation coefficients between the studied values, which have inversely proportional correlational relationships has been defined (Table 4).

Table 4 Correlation between the percentage of *Azotobacter* genus representatives and the concentration of nitrate ions in the fertilized soil before and after soya bean cultivation

Correlation coefficient			
30th day of experiment	60th day of experiment	30th day of experiment	60th day of experiment
Before soya bean cultivation		After soya bean cultivation	
Calcium nitrate			
-0.62	-0.79	-0.99	-0.85
Ammonium nitrate			
-0.99	-0.79	-0.99	-0.85

No data currently exists on the effect of biochar on free-living N fixers, however it is possible that these organisms would benefit from a reduced partial pressure of oxygen in the small pores of biochar (since oxygen destroys enzymes required for the biological fixation of N). For free-living diazotrophs, the fine pores of biochar create a habitat where reduced O_2 tensions are likely [17].

According to Thies and Rillig [18], if iron and Mn are sufficiently available for free-living N fixers, they could be favoured on and in biochar particles. If Fe and Mo are available in sufficient supply, the fixation of atmospheric N_2 will increase an organism's competitiveness in the biochar environment and, thus, their proportional representation within the biochar and soil community [17, 18].

Adding biochar to soil also appeared to stimulate the N_2-fixing activity of free-living diazotrophs. Ogawa [19] proposed that these bacteria might be poorer competitors whose survival in soil may be enhanced by their ability to colonize the biochar pores. Most biochars are very low in inorganic N content, giving diazotrophs a competitive advantage for surface colonization [17, 19].

Rondon et al. [20] in their studies found that the proportion of N derived symbiotically increased and the proportion of N derived from the soil decreased as more biochar was applied.

Conclusion

It has been established, that *Azotobacter* is a sensitive indicator to nitrogen fertilizers load, therefore a research conduction aimed at the study of quantitative composition changes of these microorganisms in fertilized soils of different types in model experiment conditions is of great importance.

Our research conducted in Ukraine has showed that the number of free-living nitrogen-fixing bacteria has been reduced under the influence of nitrogen fertilizers. According to the laboratory experiment research results concerning the nitrate fertilizers application into the soil has revealed that the percentage of nitrogen-fixing microorganisms gradually decreases with the increasing doses of applied nitrate fertilizers, and as a result the number of nitrogen-fixing bacteria decreased on average in 1.1 - 3.8 times compared to a control sample.

In addition, high inverse correlation coefficients up to -0.99 on the 30th day of experiment between the percentage of *Azotobacter* and the concentration of nitrate ions in the studied soil have been obtained.

Specific changes in the quantitative composition of nitrogen-fixing microorganisms of soil, treated by nitrate addition, allows us to confirm the high sensitivity of this kind of microorganisms to anthropogenic impact and to recommend them as an ecological indicators of the possible changes in anthropogenically transformed soil, that support the possibility of using *Azotobacter* for bioindication in our further studies with a biochar application.

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References:

[1] Downie A, Crosky A, Munroe P, Physical properties of biochar. In: Lehmann, J., Joseph, S. (eds), *Biochar for Environmental Management: Science and Technology*, Earthscan, London, 2009, pp. 13-29.

[2] Lehmann J, Joseph S, Biochar for environmental management: an introduction. In: Lehmann, J, Joseph S (eds.), *Biochar for Environmental Management: Science and Technology*, Earthscan, London, 2009, pp. 1-12.

[3] Clough TJ, Condron LM, Biochar and the nitrogen cycle: introduction, *Journal of Environmental Quality,* Vol.39, No.4, 2010, pp. 1218-1223.

[4] Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday CW, Crowley D, Biochar effects on soil biota-A review, *Soil Biology & Biochemistry,* Vol.43, 2011, pp. 1812-1836.

[5] Diehl MJ, The effect of urea-ammonium-nitrate fertilizer amended with urease and nitrification inhibitors on nitrogen cycling in highly organic agricultural soil, *The University of North Carolina at Chapel Hill*, 2007, 90 p.

[6] Lebid E, Desyatnyk L, Kotsyuban D et al., The effect of farming system elements on biological activity and yield of winter wheat in parts of the south-eastern steppes of Ukraine, Bulletin №38, *Agricultural Institute of the steppe zone NAAU*, 2014.

[7] Kizilkaya R, Nitrogen fixation capacity of *Azotobacter spp.* strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils, *Journal of Environmental Biology,* Vol.30, No.1, 2009, pp. 73-82.

[8] Line MA, Loutit MW, Occurrence of Azotobacter in some soils of South Island New Zealand, *New Zealand Journal of Agricultural Research*, 2012, pp. 628-638.

[9] Sicardia M, García-Préchacb F, Frionic L, Soil microbial indicators sensitive to land use conversion from pastures to commercial Eucalyptus grandis (Hill ex Maiden) plantations in Uruguay, *Applied Soil Ecology,* Vol.27, No.2, 2004, pp. 125–133.

[10] Zvyagintsev D, Methods of Soil Microbiology and Biochemistry, Moscow, Moscow State University, 1991, 304 p.

[11] GOST 26951-86 Soils, Nitrate determination by ion-selective method, *State All-Union standard* (*USSR State standard specification*), 1986, pp. 1-7.

[12] Andreyuk K, Iutynska D, Antypchuk A et al., The soil microbial coenosis functioning in conditions of an anthropogenic load, *Oberegy*, Kyiv, 2001, 240 p.

[13] Emtsev V, Microorganisms and their activity regulation in the soil, *Timiryazev Agricultural Academy*, Vol.6, 1982, pp. 104-113.

[14] Patyka B, Kots C, Volkogon B et al., Biological nitrogen, Kyiv, *Svit*, 2003, 422 p.

[15] Malinowska I, Microbiological processes orientation in dark gray podzolic soil under different technologies of soybean cultivation, *Electronic scientific journal Problems of environmental biotechnology*, № 1, 2012.

[16] Ohyama T, Fujikake H, Yashima H, Tanabata S, Ishikawa S, Sato T, Nishiwaki T, Ohtake N, Sueyoshi K, Ishii S, Fujimaki S, Effect of Nitrate on Nodulation and Nitrogen Fixation of Soybean Faculty of Agriculture, Niigata

University Quantum Beam Science Directorate, *Japan Atomic Energy Agency Japan InTech*, 2011, pp. 333-364.

[17] Lehmann J, Joseph S, Characteristics of Biochar - Biological Properties (Chapter 6), in: J. Lehmann and S. Joseph (Eds.), *Biochar for Environmental Management: Science and Technology*, Earthscan, London, UK, 2009, pp. 96-97

[18] Thies JE, Rillig MC, Characteristics of Biochar - Biological Properties (Chapter 6), in: J. Lehmann and S. Joseph (Eds.), *Biochar for Environmental Management: Science and Technology*, Earthscan, London, UK, 2009, pp. 85.

[19] Ogawa M, Symbiosis of people and nature in the tropics, *Farming Japan*, Vol.28, 1994, pp. 10-34.

[20] Rondon MA, Lehmann J, Ramirez J, Hurtado M, Biological nitrogen fixation by common beans (*Phaseolus vulgaris L.*) increases with bio-char additions, *Biology and Fertility of Soils*, 43:699. DOI: 10.1007/s00374-006-0152-z. 2007.