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AMMONIA-OXIDIZING BACTERIA RESPOND TO MULTIFACTORIAL GLOBAL CHANGE

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In many recent studies, the effects of climate change on the future of biodiversity have been studied. As a result of human economic activity, extraction of mineral resources, land-use management, application of fertilizers (for example, nitrogen-containing compounds), carbon dioxide emissions into the Earth's atmosphere increase. This causes a change in the air temperature in the direction of increase and a change in the quantity and quality of the sediments, causing a wetting of the soils. The reaction of bacteria capable of oxidizing ammonia, belong to the group of chemo-litho-autotrophic microorganisms and at the same time are not associative microflora, was studied. In both natural and human-induced disturbance altered ecosystems, they strongly depend on these bacteria through intensive oxidation of ammonium. In response to artificially created climate changes, we received a change in the structure of the ammonium-oxidizing bacteria (AOB) community. The addition of a large amount of nitrogen fertilizer has led to a change in the number of bacteria that oxidize ammonia, while the temperature and humidity levels have changed, which also have an impact on AOB. In response to the increase in carbon dioxide significantly reduced the number of AOB. If, in this case, the amount of precipitation also increased, then this decrease was most pronounced. Increased nitrification caused also caused changes in the composition of the community of microorganisms. These results show that microbial communities can be successively changed by climate change and that these changes may have consequences for the functioning of the ecosystem.

Keywords: *ammonia-oxidizing bacteria, agricultural soil, climate change.*

Introduction

Environmental systems are changing globally under the pressure of human activity. As a result of some studies it has been shown that numerous joint multifactorial changes can affect the diversity, quantity and functionality of microbial communities. The interrelation of such changes is seen through the increase in the amount of released atmospheric carbon dioxide due to the extraction and combustion of various fuels and combustible materials, as well as the intensification of land use. As a result of such a person's activity, the amount and quality of precipitation, temperature changes, soil-formation process changes occur, including intensive transformation of nitrogen in soils. Soil microorganisms play an unimportant role in the internal processes and actively respond to global changes. Almost nothing is known about the effect of multifactorial changes on communities of microorganisms in soils.

Bacteria capable of oxidizing ammonia belong to the group of chemo-litho-autotrophic microorganisms and, at the same time, not being an associative microflora. Both in natural and anthropogenically altered ecosystems they are strongly influenced by these bacteria through intensive oxidation of ammonium [11]. This group of bacteria is not chosen by chance. Most studies consider it an ideal model for studying the ecology of microorganisms for a number of reasons [8]. First, these bacteria are widespread in nature, they can be found in soil, fresh and sea water. Secondly, they have great ecological significance, namely: these bacteria play a central role in the nitrogen cycle, because they carry out the limiting stage of nitrification – the oxidation of ammonia. Third, the activity of the enzymes of ammonium-oxidizing bacteria participating in nitrification processes can be changed by changing the environment [10, 16]. Finally, all ammonium-oxidizing bacteria carry the amoA gene; it is a protein that catalyzes the oxidation stage of ammonia to hydroxylamine, which codes for the α-subdeitenium of the enzyme-ammonia-mono-oxygenase. This gene is widely distributed among the ammonium oxidizing bacteria and is used as a molecular marker that allows detecting them and to count without the laboratory culture. Most soil bacteria are not suitable for cultivation in the laboratory, so it is very important to use such molecular markers as a known unique gene for a more accurate assessment of the diversity and quantity of microorganisms in the soil [4]. The object of the current study was bacteria capable of oxidizing ammonia, affected by various environmental changes.

Materials and Methods

In current work, various environmental changes that can affect the ecosystem in soils were created artificially. Namely: 1) simulation of increasing atmospheric CO_2 (adding carbon dioxide to the air with a target concentration of 700 ppm); 2) imitation of precipitation (increased (by 50%) moistening of the soil); 3) imitation of global warming (increase in ambient temperature); 4) increased nitrogen content (by adding Ca (NO_3) 2 in an amount of 7 g m^{-2}). The reaction of ammonium-oxidizing bacteria (AOB) on these factors was studied both individually and in a complex. Separately, carbon dioxide amount in the airspace was studied, in experiments with simulated precipitation, temperature increase and nitrogen addition.

Results and Discussion

As a result, in response to these man-made changes in the soil ecosystem, some influences the community structure of AOBs were recorded, namely: change in the number of these bacteria. In response to the addition of a nitrogen-containing fertilizer, the number of bacteria that oxidized ammonia increased significantly, on 74.4%. Ammonia oxidizer populations are influenced by temperature. There was also an increase in the AOBs number with increasing ambient temperature, on 51.2%. With the increase in atmospheric air of carbon dioxide, a decrease in the AOBs total number, on 18.6%. The most pronounced decrease in the amount of AOB (on 30.2%) was observed in response to an increase in soil humidity. The complex impact of these factors adversely affected the AOB community, their number was decreasing on 25.6%.

The changes in the AOBs amount observed in response to artificially created changes may well be the result of the indirect influence exerted by the common microbial community. Literature review illustrates, the amount of AOB can vary under the form of humidity in the soil, since in the dock of their members, it also goes down. An increase in the number of members of the investigated group of microorganisms can occur with a relatively moderate increase in humidity, by reducing water stress [1, 5]. A significant reduction in the number of AOB occurs with a strong increase in humidity, due to a decrease in the diffusion of oxygen into the soil [1]. Therefore, in order to achieve a balance between these two effects, it is necessary to maintain optimum soil humidity. This balance can underlie the interaction that was observed in the study of a set of factors. Since the increase in humidity results in a sustained negative impact on the AOB community, due to the reduced availability of oxygen, it is likely that an increase in temperature can reduce water stress and thereby increase the access of oxygen to the soil.

The effect of carbon dioxide can also be indirectly through soil humidity. Carbon dioxide increases soil humidity due to changes in the activity of the general microbial community of the soil. The same reaction of the investigated group of bacteria on the increased soil humidity and carbon dioxide is found in current research. As the carbon dioxide content increases, the rate of competition for nutrient resources between AOB and heterotrophic microorganisms in the soil changes. The increased level of carbon dioxide increases the content and activity of heterotrophic microorganisms in the soil [7, 9]. AOBs are the lowest competitors for some resources (for example, oxygen) for heterotrophic microorganisms, and increased competition of resources can lead to an increase in CO_2 content due to a decrease in the number of AOBs [1]. The number of the investigated group of bacteria can significantly decrease with a simultaneous increase in the amount of precipitation, as these results in the elution of mobile nutrients and a decrease in the diffusion of oxygen.

Experimental data have been obtained in favor of this conclusion. Thus, increased humidity and precipitation contribute to an increase in the carbon dioxide content in the surrounding atmosphere by a factor of 2. This probably serves as a key factor to a decrease in the AOBs number in a multifactor test. When nitrogen was introduced into the soil, a change in the amount of AOB was observed, which, very likely, was mediated by the entire action of the nitrate on the soil. So, Anna Hermansson and Per-Eric Lindgren [6] point out that in the fertilized soil ammonia-oxidizing bacteria three times more than the number of bacteria in the unfertilized soil. Some researchers point out that the availability of nitrogen can decrease with a high amount of precipitation, because the losses of available nitrogen increase. A significant role in this process is played by an increased temperature, which leads to an increase in soil humidity.

The results obtained show that global multifactorial changes can consistently change microbial communities. Such changes in the microbial community can have serious consequences and disrupt the functioning of the entire system. Free living prokaryotes, to which the group of bacteria we are researching, are key participants in the functioning of ecosystems and represent a large part of the Earth biodiversity [14]. The change in microbial diversity, its abundance, can lead to a disruption in the pace and functions throughout the system. Due to the low degree of functionality, microorganisms of the nitrogen cycle and, in particular, AOB [15] are particularly vulnerable to this. Literature review shows, there are indications of a connection between the functions of denitrifying bacteria and the structure of the community [2, 3, 12].

Conclusion

An answer was received to the multifactorial global changes from the communities of free-living soil ammonium-oxidizing bacteria. A significant quantitative change in the structure of the community of these bacteria was demonstrated. The number of AOBs varied with temperature, precipitation, carbon dioxide and nitrates. The data obtained agree with the literature review, which indicate that the water balance of soils often causes global changes in ecological communities [13]. Some AOB reactions that do not carry global changes can be representative predecessors in the modeling of global changes. The insignificant but notable known reactions of these bacteria can predict the response to much more serious changes in the system. For example, the AOB reaction for the addition of nitrogen was similar to the previously studied effect of agricultural fertilizers [4]. Some changes in the community of ammonium-oxidizing bacteria observed are important for understanding possible changes in the functioning of the ecosystem. This point to the potential importance of feedback between such factors of the soil ecosystem as humidity, carbon dioxide, nitrogen, temperature and microbial groups involved in nitrogen transformations (for example, nitrogen fixing, denitrification and nitrite microorganisms). Current study shows that a full awareness of the environmental consequences of a global change requires an understanding of the response of the microbial community as one of the fastest-changing factors.

REFERENCES:

1. Belser LW (1979) Population ecology of nitrifying bacteria. Annu Rev Microbiol 33**:** 309–333.
2. Bruns M, Stephen J, Kowalchuk G, Prosser J, Pau E (1999) Comparative Diversity of Ammonia Oxidizer 16S rRNA Gene Sequences in Native, Tilled, and Successional Soils. Appl Environ Microbiol 65: 2994–3000.
3. Cavigelli M, Robertson G (2001) Role of denitrifier diversity in rates of nitrous oxide 274 consumption in a terrestrial ecosystem. Soil Biol Biochem 33: 297–310.
4. Horz H-P, Barbrook A, Field CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. Proc Natl Acad Sci USA. 101(42): 15136–15141.
5. Hastings RC, Butler C, Singleton I, Saunders JR, McCarthy AJ (2000) Analysis of ammonia-oxidizing bacteria populations in acid forest soil during conditions of moisture limitation. Lett Appl Microbiol 30: 14–18.
6. Hermansson A, Lindgren P-E (2001) Quantification of ammonia-oxidizing bacteria in arable soil by real-time PCR. Appl Environ Microbiol 67: 972–976.
7. Hungate BA, Jaeger CH, Gamara G, Chapin FS, Field CB (2000) Soil microbiota in two annual grasslands: responses to elevated atmos pheric CO_2. Oecologia 124: 589–598.
8. Kowalchuk GA, Stienstra AW, Heilig GHJ, Stephen JR, Woldendorp JW (2000) Changes in the community structure of ammoniaoxidizing bacteria during secondary succession of calcareous grasslands. Environ Microbiol 2: 99–110.
9. Luo Y, Jackson RB, Field CB, Mooney HA (1996) Elevated CO_2 increases belowground respiration in California grasslands. Oecologia 108: 130–137.
10. Niklaus PA, Kandeler E, Leadley PW, Schmid B, Tscherko D, Körner C (2001) A link between elevated CO2, plant diversity and soil nitrate. Oecologia 127: 540–548.
11. Okano Y, Hristova KR, Leutenegger CM, Jackson LE, Denison RF, Gebreyesus B, Lebauer D, Scow KM (2004) Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. Appl Environ Microbiol 70: 1008–1016.
12. Phillips CJ, Harris D, Dollhopf SL, Gross KL, Prosser JI, Paul EA (2000) Effects of agronomic treatments on structure and function of ammonia-oxidizing communities. Appl Environ Microbiol 66: 5410–5418.
13. Volk M, Niklaus P.A, Korner C (2000) Soil moisture effects determine CO_2 responses of grassland species. Oecologia 125: 380–388.
14. Whitman W, Coleman D, Wiebe W (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 95: 6578–6583.
15. Wolters V, Silver W, Bignell D, Coleman D, Lavelle P, van der Putten W, de Ruiter P, Rusek J, Wall D, Wardle D, *et al.* (2000) Effects of global changes on above- and belowground biodiversity in terrestrial ecosystems: Implications for ecosystem functioning. Bioscience 50: 1089–1098.
16. Zak DR, Ringelberg DB, Pregitzer KS, Randlett DL, White DC, Curtis PS (1996) Soil micro bial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecol Appl 6: 257–262.