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Review of the PhD thesis entitled
"The impact of simulated drought on changes in microbial biodiversity and soil biological activity"
elaborated by Kalisa Amarsingh Bogati

This PhD project was performed in the Department of of Environmental Microbiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń under the supervision of prof. dr hab. Maciej Walczak, Nicolaus Copernicus University and prof. Ali Boilarbah, Université Cadi Ayyad, Marrakech, Morocco.

Agricultural and non agricultural systems are strongly influenced by climatic factors such as temperature, solar radiation, wind and rain, so climate and its changes pose major risks for plant growth and development. The consequences of climate change are extremely serious and affect many aspects of our lives. Rising temperatures are causing water shortages and droughts in some areas of the world. Drought is a limiting environmental factor for plant growth and one of the natural phenomena that most severely affects agricultural productivity. Given that soil microbes are crucial for the regulation ecosystem functions such as primary productivity, carbon and nutrient cycling in terrestrial ecosystems, detailed understanding of the linkages between soil microbial community structure and function under scarcity of water is of great importance. Hence, the studies evaluating the response of soil microorganisms to drought stress are necessary and urgent from an environmental and agricultural perspective. Experiments carried out by Kalisa Bogati and presented in her PhD project are part of such research conducted worldwide.

***Assessment of work in formal and structural terms***

The doctoral dissertation of Kalisa Bogati consists of three scientific articles, two of which were published in Agronomy (IF 3.949) and the last one in Ecological Questions (IF 0.312). All papers are co-authored and, and importantly, Bogati is the first and corresponding author in each of them. In the statement accompanying the dissertation or described as author contributions in the publications, the authors clarified the scope and extent of their individual contribution to the works. In the review paper, Kalisa Bogati's contribution was estimated at 80%, as confirmed by the second co-author in the signed statement. According to the explanations of the co-authors, in the experimental papers Kalisa Bogati made an important contribution to the laboratory work, since she carried out all experiments related to the counting of bacteria and fungi, determining activity of the enzymes and the physiological profiling of soil microorganisms, participating in analyzes physico-chemical parameters of soil samples. In addition, Kalisa contributed to the data analysis and result presentation and was involved in the drafting and revision of the manuscripts. The publications are preceded by the abstract, introduction, study objective, hypothesis and research objectives. The final sections of the thesis include conclusions, a summary and Kalisa's CV.

From a formal point of view, the Kalisa Bogati's dissertation does not raise any objections.

***Substantive assessment of the dissertation***

The evaluated thesis addresses a relevant and current issue. It delves into one of the fundamental questions within soil microbial ecology concerning the functioning of microorganisms in drought-stressed soils.

The first publication "The impact of drought stress on soil microbial community, enzyme activities and plants" is a review paper published in Agronomy in 2022. This article highlights the threats to ecosystems stemming from a climate change and global warming and presents the outcomes of studies focusing on the the impact of drought on plant and soil microbial function. In the subsequent chapters the effects of water scarcity on the genetic and physiological structure of soil microbial communities, as well as on plant morphology, biochemistry and physiology were discussed. The authors described the mechanisms allowing microorganisms and plants to survive under. They also paid attention to the relations between plants and soil microorganisms, and the role of plant growth promoting microorganisms in alleviating the negative effects of drought stress in plants. This paper, especially its parts dealing with the impacts of water scarcity on the number, physiological and genetic diversity of soil microorganisms, is a very good justification for the research goal of the presented dissertation.

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The aim of the PhD project was to evaluate the impact of a two-month drought on the total number of bacteria, actinomycetes and fungi, selected enzymes and the metabolic and genetic diversity of microbial communities in four types of agricultural soils. The following research hypotheses were tested during the study: (1) The significant diferences in soil microbial communities are connected with the seasonal variations and changes an soil moisture content; (2) The prolonged drought conditions has strong influence on microbial enzymes; (3) Drought and changes in microbial community affects on physiological profiles of microorganisms and ecophysiology of soil environment.

The soils for study were collected in four locations Gniewkowo, Lulkowo, Wielka Nieszawka i Suchatówka near Toruń in the spring and autumn seasons. The soils were chosen on the basis of texture and they differed in the values of some soil physico-chemical parameters. Such a selection gave Kalisa an opportunity to assess the influence of soil type on the directional changes occurring in the soil under water-deficient conditions.

The outcomes of Bogati's study are presented in two experimental publications "Deciphering the impact of induced drought in agriculture soils: Changes in microbial community structure, enzymatic and metabolic diversity" (Agronmy 2022) and "Effect of changes in soil moisture on agricultural soils: response of microbial community, enzymatic and physiological diversity" (Ecological Questions, 2023). The first provides the results of soil analysis performed on samples collected in May 2021, while the second displays the analysis of soil samples gathered in October 2022. Due to the fact that certain sections of these two publications overlap (Introduction, Materials and methods, Discussion), I think that all the results obtained could have been presented in a single publication.

In order to test the validity of the hypotheses Kalisa Bogati used a spectrum of methods that could capture the values of the soil parameters, abundance, activity, metabolic and genetic diversity of soil microorganisms in the soil samples studied. The study was planned and performed correctly from the methodological point of view. Physico-chemical parameters of soil were determined by standard, commonly used methods. The total number of bacteria, Actinomycetota and fungi were determined using standard plate method and appropriate media. The soil activity was assessed by the measurements of the activity of selected enzymes i.e dehydrogenase, urease, alkaline and acid phosphatases by commonly used procedures. The metabolic potential of soil microorganisms was estimated by community-level physiological profiles (CLPP) approach. To measure the functional diversity the 96-well Biolog EcoPlates containing of 31 different carbon sources representing five guilds: amines and amides, amino acids, carbohydrate, carboxylic acid, and polymers were used. In order to show the overall metabolic potential of soil microorganisms the average well color development (AWCD) was calculated. Soil microbial community

utilization patterns of carbon sources were plotted in heatmaps. Principal component analysis (PCA) was done to show correlations among the soil physico-chemical and biological parameters measured.

The genetic diversity of bacteria and fungi was determined by sequencing the specific fragments of the 16S rDNA gene and ITS regions, respectively. The appropriate software programs were applied to analyse the raw data and for taxonomic profiling. To show relative bacterial and fungal diversity at the genus level Shannon diversity indices (α diversity) were calculated. The results obtained were subjected to appropriate statistical analyses.

Kalisaa Bogati achieved a lot of results which are clearly presented in tables, figures and heatmaps. All tables and graphs were prepared according to the requirements of the journals in which they were published. All graphics are clear and properly labeled. The main outcomes of Kalisa Bogati's studies showed that bacteria and fungi reacted strongly to decreasing amount of water in soils, however, the pattern of these changes depended on the group of microorganisms, the parameters measured and season. In all soils sampled in autumn at 8$^{th}$ week of the experiment the number of total bacteria and actinomycetes (in soil G and L) significantly decreased over the experimental period whereas the number of fungi lowered only in soil G. In contrast, in the soils collected in spring, the abundance of actinomycetes and fungi increased significantly, and for bacteria, their total numbers did not differ significantly between the first and last sampling day (except for soil G). And here I have a question for Kalisa: What do you think were the main drivers of the observed changes? Drought stress also had a strong impact on the activity of the studied enzymes and the physiological profiles of microbial communities. In general, based on the AWCD values, it can be said the metabolic potential of the microorganisms dropped along with decreasing soil moisture. A shift in the pattern of carbon use by microorganisms in soils was found. I think it was worth calculating the values of parameters like the Shannon-Wiener, richness and evenness indices. Similarly, the reduction in enzyme activities was observed; however, some fluctuations were observed. Interestingly, a low sensitivity of urease to water deficiency was noted.

Results of the Author study showed that microbial communities exposed to drought stress shifted their taxonomic diversity which was confirmed by changes in the values of Shannon index. In spring-collected soils, the relative abundance of Pseudomonadota and Bacteroidota decreased over the experiment whereas the relative abundance of Actinomycetota increased at the same period. In contrast, in three soils (G, N and S) collected in the autumn, the relative abundance of Actinobacteriota decreased over the same time. The changes in the genetic diversity suggest that microbial communities are being rebuilt and species/genera capable of surviving prolonged water deficiency are dominant. I agree with

Kalisa that findings regarding changes in microbial diversity supported by soil metagenomic studies can be a valuable source of knowledge for developing strategies to protect and maintain soil activity.

In the discussion Kalisa Bogati confronted her results with the outcomes published by other researchers. She skilfully and logical explained the obtained results and underlined the most important outcames of her study. In this part of her PhD, she also demonstrated her knowledge and in-depth understanding of microbial functioning in drought-stressed soils.

As a reviewer, I have some additional comments/questions:

- The data from Figure 2 should be presented in the form of a columns or table.
- There is a discrepancy between the data presented in the text (page 18, paper III) and the Table 2. In the text the Autor stated that the relative abundance of Proteobacteria decreased in soils L, N and S soils but the data in the table showed that the relative abundance of Proteobacteria in soils N and S increased.
- How can the huge relative abundance (36.48%) of fungi from the genus *Panaeolina* in soil type N be explained? (Table 3, paper III)
- Page 17, paper III: Incorect sentence – „It should be highlighted that that reports on the impact of prolonged drought stress on fungi in agricultural fungi are limited (Figure 3)" – this sentence refers to general knowledge, not table should be quoted here
- There are some language mistakes in the text – for example, in the sentence „Effect of Drought stress on..." (page 11, paper III) the word „Drought" should be written in lower case

I would like also say some words about Kalisa Bogati MSc. Based on the attached resume, I've learned that Kalisa is a very active and committed student, dedicated to her work and acquiring new skills. She is co-author of 8 scientific articles published in journals such as Frontiers in Plant Sciences, Agronomy, Zootaxa, Environmental Monitoring and Assessment and Symbiosis, among others. She successfully applied for student mobility grants that enabled her to complete the internships at the scientific institutions in Germany and for the participation in the scientific conference in Austria. Kalisa Bogati attended conferences and presented four posters. She expanded her skills in bioinformatics, phylogenetics, molecular biology and metagenomic analysis by attending a series of workshops and webinars.

***Final conclusion***

The PhD thesis of Kalisa Bogati MSc entitled „The impact of simulated drought on changes in microbial biodiversity and soil biological activity" is an original work which brings new and useful knowledge in a field of biology, particularly in soil microbiology. In the era of global warming such a topic

is capturing interest and attention. Finding of this work also have an application potential, as knowledge of the microorganisms that tolerate water scarcity can be helpful in developing strategies to maintain soil activity and mitigate drought stress in plants.

This project proves that Kalisa Bogati MSc has the theoretical knowledge and practical skills to plan and perform experiments. She can interpret results and draw conclusions. The PhD thesis meets the requirements for doctoral dissertations specified in the Regulation of the Minister of Science and Higher Education of 19 January 2018. In view of the above, I recommend allowing Kalisa Bogati to proceed to the further stages of the doctoral process.

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