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DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY

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DOCTORAL THESIS

"THE IMPACT OF SIMULATED DROUGHT ON CHANGES IN MICROBIAL BIODIVERSITY AND SOIL BIOLOGICAL ACTIVITY"

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PREFACE

This thesis submitted for PhD degree is based on experimental work carried out at the Department of Environmental Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University Torun Poland.

The work was carried out under the supervision of Prof. Maciej Walczak (main supervisor) and Prof. Ali Boularbah (co-supervisor).

The thesis is based on three publications:

1. **Bogati, K.,** & Walczak, M. (2022). Review- The impact of drought stress on soil microbial community, enzyme activities and plants. Agronomy 12(1):189. doi: 10.3390/agronomy12010189. [IF: 3.949; MNiSW: 100].
2. **Bogati, K.A.**; Golińska, P.; Sewerniak, P.; Burkowska-But, A.; Walczak, M. (2023). Deciphering the Impact of Induced Drought in Agriculture Soils: Changes in Microbial Community Structure, Enzymatic and Metabolic Diversity. Agronomy 2023, 13, 1417. doi: https://doi.org/10.3390/agronomy13051417. [IF: 3.949; MNiSW: 100].
3. **Bogati, K.A.**; Sewerniak, P.; Walczak, M. (2023). Effect of changes in soil moisture on agriculture soils: response of microbial community, enzymatic and physiological diversity. Ecological Questions, 34(3). doi: 10.12775/EQ.2023.0431. [IF: 0.312; MNiSW: 20].

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DEDICATION

This research is dedicated to my parents; my mother, Bhakti, my late father, Amarsingh; and my late Pastor, Peter D'Souza; all of whom gave me the foundation of something they had never enjoyed-education. Ever since then, I have been able to appreciate and recognise the value of reading and lifelong learning.

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Abstract of PhD thesis entitled: "The impact of simulated drought on changes in microbial biodiversity and soil biological activity".

Drought stress is currently the most serious impact of climate change. Drought is one of the most significant, concerning, and dangerous abiotic stresses that cause changes in the soil environment that influence soil organisms, mainly as microbes and plants. It affects the activity and functional composition of soil microorganisms, which oversee critical ecosystem processes. These unfavourable conditions reduce microbial abundance, disrupt microbial structure and activity, including enzyme synthesis and nutrient cycling, resulting in a reduction in soil fertility, poorer plant productivity, and economic loss. The purpose of this study was to investigate the effects of drought stress on the soil microbial community, enzyme activity, metabolic profile (soil respiration) and taxonomic diversity.

This research work was based on the study of the impact of short-term drought (2 months) on the microbial community structure, enzymes, metabolic profile and taxonomic diversity in four agricultural soils (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S) sites) in Poland during spring and autumn season. These four soil types were chosen based on texture and bonitation classification (gleyic luvisol Phaeozem in G (rich in clay and humus, 1st class), stagnic luvisol in L and fluvisol in N (3rd class), and haplic luvisol in S (sandy, 5th class)). The entire experiment focused on determination of (1) number of bacteria, actinomycetes (formally phylum *Actinomycetota*) and fungi; (2) taxonomic diversity (16S rRNA and ITS amplicon regions); (3) metabolic activity by community-level physiological profiling (CLPP); (4) soil enzyme activities (dehydrogenases (DH), phosphatases (acid ACP and alkaline ALP) and urease (UR)); and (5) soil chemical properties, at each time gradient (T0, T1, T2, T4, T8th week); during spring and autumn season. Soil moisture content had significant alteration in all sites during both seasons. During spring season, there was no change in total bacteria, but Actinomycetota and fungal numbers increased, while in autumn season, decrease in the number of bacteria and Actinomycetota, but not in the case of fungi was noted. Both seasons showed a

decrease in ACP, DH, UR activity (but fluctuated during spring), whereas ALP activity significantly increased. Overall, metabolic profile declined in both seasons apart from D-mannitol and L-asparagine (spring) and 4-Hydroxy Benzoic Acid, α-Ketobutyric Acid, L-Phenylalanine and α-Cyclodextrin (autumn). In the case of taxonomic diversity, a strong decrease in the relative abundance of Pseudomonadota, *Sphingomonas*, and strong increase in the abundance of Actinomycetota and *Fusarium* was noted in all sites during spring. On the other hand, relative abundance of Actinobacteriota, Bacteroidota, Acidobacteriota, *Bryobacter*, Basidiomycota, *Panaeolina*, *Plectosphaerella*, and *Kazachstania* decreased significantly but Ascomycota and *Ramophialophora* fluctuated in all sites during autumn season.

The short-term duration of drought stress had a considerable influence on soil biological activity, according to this study. This could help researchers better understand the effects of soil moisture fluctuations on soil nutrient cycle and biological activity in agricultural settings. To investigate the consequences of these catastrophic climate change events, genome editing, and molecular analyses (metagenomics, transcriptomics, and metabolomics) are required. This could potentially aid in the development of improved methods for minimizing the effects of drought and managing agricultural activities in tough conditions in a profitable manner. As a result, influence of drought conditions on soil microbial populations, enzyme activity, metabolic profile and taxonomic diversity in the soils were studied.

INTRODUCTION

Climate change is predicted to impact almost every environmental aspect and water stress cause significant impacts worldwide. As 75% of the world's cropland is covered by rain-fed areas, the water held in the soil is one of the most crucial water resources because it supports a significant amount of global food production (Grillakis, 2019). To predict and reduce potential repercussions in agriculture, it is essential to understand how soil moisture dynamics relate to the water cycle and how global warming affects them. The first part of the earth is the soil and is divided into three important phase system, that is, particles, gases, and water phases (He et al., 2023). The particle phase of the soil is largely composed of minerals and organic matter, whereas the gaseous phase is also known as soil pore space and is composed of the soil air and water. On the other hand, the third phase which is the water phase is one of the most important phases, because it’s composed of dissolved ions, gases and other water-soluble compounds that are importance for biogeochemical activities (Sparks et al., 2022). The amount of water in the top unsaturated layer of the soil is known as the soil moisture. The hydrologic cycle includes this layer of water storage as a vital and dynamic component (Grillakis, 2019) which receives precipitation and transfers it by evaporation to deeper ground or the atmosphere. One of the most significant water resources is the field capacity since it supports a significant amount of the world's food production (Grillakis, 2019). The moisture content remaining at field capacity followed the order from clay (45 to 55%) > loamy (35 to 45%) > sand (15 to 25%) (Yang et al., 2023).

The primary cause of drought is a lack of soil moisture, which is sometimes referred to as an agricultural drought because it has a direct impact on plant growth and crop output (Grillakis, 2019). Agricultural drought is the state that develops after the decline in vegetation and ecological health. This decline is caused due to low soil moisture near root zone because of increased evapotranspiration (Otkin et al., 2018). The effects of soil moisture droughts on the environment, society, and economy can be alarming because they frequently develop gradually over a long period of time and may continue for years after the precipitation drought has ended (Grillakis, 2019). Flash droughts can occur during abrupt decline or abnormally low soil moisture levels

in conjunction with brief rise in surface temperatures (Grillakis, 2019). Since the industrial revolution, the average global temperature has risen by 0.85 °C, and between 2030 and 2052, it is expected to climb by another 1.5 °C (Song et al., 2021).

In the European Union, Poland plays a key role in agricultural output, particularly in the production of cereals, rapeseed, sugar beet, apples, poultry, pig, and milk (Szczepaniak and Szajner, 2020; Piwowar, 2023). A significant amount of water is needed for agricultural production, and the main supply of water for crops is atmospheric precipitation. Precipitation varies greatly in terms of both quantity and type depending on the time and geographical location (Piwowar, 2023). The current climate changes in Poland include an increase in temperatures and frequency of extreme events. Therefore, coping with the effects of climate change in rural regions is quite difficult (Piwowar, 2023). In the years between 1951-2020, a data generated from the Institute of Meteorology and Water Management in Warsaw revealed significant increase of 0.29 °C/10 years average annual air temperature, indicating the rise of 2.0 °C temperature since the year of 1951 (Klimat Polski, 2021).

According to recent climate change forecasts, Central Europe will see warmer air and less yearly precipitation. Although global warming is anticipated, regional variability in precipitation patterns still exists. The rate of soil evapotranspiration and the depth of the water table (WTD) will be impacted by ongoing climate change (IPCC, 2013; Buras et al., 2020; Glina et al., 2021). Additionally, particularly in recent years, there have been instances of extremely high summertime temperatures. These factors, along with the relatively low rainfall (and other unfavourable shifts from an agricultural perspective, such as winters without snow), have had a negative impact on agriculture, notably the crop production (Piwowar, 2023). In Europe, particularly in Eastern Europe and the Mediterranean Basin, drought conditions are predicted to get worse. In fact, in comparison to other European nations, Poland is one of the nations with minimal water resources (Grillakis, 2019).

Soil microbes, including bacteria, fungi, and archaea, and enzymes, contribute significantly as regulators governing nutrient cycling (Adam et al., 2017; Zheng et al., 2020). Soil enzymes secreted by microorganisms are crucial for nutrient cycling, as

they regulate decomposition of organic matter and determines nutrient availability in soil (Zi et al., 2018; Meng et al., 2020). In response to availability of environmental resources, microbes can control the production and release of extracellular enzymes (Zheng et al., 2020). Enzymatic activity in the soil reflects the metabolic needs of the microbial population, making soil enzymes an indicator of microbial function in response to climate change under field conditions (Bogati et al., 2023). Seasons had a significant effect on enzyme activities, that reveal differences in environmental conditions and substrate availability with respect to seasonal variations (Bogati and Walczak, 2022; Bogati et al., 2023). Therefore, determining enzymatic activity can indicate organic matter decomposition (Xu et al., 2018).

The biogeochemical cycle and energy flow of the ecosystem are maintained by soil extracellular enzyme activity (EEA), that mediates the decomposition, transformation, and mineralization of soil organic matter (SOM) (Zheng et al., 2020). The response of EEA to temperature variations can influence the dynamics of metabolic activities, breakdown of SOM and nutrient cycles as well as substrate availability (Bernard-Jannin et al., 2018). Soil water phase has strong influence on enzymatic functioning and soil processes, as it contains vital dissolved ions, organic matter, and mineral compounds (Sparks et al., 2022). The most important chemical reactions occur in this phase because nutrients released from particles will enter in the water phase (Sparks et al., 2022; Li et al., 2023). Thus, drought conditions contribute to the relative nutrient constraints of microbial growth and nutrient assimilation as consequences of drying of the water phase. The microbial composition and activity primarily influence the carbon (C) and nitrogen (N) cycles (Bernard-Jannin et al., 2018). Machmuller et al. (2016) performed 3-year warming experiment and found significant impact of season on enzyme activity. According to the vector analysis of EEA stoichiometry conducted by Moorhead et al. (2013), showed limitations of C, N, and P on microbial processes and organic matter decomposition. But no recent studies have utilized this approach to investigate impact of experimental warming on the relative C, N, and P constraints of microbial processes. We believe that the influence of climate warming and seasonal fluctuations on soil EEA must be better understood to predict global soil nutrient cycling. The amount of water in the soil alters the microbiome because water availability is crucial for preserving microbial existence and facilitates their mobility in the soil, breakdown of nutrients, and gas diffusion.

The makeup of the microbial community was found to be significantly influenced by soil moisture (Mishra et al., 2023).

In particular, the effects of climate change have been investigated in relation to soil physicochemical characteristics and enzyme activity for a long time. In addition, recent developments in high-throughput sequencing technology, comparative metagenomics, and marker gene profiling have allowed for the investigation of the composition of the entire soil microbial population (Le et al., 2016; Stämmler et al., 2016). Despite the widespread use of these methodologies in studies, no clear trends in the diversity, composition, or organization of soil microbial communities in response to global climate change have been found (Cheng et al., 2017). Since climate change may alter the soil microbial community structure and functions, their role in nutrient cycling can provide a positive or negative response to rising global temperatures (Zheng et al., 2020).

Numerous studies have been conducted to indicate impact of soil warming on soil microbial community composition (DeAngelis et al., 2015; Siebielec et al., 2020; Zheng et al., 2020). For instance, Weedon et al. (2012) conducted 9-year summer warming experiment and concluded stable soil microbial community structure. Kuffner et al. (2012) investigated 4 years of simulated warming, wherein 16S rRNA gene pyrosequencing revealed stable and resistance by soil microbial community diversity indices, composition, and structure, and may be influenced by the taxon level. Whereas Luo et al., (2014) found significant shift in communities using metagenomic approach for 10 years. Also, Illumina sequencing of the 16S region from temperate forest soils (warmed for 20 years) enhanced significant alteration in bacterial communities (DeAngelis et al., 2015). Additionally, studies based on long-term warming experiments revealed significant lag of microbial community response to climate change (Luo et al., 2013; DeAngelis et al., 2015; Cheng et al., 2017). Several concerns regarding the response of microbial communities to climate change continue to be resolved as previous studies indicated that microbial responses to soil warming are complicated and inconsistent over time (Zheng et al., 2020).

Soil respiration constitutes respiration by the soil microbial community (via breakdown of organic matter and plant tissues in the bulk soil) and by plant roots, associated mycorrhizal fungi, and rhizosphere microbes (Preece et al., 2020). It involves release of CO_2 into the atmosphere but also indicating soil activity. Therefore, the impact of drought on soil respiration can be a complex process and could result in huge implications for global C balance (Brændholt et al., 2018; Vries et al., 2019). Drought reduces soil respiration as it diminishes the biological activities of soil microorganisms, animals, and plants (Preece et al., 2020). According to Schimel, (2018), during drought events, the microbial biomass can remain constant, hence decline in soil respiration cannot be misguided by changes in microbial biomass.

Microbial community level physiological profiles (CLPP) (Biolog EcoPlates) enable to assess the soil respiration and potential microbial communities by measuring the metabolic responses to the range of organic C substrates that vary in structural complexity (Preece et al., 2020). This method has the potential to yield a large amount of data that is excellent for identifying differences in soil bacteria that are peculiar to a certain site and assessing the relationship between soil biological activities and site characteristics (Bogati et al., 2023). Some studies found reductions in soil respiration tended to have lower soil moisture (e.g., <5% in Curiel Yuste et al. (2007) and <10% in Misson et al. (2009)) and other studies also detected changes in CLPPs (Kassem et al., 2008; Ochoa-Hueso et al., 2018). However, a lack of soil respiration may indicate that typical soil processes are not occurring, which could have long-term effects on the immediate health of an ecosystem. On the other hand, there are studies indicating the impact of increasing amount of water content on activity of microorganisms (Borowik et al., 2016; Dinter et al., 2019; Furtak et al., 2022).

Our study investigates the influence of soil moisture variations (2 months) on the microbial community (bacteria, fungi and actinomycetes), their enzymes, namely dehydrogenases, phosphatases (acidic and alkaline) and urease, and metabolic diversity in four types of agricultural soil samples collected in Poland during the spring, and autumn seasons. These sites were selected based on differences in their bonitation classification and texture such as gleyic luvisol Phaeozem in G (rich in clay

and humus, 1st class), stagnic luvisol in L (3rd class), fluvisol in N (3rd class) and haplic luvisol in S (sandy, 5th class). Our experiment was based on climatic zone and weather conditions in central Europe (Bogati et al., 2022). Drought conditions have been observed in Poland as well as in other neighbouring countries that last for almost 2 months, indicating huge implications on vegetation lands (Siebielec et al., 2020; Bogati et al., 2022). These impacts of drought stress on the soil biological activities are not well known. Hence, studies on the above parameters in response to changes in soil moisture content may provide insights to soil processes and mitigate future deleterious effect on the healthy agricultural land.

AIM OF THE STUDY

The main aim of this research was to determine the impact of drought in soil biological activities such as microbial diversity, ecophysiology, and enzymatic activity.

HYPOTHESIS

1. The significant differences in soil microbial communities (bacteria, fungi and actinomycetes) are connected with the seasonal variations and changes in 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.

RESEARCH OBJECTIVES

1. Investigation of the impact of long-term drought on changes in the number of basic groups of soil microorganisms and taxonomic biodiversity of microorganisms in agricultural soils.

2. Determination of changes in the activity level of soil enzymes of microbial origin occurring in conditions of prolonged drought.

3. Determining whether and how taxonomic changes of microorganisms occurring in drought conditions in agricultural soils affect changes in the physiological diversity of soil microorganisms.

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PUBLICATION I

The Impact of Drought Stress on Soil Microbial Community,

Enzyme Activities and Plants

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Review

The Impact of Drought Stress on Soil Microbial Community, Enzyme Activities and Plants

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Abstract: Nowadays, the most significant consequence of climate change is drought stress. Drought is one of the important, alarming, and hazardous abiotic stresses responsible for the alterations in soil environment affecting soil organisms, including microorganisms and plants. It alters the activity and functional composition of soil microorganisms that are responsible for crucial ecosystem functions and services. These stress conditions decrease microbial abundance, disturb microbial structure, decline microbial activity, including enzyme production (e.g., such as oxidoreductases, hydrolases, dehydrogenase, catalase, urease, phosphatases, β-glucosidase) and nutrient cycling, leading to a decrease in soil fertility followed by lower plant productivity and loss in economy. Interestingly, the negative effects of drought on soil can be minimized by adding organic substances such as compost, sewage slugs, or municipal solid waste that increases the activity of soil enzymes. Drought directly affects plant morphology, anatomy, physiology, and biochemistry. Its effect on plants can also be observed by changes at the transcriptomic and metabolomic levels. However, in plants, it can be mitigated by rhizosphere microbial communities, especially by plant growth-promoting bacteria (PGPB) and fungi (PGPF) that adapt their structural and functional compositions to water scarcity. This review was undertaken to discuss the impacts of drought stress on soil microbial community abundance, structure and activity, and plant growth and development, including the role of soil microorganisms in this process. Microbial activity in the soil environment was considered in terms of soil enzyme activities, pools, fluxes, and processes of terrestrial carbon (C) and nitrogen (N) cycles. A deep understanding of many aspects is necessary to explore the impacts of these extreme climate change events. We also focus on addressing the possible ways such as genome editing, molecular analysis (metagenomics, transcriptomics, and metabolomics) towards finding better solutions for mitigating drought effects and managing agricultural practices under harsh condition in a profitable manner.

Keywords: climate change; water scarcity; soil microbiome; microbial activity; plant growth and development

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1. Introduction

An increase in greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), and atmospheric temperature, and depletion of water resources, being a consequence of anthropogenic activities, have driven climate change [1–5]. One of the important consequences of climate alteration is the occurrence of drought stress conditions. Drought stress in soil occurs when the water content or humidity in the soil and air is significantly low along with the high atmospheric temperature. This happens due to an uneven balance between the soil surface evaporation and plant transpiration followed by low soil water content [1]. Khan et al. [6] monitored global drought for almost two decades (2001–2019) using big geospatial datasets from Google Earth Engine and calculated drought indices, namely vegetation condition index (VCI), temperature condition index

(TCI), soil moisture condition index (SMCI), and precipitation condition index (PCI). These indices showed continuous fluctuation of soil moisture and severe vegetation drought that affected 70% of the land globally. To date, many countries, including the USA, Australia, France, Russia, Turkey, Afghanistan, Iran, Mongolia, China, Brazil, Thailand, and Africa, have had historic events on the negative impact of drought, mainly on the agriculture and economy sector [6–10]. According to ICCP [2], each of the last four decades has been successively warmer than any decade that preceded it since 1850. Global surface temperatures were higher by 0.99 and 1.09 °C in 2001–2020 and 2011–2020, respectively, than in 1850–1900, with larger increases over land (1.59 °C) than over the ocean (0.88 °C). In Europe, according to European Environment Agency (EEA; https://www.eea.europa.eu/ims/global-and-european-temperatures; 29 December 2021), land temperatures have increased even faster over the same period by around 1.9–2.02 °C [11,12] compared to the average values. Moreover, climate change scenarios predict a further decrease in average precipitation from May to October [12]. These trends show that the risk of soil drought in the vegetation growing season is high and may negatively affect crop yields. In Poland, a serious threat of agricultural drought was reported in the last few years, namely in 2010, 2011, 2013, 2015, 2017, and 2018 [13].

Drought is defined as a state of the total water capacity being within the range of 12–20% for a period of 16 days and can be distinguished from the water deficit, which is the state of water capacity falling below 30% [14–17].

Drought is one of the most prevalent stresses that impact microbial community and activity, crop development, yield production, and quality [5,12]. A negative impact of drought on soil microbes can lead to a decrease in enzymes activity, loss in nutrient cycling (e.g., C, N, P), and soil fertility, thus plant productivity, especially of drought susceptible crops, and consequently economic outcomes [5,18]. The condition of the soil under drought stress strictly corresponds to plant growth and development. Drought directly affects plant morphology, physiology, and biochemistry [19]. It also reduces seed germination and seedling growth [20]. Plant responses to drought stress were also observed at transcriptomic and metabolomic levels [21]. Severe and long-term drought stress disturbs the availability of soil microbiota to the plant roots, significantly affecting their microbiome composition leading to modification of root structure and release of root exudates and disturbance of useful nutrients. The microbiome of plant roots changes during drought, favoring *Actinobacteria* and many other Gram-positive species, which substitute the Gram-negative taxa that are predominantly present in the rhizosphere [22]. Generally, soil microorganisms can enhance plant resistance to drought via different mechanisms, including the production of polysaccharides that improve soil structure and water holding capacity, synthesis of deaminase, indoleacetic acid (IAA), and proline (Pro), which induce drought stress tolerance in plants and improved water circulation through fungal mycelia [23]. Interestingly, the negative effect of drought on microorganisms can be mitigated by the addition of organic matter to soil [24]. Moreover, microbial activity (respiration rates) and soil microbial community structure can be modified by drought-associated phytohormones such as abscisic acid (ABA), jasmonic acid (JA), and 1-aminocyclopropane-1-carboxylic acid (ACC), but adaptation to prolonged drought modifies the responses of soil microbial communities to these hormones [25].

Despite negative aspects of changes caused by drought, such severe environmental conditions can induce interesting adaptations in microbes and plants that allow them to survive and reproduce. These adaptations can lead to the emergence of new functional groups in the ecosystem or serve as an important tool for improving agricultural practices and plant breeding programs [4,25]. Such drought-tolerant microorganisms and their traits could be used in the search for efficient compounds of biopreparations supporting plant growth [19].

This review was undertaken to discuss the impact of drought stress on soil microbial community abundance, structure, and activities, thus soil enzyme activities, pools, fluxes, and processes of terrestrial carbon and nitrogen cycles, and plant growth and development,

including the role of soil microorganisms in this process. A deep understanding of many aspects is necessary to explore the impacts of these extreme climate change events.

2. Impact of Drought Stress on Microbial Communities and Enzyme Activities

Prolonged drought has a significant impact on the abundance, structure, and activity of the soil microbiome [12]. The potential metabolic microbial activity decreases with a reduction in soil water potential, followed by lowering nutrient mineralization and respiration [26]. However, structural and/or functional adaptation of microbial communities in response to drought depends on soil type, farming system, and plant cultivars [22], as discussed later.

In this section effect of drought in soils on microbial structure and enzyme activity, and consequently on nutrient cycling and soil fertility, is discussed (Figure 1).

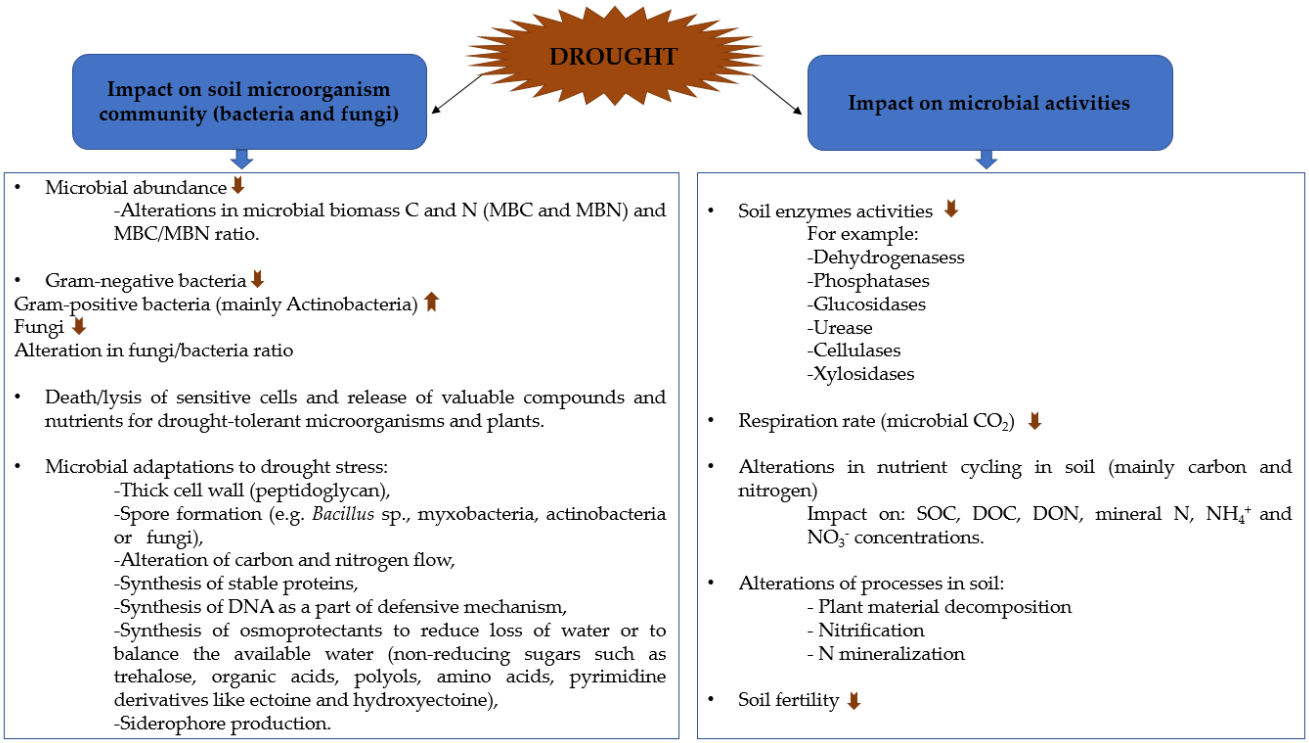

Figure 1. Schematic representation on the impact of drought stress on microbial communities and activities.

2.1. Effect of Drought on Microbial Communities

2.1.1. Microbial Adaptations to Drought Stress

It is known that thick peptidoglycan in the cell wall of Gram-positive bacteria is responsible for their higher resistance towards drought when compared to Gram-negative bacteria. Microbes either die or become dormant during immediate stressful environments, whereas on the arrival of favorable conditions, the dormant forms regain their activity. In addition, dead microbial cells release many valuable compounds such as nitrogen, phosphates, amino acids, polyols, nutrients that can be beneficial to other microbes and plants. These microorganisms that form spores (e.g., *Bacillus* sp., myxobacteria, filamentous actinobacteria, or fungi) are resistant to abiotic factors, including drought stress, and able to survive for a long time in dormant forms. However, microbes might develop many tolerance mechanisms, some of which are energetically expensive, such as these related to the regulation of resistance genes, alteration of carbon and nitrogen flow, synthesis of stable proteins and osmolytes, which reduce loss of water or balance the available water [27,28].

Desiccation-tolerant microorganisms, e.g., *Rhodococcus jostii* RHA1 [29], *Microbacterium* sp. 3J1, *Arthrobacter siccitolerans* 4J27, *Rhodococcus* sp. 4J2A2 [30], *Rhodococcus opacus*

PD630 [31], *Lactobacillus paracasei* [32], and *Pseudomonas putida* KT2440 [33] are known to overproduce various molecules such as non-reducing sugars (trehalose), organic acids, polyols, amino acids, (rich in hydroxyl groups), pyrimidine derivatives such as ectoine and hydroxyectoine [30,33–38] responsible for the protection of cells from drought stress that are called xeroprotectants. These molecules can also be taken up by non-synthesizing microorganisms in drought conditions [39,40]. A study by García-Fontana et al. [41] on desiccation-tolerant microorganisms of *Microbacterium* sp. 3J1 showed overexpression of genes encoding for enzymes involved in DNA syntheses such as topoisomerases, DNA polymerases, and gyrases. This resulted in an increase in DNA production in cells as part of their defensive mechanisms to protect protein structures and functions from drying, confirmed by RNA-seq analysis. In addition, siderophores, secondary metabolites that scavenge iron from environmental stocks and deliver it to cells via specific receptors, are believed to help bacteria to thrive in such environments [42]. It was proved that siderophore producing *Azospirillum* sp. strain B2 was most resistant to drought stress and used as an inoculant for wheat can alleviate drought stress on plant growth and yield [42].

2.1.2. Effect of Drought Stress on Microbial Community in Different Type of Soils or under Soil Modifications

Severe drought conditions modify the microbial community structure, size, and activity in soils. However, their effect on the microbial structure is more significant in soils with low organic matter content [12,43]. For example, controlled conditions of drought stress-induced changes in the relative abundances of particular phyla present in sandy and loamy soils. Among six phyla, namely *Actinobacteria*, *Bacteroidetes*, *Firmicutes, Planctomycetes*, *Protecobacteria,* and *Verrucomicrobia* that accounted for >95% of the total bacterial abundance, the *Actinobacteria* (especially genera *Gaiella* and *Nocarioides*) were most prevalent in analyzed samples. While relative abundance of *Proteobacteria*, being corticotrophs, and *Verrucomicrobia* decreased significantly [12]. Baldrian et al. [44], based on phospholipid fatty acid (PLFA) analyses, recorded that with the decrease in soil moisture content, the microbial (bacterial and fungal biomass) also decreased. In contrast, Bastida et al. [45] showed that drought influenced the fungal PLFA biomarker but not the bacterial PLFA biomarkers. Interestingly, in other studies on drought impact, a higher abundance of *Firmicutes* in soil that has previously been exposed to such stress conditions was observed [46]. This indicates that soils under repetitive drought events exhibit lower stress mortality. This can be a consequence of microbial adaptation that has been developed.

Castro et al. [47] reported that drought-induced reductions in labile carbon and nitrogen entering the rhizosphere might be a contributing factor in the loss of microbial phyla such as *Verrucomicrobia*, *Proteobacteria* and *Acidobacteria*, which are heterotrophs and sensitive to nitrogen ratios. It has been well-documented that exogenous organic matter in the form of sludge or compost amendments plays an important role in protecting soil microbial community and microbially-mediated processes in semiarid or arid soils [43]. They recorded that the total and Gram-positive bacteria and total monounsaturated PLFA were affected significantly by drought only in the unamended soil. The alterations in bacterial community composition under drought stress were also observed in two types of wheat rhizosphere soils (Chernozem and Luvisoil) [22]. The Luvisol soil showed a decrease in Gram-negative bacteria and persistence of actinomycetes under drought conditions. However, at the same time, actinobacteria diversity was lower in Luvisol than in Chernozem soil. Therefore, in the case of Luvisol soil, which is less rich in organic matter content, the effect of drought was more prominent [22]. It is another proof that the application of organic residues can be proposed as an adequate strategy against soil degradation in semiarid environments. However, the interactions between organic amendments and drought are not fully known [12,43,45].

Furthermore, microbial community structure and microbial activity (respiration rates) in soil under drought stress can be modified by drought-associated phytohormones (ABA, JA, and ACC) [25]. However, the authors demonstrated the adaptation of soil microbial

communities to the long-term drought that was observed by their response to plant stress hormones. All three phytohormones significantly declined Gram-positive biomarkers in drought soils. That is interesting, as these bacteria are generally assumed to be more stress-tolerant than Gram-negative bacteria [48]. Overall, the adaptation of microbial community-level to drought stress often involves shifts towards organisms with greater tolerance to water deficit and slow-growing taxa with a reduced metabolic capacity [12,25,49]. Authors speculated that the decrease in Gram-positive biomarker abundance after treatment with all three phytohormones indicates drought-adapted bacteria that responded negatively to stress signaling by investing resources in survival strategies such as dormancy, osmolytes, or spore production instead of growth and turnover. Further, they claimed that drought-adapted soil microbial communities might perceive these phytohormones as signals of impending water stress rather than as a substrate for growth [25]. Moreover, they found that the abundance of Gram-negative biomarkers remained unchanged in droughted soils but increased with ABA, ACC, and JA addition to control soils. Therefore, the Gram-positive:Gram-negative bacteria ratio increased in drought stress. Furthermore, they also found that in response to all phytohormones, the relative abundance of saprophytic fungal and arbuscular mycorrhiza (AM) biomarkers increased in droughted soils [25].

Drought stress is known to hamper the availability of soil microbiota to the plants and significantly affect root microbiome composition. The differences in the abundance of root-associated microbial communities at endosphere and rhizosphere levels during monsoon and dry seasons were reported by Naylor et al. [50]. They found that fungal communities in soils and rhizosphere were unaffected, whereas the class *Actinobacteria* decreased significantly in α-diversity in roots with also a decline in *Acidobacteria* and *Deltaproteobacteria*. On the other hand, they also noticed an increase in enrichment of *Actinobacteria* (*Saccharopolyspora, Glycomyces,* and *Actinopolymorpha*) under drought in the root endosphere than in rhizosphere or bulk soil itself.

2.2. Effect of Drought on Soil Enzyme Activities

Soil enzymes are produced by animals [51], plants [52], and microorganisms. They are prominently secreted by microbes and reflect microbial activity in this biome [53]. Soil microorganisms mainly synthesize extracellular enzymes such as β-glucosidase, hydrolases urease, phosphatase, glycosylating enzymes, cellulase, amylase, cyclomaltodextrinase, chitinase, and many more [43,54–56]. Other enzymes such as dehydrogenases or catalase are intracellular. Therefore, soil enzymes play a vital role in the biodegradation of organic compounds in soil and become the most delicate indicator of changes in microbial activities (termed as "sensors") that occur in the soil environment in response to different factors including drought [43,57].

The reports on the effect of drought on different soil enzymes are limited. Generally, these effects are uncertain or not easy to predict [58]. However, a decrease of moisture content in soils decreases microbial biomass and thus soil enzyme activities [44,59,60]. Baldrian et al. [44] observed seasonal changes in the activity of enzymes and the abundance of microorganisms correlated with the water content in the soil. The significant decrease in soil microbial communities and reduction of >50% activity of laccase, Mn-peroxidase, endo-1,4-β-glucanase, endo-1,4-β-xylanase, cellobiohydrolase, β-glucosidase, β-xylosidase, chitinase, and acid phosphatase enzymes were recorded in soils with low water content (0.30–0.40 g g^{-1}) when compared to control samples with higher water content (0.60–0.70 g g^{-1}). In other studies, it was found that drought-affected activity of C-cycling enzymes (cellulases, glucosidases, and xylosidases) in the rhizosphere of two wheat cultivars in loamy Chernozem and sandy Luvisol soils in organic and conventional farming. For 'Dichter' wheat cultivar, enzymatic activities were decreased in Chernozem soil in both farming types. While in Luvisoil, increased activity of glucosidases and xylosidases was recorded in organic farming. In the case of 'RGT-Reform' wheat cultivar, drought negatively affected xylosidase and glucosidase activities in the Chernozem soil but promoted glucosidase activities in Luvisol [22]. Drought impact on sediment revealed de-

crease in esterase (0.5%/day) [61], β-glucosidase (>50%) [62], leucine aminopeptidase [63], phosphatase (>50%) [64], and phenol oxidase (PO) [63] activities. In the case of Mediterranean evergreen oak forests, a reduction of 10 and 21% of soil moisture in plots (by water runoff) decreased urease activity by 10–67% and 42–60%, protease activity by 15–66% and 35–45%, and β-glucosidase activity by 10–80% and 35–83% depending on the annual period (spring and autumn) and soil depth (0–15 and 15–30 cm). The lowest activities of these enzymes were observed in autumn and at a greater depth. The significant reduction of acid phosphatase activity (by 31–40%) was observed only when the moisture content in the soil was reduced by 21%. The phenolooxidases (laccases, Mn-peroxidases, lignin-peroxidases, and tyrosinases) activities in evergreen oak forests were studied by Cricquet et al. [65]. They observed a significant increase in laccase and Mn-peroxidase activities in autumn when compared to other months, while other phenoloxidases, lignin-peroxidases, and tyrosinases were never detected in analyzed samples. However, in this study, analyzed forest plots were not artificially dried by water runoff [65]. Similarly, in other studies by Criqet et al., acid phosphatase activities were significantly higher from November to January and decreased in other months [66]. These highest activities (>6 $\times$ 10^{-2} U g^{-1} DM) correlated with high soil moisture content (around 65–70%). The lowest enzyme activities (1.2 $\times$ 10^{-2} U g^{-1} DM) were recorded in July, when soil moisture content was found to be nearly 15%. The alkaline phosphatase was undetectable or at low activity when compared with acid phosphatase activities but highest between May and October. A significant decrease in enzymatic activity of dehydrogenases and phosphatases in both loamy and sandy soils was observed during the first month under drought stress, while after two months of stress conditions, activity of dehydrogenases was even three times less when compared to the control sample under the optimal moisture level [12]. However, the level of dehydrogenase activity was considerably higher in the loamy soil than in sandy soil, which can be related to higher organic matter content in the former one [12,67], but the aspect of the presence of organic residues on microbial abundance and activity in soil will be discussed later in this section.

Moreover, relatively high soil exoenzyme (β-glucosidase, β-xylosidase, α-glucosidase (AG), β-D-cellobiosidase (CBH), N-acetyl-β-glucosaminidase, acid phosphatase, leucine amino peptidase, phenol oxidase, and peroxidase (PER)) activities were observed during summer girdling of lodgepole pine (that was complete removal of the bark from around the trunk of a tree) but decreased after girdling in response to drought [68].

2.3. Effect of Drought on Microbial Activity

Severe drought conditions, despite enzyme activities, may compromise nutrient availability in the soil, as proved in the study by Hueso et al. [43]. They found high soil dehydrogenase activity (34% of the total variability), a decrease of other enzyme activity, high basal respiration, and water-soluble carbon in stressed soil compared to well-watered soils. Increased levels of water-soluble carbon may be linked to an increase in carbohydrate production (52% of the total variability) due to the fact that few soil microbes produce biological polymers in response to low water conditions. Perhaps, drought can cause the death of sensitive microorganisms unable to thrive under such harsh conditions, which results in the release of substrates from dead cells into their surroundings, in turn providing accessible nutrients to the drought-resistant microorganisms or survivors. In addition, decreased metabolic activity of the soil microbial community was also observed under drought conditions, in turn leading to a decrease in mineralization of carbon, nitrogen, and phosphorus biological cycles or pathways [43]. This suggests that a large community of active microbial biomass did not survive during long drought stress periods, resulting in the decline of overall soil microbial functional diversity [12,18,69].

Drought strongly affects soil nitrogen cycling by inhibiting nitrification [12]. The soil nitrification potential (NP) is a highly sensitive parameter, which reflects the response of soil microorganisms to environmental factors, e.g., temperature or moisture content [70]. It describes the potential activity of a specialized group of autotrophic bacteria, namely

ammonia-oxidizing bacteria (mainly genera *Nitrosomonas* and *Nitrosospira*) that are responsible for the first phase of nitrification [71]. Siebielec et al. [12] showed that after one month drought period, the NP activity was reduced by 70 and 80% in the loamy and sandy soils, respectively. These results indicate that the resistance of sandy soil with low organic matter content to drought stress was lower than that in loamy soil. Interestingly, the compost application to the sandy soils only slightly reduced the negative effects of drought on soil nitrifying bacteria [12].

Deng et al. [72] investigated soil C and N pools and fluxes in response to drought in three different ecosystems, namely forests, grasslands, and shrublands, conducting meta-data analyses, wherein a huge amount of data was collected. These data were taken from 148 reports and included 1815 sampling data at 134 sites across the globe and were mainly focused on short-term drought. They analyzed vegetation-related properties, soil pools of C and N and their fluxes, soil microbial biomass, and activity of enzymes (Table 1). Overall, the effects of drought on soil C and N cycles were regulated by the ecosystem type, drought duration, and intensity. The latter two parameters intensified all effects. The soil organic carbon (SOC) concentration was decreased across the globe as a consequence of decreased litter input and decomposition under drought conditions. Drought also negatively affected root biomass. Microbial respiration (microbial CO_2) was increased, especially in forests and grasslands. Moreover, drought increased the contents of dissolved organic carbon (DOC) by 59% and dissolved organic nitrogen (DON) by 33% due to decreased mineralization and higher stability of dissolved organic matter. Although the soil mineral nitrogen (SMN) content increased (by 31%), the nitrogen mineralization and nitrification rates decreased (by 5.7 and 13.8%, respectively), thus total nitrogen concentration unchanged. Globally, NH_4^+ and NO_3^- increased (52 and 16%, respectively), but in forest soils, an increase of NH_4^+ concentrations corresponded to an 11.3% decrease of NO_3^-, thus reflected the increase of N mineralization rate, but a decrease of nitrification rate. In shrublands, unlike forest soils, the concentration of NH_4^+ slightly increased, but NO_3^- increased significantly (69.2%). Since nitrogen mineralization rate was unaffected, but nitrification rate strongly decreased (by 56.4%), this might be a consequence of less N uptake by plants under drought. Drought negatively affected microbial biomass nitrogen (MBN) content. Thus, the ratio of microbial biomass carbon MBC to MBN increased, similarly to fungal/bacterial ratio and enzyme activities across the globe (Table 1).

Table 1. Effects of drought on soil C and N dynamics in three different ecosystems according to the study of Deng et al. [72].

Analyzed Parameters	Ecosystem Type			Mean Changes in All Ecosystems [%]
	Forests	**Grasslands**	**Shrublands**	
Litter and Root Biomass Response				
• Litter input	-	↓ +++	↓ +++	−8.7
• Litter decomposition rate	↓ +++	-	↓ +++	−12.7
• Litter C content	↑ +++	↓ +	↑ +++	+23.4
• Litter N content	↑ +++	↓ +	-	+13.8
• Root biomass	↓ +	-	↓ +	−6.7
Soil pools and fluxes of C				
• SOC concentration	-	↓ +++	-	−3.3
• DOC concentration	↑ +++	↑ +++	↑ +	+59.2
• Total CO_2 efflux from soil	-	-	↓ +++	-
• Soil microbial respiration	↑ +++	↑ +++	-	+15.8
Soil pool (concentration) and fluxes of N				
• Total N	-	-	↑ +++	-
• DON	↑ +	-	↑ +	+33.0

Table 1. *Cont.*

Analyzed Parameters	Ecosystem Type			Mean Changes in All Ecosystems [%]
	Forests	**Grasslands**	**Shrublands**	
• NH_4^+	↑ +++	↑ +	↑ +	+52.5
• NO_3^-	↓ +++	↑ +	↑ +++	+16.0
• SMN	↑ +++	↑ +	↑ +	+31.0
• nitrification	↓ +	-	↓+++	−13.8
• N mineralization	↓ +	-	↓ +	−5.7
• Soil microbial biomass	-	-	-	-
• MBC	-	-	-	+2.2
• MBN	↓ +	↓ +	↓ +	−10.4
• MBC/MBN ratio	↑ +++	↑ +++	↑ +	+29.7
• F/B ratio	↑ +	↑ +	-	+15.6
Soil enzyme activities				
• β-glucosidase	↓ +	↑ +	↓ +	+3.5
• urease	↓ +++	↑ +++	↓ +	+12.7

Key: C, carbon; N, nitrogen; SOC, soil organic carbon; DOC, dissolve organic carbon; DON, dissolve organic nitrogen; SMN, soil mineral nitrogen; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; F, fungi; B, bacteria. -, no effect or not significant response; ↑, increase; ↓, decrease; +, small effect; +++, strong effect.

2.4. Mitigation of Drought Effects on Microbial Activity by Soil Amendments

The negative effect of drought in the soil can be minimized by adding organic substances. The addition of different types of organic matter, namely compost (COM), sewage sludge (SS), and municipal solid waste (MSW) to the arid soil increased the activity of soil enzymes such as oxidoreductases, hydrolases, dehydrogenase, catalase, urease, phosphatases, β-glucosidase, casein- and N-α-benzoyl-L-argininamide (BAA)-hydrolyzing proteases [24], probably as a consequence of the increase in microbial biomass [43], as mentioned previously.

Moreover, microbial activity (soil respiration) in drought soils can be modified by the addition of plant hormones, namely ABA, JA, and ACC. However, soil respiration response to phytohormones depended on their dose. For example, the addition of 1 mM ABA two times increased microbial activity in droughted soil when compared to control soil, but lower ABA concentrations, namely 1 µM and 1 nM, did not affect the respiration rate of soil samples. Interestingly, both ACC and JA in low and high concentrations increased microbial activity but not in intermediate concentrations. Respiration from droughted soils was 1.5 times higher than from the control samples treated with ACC. In the case of JA, the lowest concentration of JA (1 nM) had the largest effect on respiration in droughted and control soils [25]. Authors suggested that increased respiration rates in soils in response to phytohormones could be a result of hormone utilization as sources of C and N by bulk soil microorganisms. However, such a relationship was confirmed only in the case of ABA addition [25]. Therefore, further studies are required.

3. Plant Morphological, Physiological, Biochemical Responses towards Drought Stress

In general, plants are exposed to numerous environmental stresses in the course of their different stages of growth irrespective of the natural or agricultural environment [73]. The occurrence of environmental alterations has developed various adaptations in plants for their survival in harsh conditions [1]. Common prominent environmental stress such as drought caused due to dwindling of water resources is the main foundation in affecting overall plant productivity [73]. The plant responds to drought stress at different levels—morphological, anatomical, biochemical, physiological, and molecular [73–76] (Figure 2).

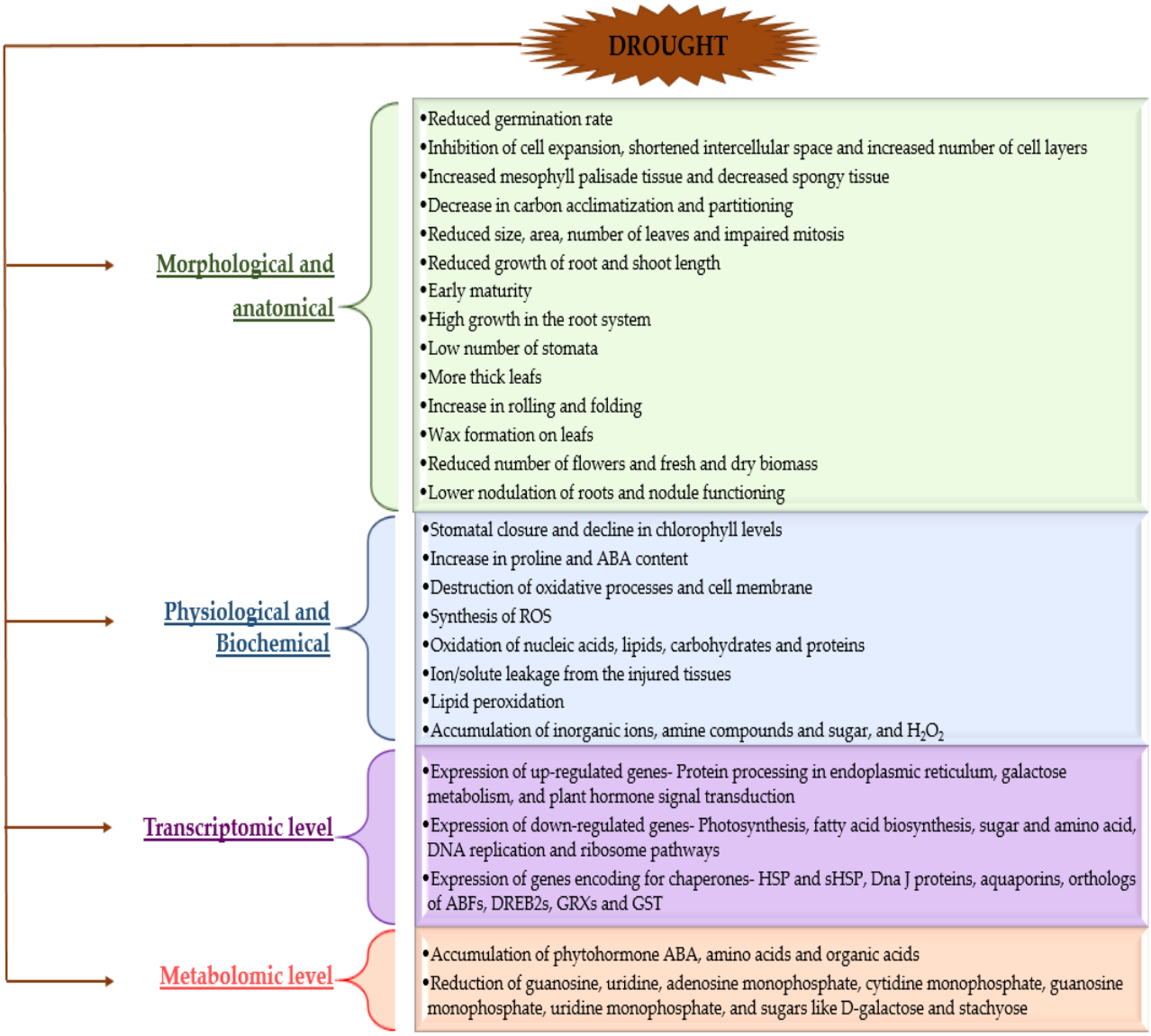

Figure 2. Morphological, anatomical, physiological, biochemical, and molecular responses of plants to drought stress.

3.1. Morphological and Anatomical Changes in Plants in Response to Drought Stress

Drought might affect plants at each stage in their growth cycle, including seed germination. Water scarcity or drought reduces germination rate leading to reduction of seedling emergence or inhibition and further growth in plants [20]. Drought stress disturbs the seedling growth because of cell expansion inhibition, decrease in carbon acclimatization, and partitioning [77]. The decrease in plant height is mainly due to decreased cell expansion, increased leaf shedding, and impaired mitosis under drought conditions [78]. Reduction in seedling characteristics in drought-stressed plant seeds such as *Brassica napus* was reported by many authors [79–81]. Morphological changes in plant under drought stress involve reduced size, area, and number of leaves as well as the growth of root and shoot length due to stimulation of the ABA precursor called ACC that prevents the growth of root, with early maturity, high growth in the root system, low number of stomata, more thick leaves with an increase in rolling and folding and wax formation in order to prevent loss of water from leaves as well as from roots [1,25,73]. In addition, plant leaves tend to increase mesophyll palisade tissue, decrease spongy tissue, increase the number of cell layers, but decrease the volume and shorten the intercellular space to adopt drought, as

found in two avocado cultivars [82]. Overall, the change of leaf area, which directly affects plant photosynthesis and yield, is one of the most easily observed features of plant leaves under drought stress [78]. Plants under drought stress have reduced the number of flowers and fresh and dry biomass [19]. Drought stress causes a decrease in nodulation [83] and nodule functioning [75]. In plants, the water deficit conditions are recognized in roots, and then several molecular signals move from roots to shoots. Consequently, abscisic acid (ABA) phytohormone mediates resistance to drought stress by regulating stomatal closure and synthesizing stress-responsive gene expression in leaves, thereby affecting reduction in transpiration [84–87].

3.2. Physiological and Biochemical Changes in Plants in Response to Drought Stress

Photosynthesis, as mentioned above, is one of the main processes affected by water stress leading to a decrease in plant growth. The decrease of photosynthetic rate under drought stress is the result of stomatal and non-stomatal limitations. The stomatal limitation is believed to be the main factor of photosynthetic rate decrease under mild drought while non-stomatal limitation under severe drought conditions. Stomatal closure limits leaf absorption of CO_2 and prevents transpiration water loss due to turgor pressure and/or reduced water potential [78]. Non-stomatal factors are related to the decrease of activity or content of component, such as ribulose-1,5-bisphosphate (RuBP) or ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) involved in the photosynthetic assimilation process and the efficiency of photosystem II (FPSII), respectively [88,89].

Biochemical changes include the decline in chlorophyll levels, increase in Pro content, destruction of oxidative processes by the production of antioxidative enzymes, increase in ABA levels, and synthesis of reactive oxygen species (ROS) [73]. Drought stress inhibits the chlorophyll synthesis (chlorophyll 'a' and 'b'), changes in chlorophyll 'a'/'b' ratio, especially in drought-sensitive plant cultivars, thereby the efficiency of photosynthetic apparatus, resulting in the production of ROS. They are formed as a consequence of photosynthetic electron transport reactions in the circumstances of a flooded electron flow system with a decreased pool size of electron acceptors [90–92]. Therefore, chlorophyll decline is one of the most injurious concerns of drought stress. In response to the deterioration of photosynthetic apparatus, synthesized ROS causes oxidation of carbohydrates, proteins, nucleic acids, lipids, as well as the destruction of the cell membrane [90,93]. Lipid peroxidation leads to overproduction of malondialdehyde (MDA), which is one of the indicators of oxidative damage [21,94–96]. Moreover, the accumulation of metabolites such as inorganic ions (Na^+, K^+, H^+), amino acid (Pro), amine compounds (glycine betaine and polyamines), and sugar (trehalose, fructan, mannitol, etc.) in plants occurs for the regulation of osmotic potential under drought stress conditions. These substances are usually of small molecular weight, highly soluble, and have little toxicity to cells. They can maintain the normal osmotic pressure level, protect the protein activity and cell membrane structure, and so on [97–100]. For example, the contents of Pro, glycine betaine, total soluble carbohydrate, and sucrose were significantly increased due to drought stress in several pistachio genotypes, as shown in the study of Khoyerdi et al. [101].

Pro is well-known for its acclimatizing roles in plant stress tolerance, works as a molecular chaperone to alleviate the configuration of proteins, and its high concentration is considered an indicator of tolerance to water stress [102–105]. These amino acids are preferentially stored in plant vacuoles and transported to the cytoplasm during osmotic stress. Increase of its concentration in cytoplasm leads to reduction of the osmotic potential, thus that the cell can still absorb extracellular water thus, maintaining the cell protoplasm and the external environment of osmotic balance. Proline can reduce the oxygen damage caused by stress through chelating singlet oxygen and hydroxyl radical or stimulate the activity of POD, catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and other enzymes in plants to remove drought stress generated ROS. It has a strong ability to hydrate and bind to proteins, therefore, stabilizing and protecting biological macromolecules, maintaining their structure and activity, and cell membrane structures [78].

Similarly, glycine betaine, as a water-soluble substance with amphoteric properties, can bind to both hydrophilic and hydrophobic regions of biological macromolecules such as enzymes and acts as an osmotic regulator. It was found that glycine betaine protects the key enzymes of the dicarboxylic acid cycle, terminal oxidases, and the photosystem, which have important physiological significance in maintaining proper respiration and photosynthesis of plants [106]. However, the osmotic regulations can only temporary improve plant resistance to drought stress. If drought stress is severe, the turgor pressure of plants cannot be maintained [78].

In addition, plants under drought stress can synthesize drought-induced functional (e.g., late embryogenesis abundant proteins, including dehydrins and aquaporins) and regulatory proteins (protein kinases, phospholipase C, phospholipase D, G protein, calmodulin, transcription factors, and some signaling factors). They are involved in the protection of ion channel functioning, ROS scavenging, water molecule binding, enzyme activity (e.g., SOD, POD), PSII proteins, membrane structure, and water transport at cellular or subcellular levels or in signal transduction or gene expression regulation, and play indirect protective roles in water stress [78].

Reactive oxygen species (ROS) include superoxide radical O_2^-, H_2O_2, singlet oxygen $^1O_2$, hydroxyl radical ·OH, and organic oxygen radical (RO, ROO), etc. They can be produced in plants through many metabolic pathways, namely in the process of photosynthesis and respiration, as well as in other processes in mitochondria, chloroplasts, and peroxisomes. However, some other organelles or parts with high oxidation activity or strong electron transfer function may also be involved in ROS production. In plants under drought stress, ROS are overproduced mainly by chloroplasts and mitochondria [107–109]. For example, ·OH can directly induce the peroxidation decomposition of the unsaturated fatty acid chain in phospholipids, thus destroying of membrane structure. In addition, ROS can destroy almost all proteins or enzymes, break, degrade, or modify single or double strands of DNA [110–112].

For scavenging the high levels of ROS, plants have developed complex enzymatic and non-enzymatic systems. The former includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR), while the latter a low molecular weight antioxidant, namely reduced glutathione (GSH), ascorbic acid (AsA), vitamin E, mannitol, flavonoids and carotenoids [78,113,114]. SOD and APX are mainly localized in cytoplasm and chloroplasts, CAT in peroxisomes, GPX mainly in cytoplasm and mitochondria, and GR in chloroplasts [78]. The high amount of H_2O_2 in drought-stressed plants is generated as a consequence of SOD activity, which transforms superoxide radicals (O_2^-) to H_2O_2 and O_2. Generally, SOD activity increased under mild or short-term water stress but decreased under severe or long-term water stress [78]. Generated H_2O_2 being toxic to plant cells is reduced to water by POD and APX or converted to oxygen and water by CAT [97]. Moreover, GR, DHAR, and MDHAR are also very important H_2O_2 scavenging enzymes [78]. All of these scavenging systems normalize H_2O_2 levels in plants [115]. Non-enzymatic ROS scavenging system involves substances that can react directly with ROS (GSH, AsA), act as substrates of enzymes in the ROS scavenging mechanism, or, as in the case of vitamins, by scavenging oxygen free radicals, preventing lipid peroxidation directly. GSH-AsA cycle is the main pathway of GSH and AsA regeneration and antioxidant system in plants, which stabilizes the ROS level in the chloroplasts of plants [116].

Sun et al. [117] analyzed the response of nearly 60 different plant species and their cultivars (e.g., oilseed rape, fava bean, maize, wheat, mustard, mung bean, soybean, *Euphorbia tirucalli*, *Coffea arabica*, oak, cedar, Norway spruce, apple plants, and many more) to water stress (the median water stress intensity of 0.52 and experimental duration of 36 days) based on several independent variables of plant tissues such as leaf, shoots, roots, and whole plants. Water stress intensity was calculated as the proportional reduction in soil moisture (reduced soil moisture under water stress treatment/soil moisture in the control

groups). They conducted meta-data analyses wherein a huge amount of data was collected from 1301 paired observations of 84 studies across the globe and estimated the impact of short-term drought on plants. Based on the morphology, physiology, and functionalities of plants, they analyzed plant growth (dry weight and protein), photosynthetic characteristics (chlorophyll; maximal efficiency of PSII photochemistry; photochemical quenching coefficient), plasma membrane permeability (ROS, MDA, and electrolyte leakage), enzymatic antioxidants (APX, GR, glutathione reductase, CAT, POD, SOD) and nonenzymatic antioxidants (ABA, AsA, Pro, carotenoids, soluble sugars). Overall, a significant increase in plasma membrane permeability (PMP), enzymatic antioxidants (EA), and nonenzymatic antioxidants (NEA) under water stress, but a decrease in plant growth and PS globally was observed, as shown in Table 2. An increase in ROS (by 65.7%), MDA (by 44.2%), and EL (99.4%) shows malfunctioning of the plasma membrane and lipid peroxidation, thereby causing oxidative stress while decrease in chlorophyll (Chl) content (by 24%), maximal efficiency of PSII photochemistry (Fv/Fm; by 13%) and photochemical quenching coefficient (qP; by 26.4%) indicates damage of photosynthetic organs and altered leaf structure under water stress conditions. An increase in EA (CAT, POD, and SOD) reveals the ability of the plants to manage water stress by maintaining normal metabolic processes. High NEA levels, especially ABA (+126%), Pro (+136.8%), and soluble sugar (+116.9%) contents under water stress suggest the plant adaptation mechanisms in order to endure the adverse effect of ROS under water stress. Thus, this meta-analysis proved the negative impact of water stress on overall plant growth and performance.

Table 2. Plant morphology, physiology, and functionalities in response to drought, according to studies by Sun et al. [117].

Drought Indices (Groups)	Average Values (%)	
Plasma membrane permeability (PMP)		
• ROS	↑ ++	65.7
• MDA	↑ +	44.2
• EL	↑ ++	99.4
Enzymatic antioxidants (EA)		
• CAT	↑ +	28.8
• POD	↑ +	28
• SOD	↑ +	29.8
• APX		
• GR	—	
Non-enzymatic antioxidants (NEA)		
• ABA	↑ +++	126.6
• AsA	↑ +	19.3
• Proline	↑ +++	136.8
• Soluble sugar	↑ +++	116.9
• Car	—	
Plant growth (PG)		
• Leaves	↑ +	17.1
• Shoots	↓ +	−20.5
• Whole plants and roots	—	
• Dry weight	↓ +	−28.8
• Protein	—	
Photosynthesis (PS)		
• Chl	↓ +	−23.9
• Fv/Fm	↓ +	−13.1
• qP	↓ +	−26.4

Key: ROS, reactive oxygen species; MDA, malondialdehyde; EL, electrolyte leakage; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; APX, ascorbate peroxidase; GR, glutathione reductase; ABA, abscisic acid; AsA, ascorbate; Car, carotenoid; Chl, chlorophyll; Fv/Fm, maximal efficiency of PSII photochemistry; qP, photochemical quenching coefficient; -, no effect or no significant response; ↑, increase; ↓, decrease; +, small effect; ++, medium effect; +++, strong effect.

3.3. Transcriptomic and Metabolomic Changes in Plants under Drought Stress

Response of plants to water-deficient conditions can be observed at transcriptomic and metabolomic levels (Figure 2) [21,86,118]. You et al. [21] studied drought-tolerant (DT) and drought-susceptible (DS) sesame genotypes under drought stress and found that DS plants were more disturbed by stress conditions that was confirmed at both transcriptional and metabolic levels. Such plants contained more drought-responsive genes and metabolites when compared to DT genotype. Transcriptomic analyses of DT and DS sesame plants revealed the presence of a total 2782 and 3542 up-regulated and 4163 and 4519 down-regulated genes, respectively. Among them, a set of core drought-responsive genes (a total 2030 genes including 648 up-regulated and 1346 down-regulated genes) that were differentially expressed in both sesame genotypes under drought conditions was found. Upregulated core drought-responsive genes were involved in protein processing in the endoplasmic reticulum, galactose metabolism, and plant hormone signal transduction, while down-regulated core drought-responsive genes in photosynthesis, fatty acid biosynthesis, sugar, and amino acid metabolism (amino sugar and nucleotide sugar metabolism, glycine, serine and threonine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis), DNA replication, and ribosome pathways. Transcriptomic analyses of both genotypes showed that although they share common pathways to cope with drought stress, some unique ones, such as these related to alpha-Linolenic acid metabolism, valine, leucine, and isoleucine degradation, photosynthesis and peroxisome were recognized in DT sesame plants [21]. Many other transcriptomic and metabolomic analyses in plants under drought stress highlighted the important role of amino acid metabolism and ABA metabolism and signaling pathways for drought tolerance in plants [119–125].

The most important metabolites, which are accumulated in plants under drought stress include phytohormone ABA, amino acids such as tryptophan, phenylalanine, valine, leucine, tyrosine, saccharopine, Pro and 2-aminoadipate, 4-aminobutanoic acid (GABA), and organic acids, namely glutaric acid, and 2-methylcitric acid. Accumulation of tryptophan is thought to play an important role in the regulation of stomata, osmotic adjustment, and ROS scavenging [126]. Especially, higher levels of ABA, Pro, arginine, lysine, aromatic and branched-chain amino acids, GABA, saccharopine, 2-aminoadipate, and allantoin were found in drought-tolerant sesame plants under drought conditions [21]. Similarly, drought-tolerant chickpea plants showed a lower level of oxidative damage than their drought-sensitive counterparts and higher activity of POD, CAT, AsA, and GSH and accumulation of Pro and consequently lower production of H_2O_2 or MDA [20]. In contrast, some nucleosides and nucleotides such as guanosine, uridine, adenosine monophosphate, cytidine monophosphate, guanosine monophosphate, and uridine monophosphate), and sugars such as D-galactose and stachyose were reduced under drought stress in both DS and DT sesame genotypes [21].

In addition, other genes encoding for chaperones, including heat shock proteins (HSP) and small heat shock proteins (sHSP), Dna J proteins, aquaporins, orthologs of ABFs (ABA-Responsive-Element binding factors), and DREB2s (Dehydration-Responsive-Element-Binding Proteins 2), glutaredoxins (GRXs), glutathione S-transferase (GST), and many more can also be involved in plant response to drought stress [127–136]. Transcriptome analysis of the many other plants or crops such as pine (*Pinus massoniana*) [137], tea oil camellia (*Camellia oleifera*) [138], maize (*Zea mays* L.) [139], peanut (*Arachis hypogaea* L. varieties) [140], endemic orchid species (*Dendrobium sinense*) [118] under drought stress also provided insights into the molecular mechanism such as expression of drought stress genes and certain functional genes that helps the plant to cope with drought stress. Tahmasebi et al. [141] investigated the transcriptional response of two different plant species (*Oryza sativa* (rice, C_3 plant) and *Zea mays* (maize, C_4 plant)) to drought stress based on 172 arrays in total from 11 drought stress studies. This meta-analysis also took into account the transcriptional response of shared differentially expressed genes (DEGs) in sorghum and barley, with respect to maize and rice, respectively, to drought stress, as shown in Table 3. In this meta-analysis, gene ontology (GO) analysis showed DEGs associated with photosynthesis,

metabolic pathways, and stress response. In maize, genes encoding for cytochrome c oxidase protein (COX19-like), metabolic processes, osmotic stress, photosynthesis, antioxidant activity, DNA complex, defense signaling pathways, heat shock protein, photosynthetic gas exchange as well as other DEGs play an important role in drought tolerance. In rice, dehydrin protein (RAB16B) genes that are responsible for drought tolerance were highly upregulated, and also genes responsible for stress tolerance, plant transduction, photosynthesis, metabolic processes, oxidoreductase activity, and other DEGs, as mentioned in Table 4, were expressed. Several DEGs were identified under stress conditions in both plant species, as listed in Tables 3 and 4. In both C_3 and C_4 plants, genes such as transcription factors, plastid translation, DNA replication and repair, antioxidant activity, and antioxidant defense genes were detected, as well as genes related to the hormone cytokinin, plant hormone signal transduction, and carbon fixation were differentially co-expressed between species under stress conditions. This study reflects on similarities and differences among the two plant species, such as the response to stress, small molecule metabolic process, response to cytokinin, and photosynthesis. Therefore, this meta-analysis indicated the influence of drought on the biological processes of both plants at a larger scale, thereby reflecting the importance of transcriptomic studies for obtaining precise information on plant response to drought stress.

Table 3. Meta-analysis on the transcriptional response of Sorghum and Barley to drought stress, according to a study by Tahmasebi et al. [141].

Variables	Sorghum	Barley
Total DEGs	300 genes (2% orthologous with one of maize)	2065 genes (7.2% orthologous with one of rice)
DEGs associated genes	Alkaloid biosynthesis, plant hormone signal transduction, MAPK signalling pathway, response to abiotic stimulus, and carbon metabolism.	
Transporter genes	Transmembrane transporter activity genes-SPX (Sb06g025950) and MS channel gene (Sb10g006710)	ABC transporter system- Contig18416_at and Contig13030_s_at
Shared DEGs	With maize- ASR protein	With rice- biosynthesis of secondary metabolites

Key: DEGs, differentially expressed genes; MAPK, mitogen-activated protein kinases; ABC, ATP-binding cassette; ASR, abscisic acid- stress- ripening-induced.

Table 4. Meta-analysis on the transcriptional response of Rice and Maize to drought stress, according to a study by Tahmasebi et al. [141].

Variables	Maize	Rice
Total DEGs	4915	7291
Upregulated genes	2532	3491
Downregulated genes	2383	3800
Highly upregulated identified DEGs	Probesets related to Cox family, fasciclin-like arabinogalactan proteins Three genes encoding for Di19 drought-induced 19	RAB16B and RAB21 genes
Most highly downregulated identified DEGs	Histone H3-like proteins	PMEI-like and PEAMT2 genes
Stress tolerance genes	Heat shock proteins	LEA, HSP70, WSI76, and DREB1C
Predictive accuracy	97.22%	98.72%
1. Gene Ontology Enrichment Analysis in Each Species:		
DEGs	Small molecule metabolic process Response to chemical Carbohydrate metabolic process Organic acid metabolic process	Plant hormone signal transduction
Upregulated DEGs	Response to osmotic stress	Response to temperature stimulus Response to salt stress Response to osmotic stress

Table 4. *Cont.*

Variables	Maize	Rice
Downregulated DEGs	Photosynthesis Cofactor metabolic process	Photosynthesis Light reaction
GO terms:		
Biological processes	47 DEGs	Photosynthesis Small molecule metabolic process Oxidation-reduction process Response to abiotic stimulus 30 DEGs
Species-specific enriched biological processes	34%	25%
Molecular function	Cation binding Metal ion binding Antioxidant activity 8 DEGs	Oxidoreductase activity Catalytic activity 8 DEGs
Cellular component terms	DNA packaging complex Nucleosome Thylakoid 28 DEGs	Chloroplast Plastid 31 DEGs
Common biological processes	Metabolic process and upregulated response to stress (41% common)	
2. Pathway Enrichment:	Enriched with metabolic pathways Carbon metabolism-related terms Photosynthesis Biosynthesis of secondary metabolites pathways	13 KEGG pathways Downregulated photosynthesis pathway Carbon fixation in photosynthesis genes Expression of hormone signal transduction
Upregulated metabolic pathways and genes	Asparagine synthetase, acyl-CoA oxidase and peroxidases, with 1 and 9 unique pathways in maize and rice.	
3. Identification of Consensus Modules:		
Genes related to response to water deprivation and small molecule metabolic process	BP-10, KEGG-5	
Cell wall organization and cell cycle	BP- 18, KEGG- 3	
Photosynthesis	BP- 6, KEGG- 6	
Biogenesis and biosynthesis	BP- 6, KEGG- 2	
4. Identification of Hub Genes:	Mainly enriched in pyrimidine metabolism	
Cell wall organization and cell cycle	FOR1 and PV72	
Biogenesis and biosynthesis	PDHE1-A and HyPRP18	
Genes related to response to water deprivation and small molecule metabolic process	Protein of unknown function DUF676 and PDX1	
Photosynthesis	Ankyrin-like protein and UBC37	
5. Identification of Differential Co-expression Modules:		
Photosynthesis and response to cytokinin	MF- 5, BP- 8, KEGG- 3	
Organic acid catabolic process	MF- 2, BP- 17, KEGG- 6	
Response to stress	MF- 2, BP- 9, KEGG- 1	
Cell wall organization	MF- 8, BP- 30, KEGG- 3	
Alanine, aspartate, glutamate metabolism (KEGG: 00250) enriched pathways among genes	MF- 1, KEGG- 1	
6. Co-localization of DEGs with QTL Intervals:		
DEGs localized within QTLs regions	801 DEGs	1724 DEGs
Drought tolerance	141 (2.8%)	122 (1.6%)
Photosynthetic gas exchange	444 (10.5%)	139 (1.9%)
$d^{13}C$	59 (1.2%)	105 (1.4%)
Root characteristics traits	157 (3.19%)	1358 (18.6%)

Key: DEGs, differentially expressed genes; Cox, cytochrome c oxidase protein; Di19 drought-induced 19; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular functions; $d^{13}C$, C isotope signature.

4. Plant Growth Promotion and Protection by PGPB and PGPF under Drought Conditions

For the functioning of ecosystems, interactions between plants and soil microorganisms are of utmost importance, along with their response to climate changes [142,143]. Root exudates composed of enzymes, mucilage, ions, sugars, organic acids, amino acids, and so on actually decide the overall selection of root microbiome, as they attract beneficial bacteria. Changes in the composition of root exudates (e.g., presence of stress signals) during stressful conditions may significantly affect the root microbiome [50,144], as discussed previously. Under drought conditions, mucilaginous material secreted by the roots becomes the major carbon nutrient source for microorganisms, thereby enhancing the nearby microbial biomass and altering the soil microbiome composition [145–147]. Therefore, efficient management and maintaining rhizospheric microorganisms are vital for the well-functioning of cropping practices [148]. Rhizosphere microbial communities adapt their structural and functional compositions to water scarcity and have the potential to substantially mitigate the drought stress of crops. Metagenomic analysis of the rhizobacterial communities revealed changes at the genome level in order to cope with drought stress. Such changes included enhancement of functional genes encoding for enzymes responsible for the breakdown of complex carbohydrates such as fructan and dextran, decrease in biofilm, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production, and reduction in genes encoding for spermidine (R,R)-butanediol dehydrogenase and glutathione. [22]. The plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF) by colonizing the root areas are known to contribute towards the plant adaptive mechanism in order to help the plant to survive the adverse effects of drought stress, as shown in Table 5. Microorganisms associated with plant roots minimize the harmful effects of stresses by delaying wilt and drought-induced changes (stomatal conductance, photosynthesis, and leaf discoloration), increasing amino acid content and Pro production, reducing H_2O_2 content, and increasing total phenolics in plants [1,149–153], increase nitrogen fixation, nutrients uptake, siderophore, phytohormone and secondary metabolite production, synthesis of exopolysaccharides (EPS), and many other organic compounds, and enhance enzyme activities [154–164], and many more (Table 5).

Table 5. Plant growth-promoting mechanisms by PGPR in drought stress conditions.

Microorganisms	Plants	Protection/Effect/Mechanisms	References
Azospirillum lipoferum	Maize (*Zea mays.* L)	Improves growth of the plant, increase in amino acid contents, sugar accumulation, and Pro production.	[165]
Bacillus spp.	Maize (*Zea mays.* L)	Improves intake of soluble sugar, amino acids, and Pro reduces loss of electrolytes and activity of catalase and glutathione PER enzymes.	[166]
Pseudomonas putida strain GAP-P45	Sunflower (var. Sunbred *Helianthus annuus* L.)	Improved uptake of nutrients in plant and increased growth of the plant.	[167]
Bacillus licheformis strain K11	Pepper *(Capsicum annum*)	Promotes growth of pepper, produces ACC deaminase, and prevents phytophthora blight.	[168]
Rhizobium tropici and *Paenibacillus polymyxa*	Bean *(Phaseolus vulgaris)*	Improved growth, nodulation, and nitrogen content.	[169]

Table 5. *Cont.*

Microorganisms	Plants	Protection/Effect/Mechanisms	References
Sinorhizobium medicae	Barrel medic *(Medicago truncatula)*	Delay in drought-induced leaf senescence, increase in potassium, drought-responsive proteins, and osmolyte production.	[170]
Bacillus amyloliquefaciens 5113 and *Azospirillum brasilense* NO40	Wheat (*Triticum aestivum* L.)	Improves plant growth, enhances enzyme activities (mono-dehydroascorbate reductase (MDHAR), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) and APX), lower antioxidant enzyme activities, and increases photosynthesis.	[171]
Pseudomonas libanensis TR1 and *Pseudomonas reactans* Ph3R3	Smooth-stem turnip *(Brassica oxyrrhina)*	Enhances plant growth, leaf relative water content (RWC), resistance to heavy metals and antibiotics, increased chlorophyll content, and decrease in malondialdehyde content.	[172]
Pseudomonas putida MTCC5279 (RA)	Chickpea *(Cicer arietinum* L.)	Improves plant growth, nodule formation, low antioxidant enzymes, and increases biochemical responses.	[173]
Piriformospora indica	Thale cress *(Arabidopsis thaliana)*	Expression of stress-related genes.	[174]
Rhizobium leguminosarum (LR-30), *Mesorhizobium ciceri* (CR-30 and CR39), and *Rhizobium phaseoli* (MR-2)	Wheat (*Triticum aestivum* L.)	Improved root colonization, nutrient or water holding capacity of the rhizosphere, Improved drought tolerance index of the wheat seedlings, enhanced the root or shoot lengths and fresh or dry biomass of the seedlings, production of phytohormones (IAA), exopolysaccharides or catalase, osmolytes and antioxidants in the rhizosphere.	[175]
Azospirillum sp.	Wheat (*Triticum aestivum* L.)	Plant growth enhancement.	[42]
Trichoderma harzianum	Rice (*Oryza sativa* L.)	Delay to wilt, drought-induced changes (stomatal conductance, photosynthesis, and leaf discoloration), promote plant growth, increase in Pro content, reduction in H_2O_2 content, and increase in total phenolics.	[153]
Glomus etunicatum	English walnut (*Juglans regia*)	Improve height, fresh weight, and the number of leaves of the walnut plant increased the biosynthesis of some metabolites, including soluble sugar and Pro, total phenolic content, peroxidase activity, and starch content as well as peroxidase enzyme activity.	[176]
Ampelomyces sp.	Tomato (*Solanum lycopersicum* var. Better Boy)	Enhancement of plant growth, fruit yield, drought tolerance and resistance to pathogens.	[177]

Table 5. *Cont.*

Microorganisms	Plants	Protection/Effect/Mechanisms	References
Glomus lamellosum and *Glomus etunicatum*	Cinnamon (*Cinnamomum migao*)	Improvement in seedling growth, higher POD and CAT activity, decrease in sugar and osmoreceptor content, reduction of accumulation of MDA, and enhancement of water-use efficiency in the plant.	[178]
Alternaria sp. and *Trichoderma harzianum*	Tomato (*Solanum lycopersicum* var. Rutger)	Improvement in root and shoot biomass, enhancement of water-use efficiency, and better photosynthetic efficiency.	[179]

5. Conclusions and Further Perspectives

According to the recent studies [6,10,11] and last reports of ICCP [2] and EEA, anthropogenic activities have significantly contributed towards climate change and drought that is the most important consequence of climate change in recent decades with a negative influence on our ecosystem, agriculture, and economy [6–10]. In this review article, we discussed various impacts of drought on soil microbial communities and plants. Although in previously published papers, the impact of drought on microbial community abundance, structure, and activity was described, our current knowledge is still incomplete. Soil is a very complex environment. Therefore, microbial community structure and its activity are not easy to explore and predict. The type of soil, especially the content of organic matter and the accompanying type of vegetation, is a very important aspect in determining changes in the structure and activity of soil microorganisms and subsequently enzyme activities, pools, fluxes, and carbon and nitrogen cycles. There are still relatively few metagenomic studies of microorganisms inhabiting drought-affected soil environments, and this scientific problem should be solved. Comprehensive analyses of microbial genome sequences, especially drought-tolerant ones, for genes encoding for drought stress-related compounds seem to be a promising tool to broaden our knowledge on how microorganisms cope with such harsh conditions. To date, synthesis of polysaccharides, xeroprotectants such as soluble sugars, proteins, and amino acids, as well as phytohormones (ABA, JA, ACC, IAA), and many more were described to increase microbial and plant resistance. An especially interesting solution to mitigate the negative effects of drought stress in the soil, mainly semiarid and arid ones, and improve microbial abundance, activity, and soil fertility by amendment of soil with various types of organic compounds, including compost, sewage sludge, and municipal solid waste [43]. In addition, the use of specific compounds such as plant hormones was also proposed [25]. However, the response of microorganisms to phytohormones is ambiguous and further research is required.

Plants respond to water scarcity in different ways, and this is a complex process that we still need to work on to unravel completely. In past studies, changes in plant morphology, anatomy, biochemistry, and physiology under drought stress were described in detail. However, plant response to such extreme conditions at the trancriptomic and metabolomic levels is still deficient. Therefore, more studies on the response of different plant species to drought stress are required to understand various adaptive mechanisms evolved in these organisms. In addition, meta-analysis studies seem to be a valuable approach to know global patterns of changes in the microbial community, its activity, and plants affected by stress conditions.

Based on the current knowledge, some improvement in crop management skills such as handling or selection of appropriate crops and soil, maintaining good levels of soil water content, insertion of abiotic stress tolerance traits into the plants using genetic engineering or genome editing technologies were proposed to increase plant productivity in such abiotic stress. Moreover, application of osmolytes, potassium, hydrogels, nanoparticles, mineral

nutrient (silicon), antioxidative protectant (selenium), and plant growth regulators such as uniconazole and salicylic acid were considered to increase plant yield under abiotic stress [1,73,129,180–183]. In addition, the application of plant hormones such as ABA, gibberellic acid (GA), ethylene, auxins, JA, cytokinins, and brassinolide (BR) that regulate different beneficial mechanisms in the plants can help them to cope with the adverse effects of droughts [1,73]. A summarized outline of possible approaches for managing crop practices under drought stress is represented in Figure 3.

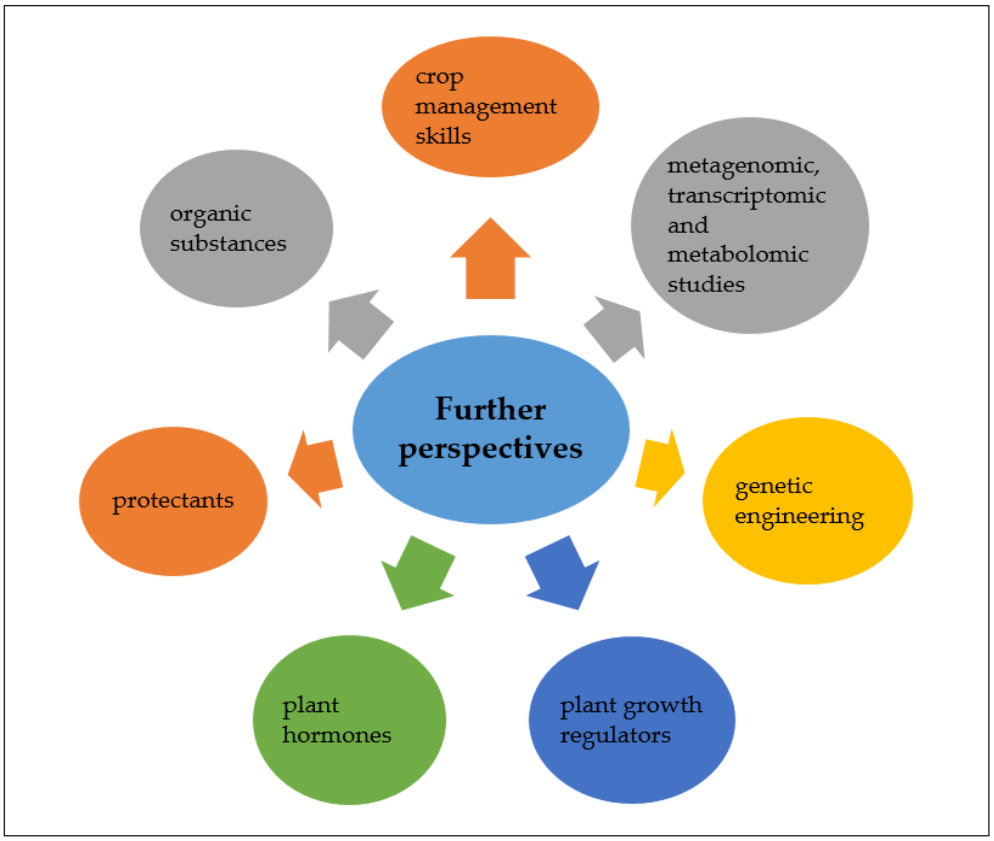

Figure 3. Future possible perspectives in order to mitigate drought stress.

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PUBLICATION II

Deciphering the Impact of Induced Drought in Agriculture

Soils: Changes in Microbial Community Structure, Enzymatic

and Metabolic Diversity

agronomy

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Article

Deciphering the Impact of Induced Drought in Agriculture Soils: Changes in Microbial Community Structure, Enzymatic and Metabolic Diversity

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Abstract: Prolonged drought stress may have a significant impact on the structure and activity of the soil microbial community. Our study aims to investigate the impact of short-term drought (2 months) on the microbial community structure, enzymes, and metabolic diversity in four agricultural soils (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S) sites) in Poland. These four types of soil were selected based on differences in their texture (gleyic luvisol Phaeozem in G (rich in clay and humus), stagnic luvisol in L, fluvisol in N and haplic luvisol in S (sandy)). We investigated the (1) number of bacteria, actinomycetes (formally phylum *Actinomycetota*) and fungi; (2) microbial community (16S rRNA and ITS amplicon regions); (3) biological activity by community-level physiological profiling (CLPP); (4) soil enzyme activities (dehydrogenases (DH), phosphatases (acid ACP and alkaline ALP) and urease (UR)); and (5) soil chemical properties. At the end of our experiment, we observed a significant decrease in soil moisture content with the highest in the soil from the S site. Overall, there was no change in total bacteria, but actinomycetes and fungal numbers increased after the 1st week with a decrease in moisture content. ACP activity decreased in three out of four analyzed soil samples. The exception was in sample G, where activity increased for 1–2 weeks and then decreased. ALP activity significantly increased with a decrease in moisture in the 1st week and was lowest at the end of the experiment. DH activity increased up to the 4th week in the G and N samples and up to the 2nd week in the L and S samples. UR activity showed variations in the analyzed samples. A reduction in the utilization of carbon sources (except D-mannitol and L-asparagine) was noted with the highest reduction in the G sample followed by the L, N and S samples. Thus, the pattern of changes was different depending on the analyzed soil type. The 16S rRNA and ITS amplicon sequencing revealed a decrease in the relative abundance of *Pseudomonadota*, *Basidiomycota*, *Apicomplexa*, and increased abundance of *Actinomycetota*, *Bacillota* and *Ascomycota* under prolonged drought conditions. With this, we concluded that drought conditions resulted in a significant alteration of soil microbial communities, enzyme activities, and metabolic diversity in the investigated soils.

Keywords: water stress to soil impact; soil microbial function; extracellular enzymes; soil respiration; soil fertility; soil microbial diversity; agronomics

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1. Introduction

Drought, a consequence of climate change, is defined as a lack of precipitation in an area/region causing a decrease in soil moisture alongside surface and groundwater sources or dehydration events that simultaneously increase with variations in precipitation events [1]. Industrialization causes a boost toward climate change and is responsible for the emission of greenhouse gases (GHGs). This not only brings forth the occurrence of drought conditions but also global warming (rise in atmospheric temperature; 0.07 °C/decade since 1880), strong heat waves, floods, intense summers, and other climate-related hazards [2]. The Intergovernmental Panel on Climate Change [3] reported that the significant factors contributing to decreased precipitation are the recurrence of El Nino events, water stress, decreased atmospheric moisture, and rise in temperature, specifically in arid and semi-arid regions. The same report claims that drought affects global agricultural production and soil quality, which are vital for agricultural sustainability. On the other hand, Eastern Europe and Mediterranean regions are mainly affected by drought, and it is predicted that in the future, Europe will be experiencing further drought events [4]. According to the Institute of Soil Sciences and Plant Cultivation State Research Institute of Poland (http://www.susza.iung.pulawy.pl, accessed on 17 March 2022), for the last ten years, agricultural lands in Poland have been in danger of drought or significantly affected by drought that caused severe damage to crop, but they were the strongest during 2015. Reasons behind the multi-faceted impact of drought on soil biological activity are often not well understood, but we cannot exclude that it is site specific [4]. Adverse effects of drought on agricultural soils lead to soil degradation, loss of biodiversity and agricultural yields/lands, and decreased surface and groundwater levels. In addition, drought stress is not only known to affect the physical and chemical parameters of soil fertility but also its microbiological parameters [4].

Soil microbes play a vital role in ecosystem function as they are responsible for the biogeochemical cycling of nutrients (macronutrients and micronutrients) by the secretion of enzymes and transform other elements crucial for plant growth [5]. In this context, short-term or prolonged drought can affect microbial community composition and consequently its activity in the soil [4,6]. As exemplified by studies of Schimel et al. [7], the one month of drought conditions caused a reduction in microbial populations and their activity in litter soil under birch trees. However, among bacteria, the Gram-positive ones, such as actinomycetes and *Bacillus* sp., are grouped as drought-tolerant members [5]. Siebielec et al. [6] showed that bacterial communities of the loamy soil samples in Poland were dominated (>95%) by six phyla, namely *Actinobacteria* (now *Actinomycetota*; https://lpsn.dsmz.de/phylum/actinomycetot, accessed on 17th May 2023); commonly known as actinomycetes), *Bacteroidetes* (now *Bacteroidota*; https://lpsn.dsmz.de/phylum/bacteroidota, accessed on 17 May 2023), *Firmicutes* (now *Bacillota*; https://lpsn.dsmz.de/phylum/bacillota, accessed on 17 May 2023), *Planctomyces* (now *Planctomycetota*; https://lpsn.dsmz.de/phylum/planctomycetota, accessed on 17th May 2023) and *Verrucomicrobia* (now *Verrucomicrobiota*; https://lpsn.dsmz.de/phylum/verrucomicrobiota, accessed on 17th May 2023), where *Actinobacteria* and *Proteobacteria* were present in relatively equal percentages under optimal soil moisture. However, the prolonged drought stress, up to two months, influenced the dominance of *Actinobacteria* while the relative abundance of *Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* decreased. In contrast, such soil conditions increased the abundance of *Firmicutes*. In addition, these loamy soils were dominated by ten genera, namely *Aquihabitans*, *Brevundimonas*, *Flavobacterium*, *Gaiella*, *Kribbella*, *Marmoricola*, *Nocardioides*, *Pseudomonas*, *Solirubrobacter* and *Sphingomonas*. The prolonged drought stress led to a decrease in abundance of *Pseudomonas*, *Sphingomonas*, *Brevundimonas* and *Flavobacterium* whereas *Gaiella*, *Kribbella*, *Nocardioides*, *Marmoricola* and *Solirubrobacter*, which belong to actinomycetes, increased [6]. Similarly, Santos-Medellín et al. [8] found that drought negatively affected microbial community composition in rice agricultural soil, while Xu et al. [9] observed a decrease in bacterial community diversity in the rhizosphere and root endosphere of sorghum cultivars. In the case of

fungal community, Hayden et al. [10] observed a decrease in fungal abundance in response to warming in grassland soil. Oliveira et al. [11] found that phytopathogenic fungi which belonged to the genera of *Curvularia*, *Thielavia* and *Fusarium* were more prevalent in water deficit conditions. The investigations on drought in soils and the corresponding meta-analyses often reflect detrimental effects of drought on the variety and abundance of soil microbial communities, with bacteria being claimed to be more vulnerable than fungi [12–14]. Since different groups of bacteria and fungi are susceptible to soil moisture change, as a result, generalizations about various agricultural areas are challenging, and it is unclear what impact these modifications will have on certain functions over time.

Soil nutrient cycling is greatly dependent on extracellular enzymes [15]. These enzymes are produced by soil microbes via the breakdown of complex organic matter, polymeric carbon, and nitrogen substrates (for example lignin, cellulose, pectin, hemicellulose, and microbial debris). These products become ultimately useful for microbial metabolism and growth [15]. The balance between the synthesis and degradation of these complexes in soil determines the soil fertility and quality, microbial composition, nutrient availability, and microbial enzyme activities. Moisture stress causes a decline in the decomposition of soil organic carbon (SOC) and its respiration to CO_2 and nitrification rates in the soil [16]. The presence of extracellular enzymes in the soil indicates healthy soil microbial function, which in turn shows the response pattern of microbial communities to environmental changes [4]. Many reports revealed different significant consequences of drought on soil enzymes [17–19]. Decreases in precipitation (drought) significantly suppressed phenol oxidase (POX) (−47.2%), urease (−30.6%), β-1,4-glucosidase (BG) (−4.6%), and acid phosphatase (AP) (−5.1%) in the soil. In addition, the overall activity of carbon, nitrogen and phosphorus acquisition enzymes were negatively affected by −4.6, −17.6% and −5.1%, respectively [17]. Similarly, a decrease in urease activity during the reduction in soil moisture levels was confirmed by other authors [19,20]. Dehydrogenase, an intracellular enzyme synthesized by viable cells, was also negatively affected under drought stress [6,21].

It is claimed that drought events have harmful effects on extracellular enzymes and oxidative activity in the soil, which is probably due to the decrease in the diffusion of substrates in the soil [22]. Although by the end of this century, the frequency of drought is expected to increase, this trend may gradually change the underground characteristics of the agricultural ecosystems [22]. Altogether, there has been no coordinated effort to dissect the impacts of extreme conditions in soils (e.g., drought or heat) on the microbial community composition and activity in different agricultural soils.

Our study was designed to investigate the influence of prolonged drought (2 months) on the microbial community (bacteria, fungi and actinomycetes), their enzymes, namely dehydrogenases, phosphatases (acidic and alkaline) and urease, and metabolic diversity in four types of agricultural soil samples collected in Poland during the spring season. These four types of soil were selected based on differences in their texture and bonitation classification (gleyic luvisol Phaeozem in G (rich in clay and humus, 1st class), stagnic luvisol in L (3rd class), fluvisol in N (3rd class) and haplic luvisol in S (sandy, 5th class)). The entire experiment was planned for 8 weeks (almost two months). Such a decision was justified by the climatic zone and weather conditions in central Europe [4]. In Poland and neighboring countries, there are periods of drought lasting from one to a maximum of two months. Such a drought in this region of Europe is already considered catastrophic, shortening the vegetation period of crops to a maximum of 5 months [4,6]. In addition, our hypothesis assumes that the lack of rainfall in the following weeks should lead to radical changes in soil biological activity and changes in the composition of the soil microbiome. As impacts of drought stress on the soil environment are still not well understood, therefore, comprehensive studies on the above parameters in response to drought may provide new opportunities to mitigate the impact of such abiotic stress on the healthy functioning of agricultural land in the future [4].

2. Materials and Methods

2.1. Sample Collection and Physicochemical Analyses of Soil Samples

The agriculture soil samples (20 cm in depth from the soil surface; n = 5 per site) were collected on 29 May 2021 (spring season) from four sites, namely in Gniewkowo (G; 52.901355° N, 18.432330° E), Lulkowo (L; 53.090675° N, 18.580300° E), Wielka Nieszawka (N; 53.006132° N, 18.466123° E) and Suchatówka (S; 52.907623° N, 18.467457° E) near Toruń, Poland (Figure 1) into plastic containers (high = 23 cm and Ø = 28 cm). For each site, five plastic containers were filled with soil for the 0, 1st, 2nd, 4th and 8th week treatments. In total, 20 containers were exposed to drought conditions by placing them outside but under the roof for up to 8 weeks. Therefore, soil samples were protected against rainfall but not maintained in strictly controlled conditions of humidity and temperature. We selected these sites based on soil bonitation classification (G (1st class), L (3rd class), N (3rd class), S (5th class)). At each treatment time, soil samples were collected from their corresponding container (in five replicates) into the plastic bag, mixed well, and subjected to further tests in the laboratory.

Figure 1. Four locations of the research sites are at Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S).

Soil samples for the study of microbial community and activities were analyzed at time (T) intervals (0, 1, 2, 4 and 8 weeks, where "0" is sampling day). For this purpose, soil samples were collected from the containers after 0, 1, 2, 4 and 8 weeks using a stainless-steel soil sampler probe (Ø 50 mm) to the plastic bags and analyzed immediately, as described below. The mean soil moisture at time intervals was determined, in five replicates, by calculation of the difference in soil mass between the collected samples and dried samples (100 °C for 4 days). The soil pH was measured, in five replicates, in distilled water at the ratio of 1:2.5 using a pH meter CP-401 (ELMETRON, Zabrze, Poland). Total organic carbon (TOC) and total nitrogen (TN) were determined using organic elemental analyzer Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The graining of the soil and its texture were determined according to the Bouyoucos areometric method, modified by Casagrande and Prószyński [23], and the sieve method [24].

2.2. Determination of the Number of Bacteria, Actinomycetes and Fungi in Soil Samples

Bacteria, actinomycetes (*Actinomycetota*) and fungi were isolated from four agricultural soil samples using a standard ten-fold dilution plate procedure. First, 1 mL of serial dilutions (10^{-4}–10^{-6} and 10^{-2}–10^{-4}) of each soil sample was placed into sterile Petri plates and poured with Plate Count Agar (PCA, Biomaxima, Lublin, Poland) for bacteria enumeration and Rose Bengal Agar (Biomaxima) for fungal enumeration, respectively. The aliquots (100 µL) of serial dilutions (10^{-3}–10^{-5}) of soil samples were spread over the surface of Actinomycete Isolation Agar (Becton Dickinson, Franklin Lakes, NJ, United States). The media for the isolation of bacteria and actinomycetes were supplemented with cycloheximide (0.1 g L^{-1}), whereas chloramphenicol (0.1 g L^{-1}) for fungal isolation was used to prevent fungal and bacterial growth, respectively. The inoculated plates (5 replicates per dilution) were incubated at 28 °C for 2 weeks. The number of colonies was counted using colony counter LKB 2002 (Pol-Eko, Wodzisław Śląski, Poland) after 7 and 14 days of incubation. The number of microorganisms were expressed as log10 of colony-forming unit (CFU) per gram of dry soil.

2.3. Soil Enzymatic Activities

The dehydrogenase (DH) activity in soil samples was determined colorimetrically according to Furtak et al. [25]. Absorbance measurements of the triphenylformazan (TPF) at 490 nm were performed using the spectrophotometer Marcel Pro Eko (Warsaw, Poland). The urease (UR) activity was determined using the spectrophotometric technique according to Nakano et al. [26], modified by Kandeler and Gerber [27]. The absorbance at 420 nm was measured using the spectrophotometer Marcel Pro Eko (Poland). The acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were determined according to the method described by Tabatabai [28] and modified by Furtak et al. [25] using sodium p-nitrophenylphosphate (PNP). Absorbance at 410 nm was measured using the spectrophotometer Marcel Pro Eko (Poland). All analyses were performed in five replicates.

2.4. Metabolic Diversity of Soil Microbes

This diversity based on ability to oxidize carbon substrates was estimated using 96-well Biolog EcoPlates (Biolog Inc., Hayward, CA, USA), as described by Weber and Legge [29]. Biolog Ecoplates consisting of 31 carbon sources, including carbohydrates (10), carboxylic and acetic acids (9), amino acids (6), polymers (4), and amines (2), all in triplicate, were inoculated with 100 µL of suspension (dilution of 10^{-2}) of soil sample, incubated for 4 days at 28 °C and read for absorbance at a wavelength of 590 nm using a microplate reader Multiskan FC photometer (Thermo Fisher Scientific, Waltham, MA, USA). The changes in color from colorless to purple resulted from a reduction in water-soluble triphenyl tetrazolium chloride to triphenyl formazan, thus indicating the degradation of carbon sources. The average well color development (AWCD) was determined after the incubation time for individual plates using the method described by Garland and Mills [30].

2.5. Statistical Analysis

All biological and chemical parameters were measured using five repetitions. The Biolog Ecoplate-derived metabolic diversity indices, AWCD, variations in the impact of drought stress on carbon source utilization and heatmaps were analyzed using a Morpheus heatmap (https://software.broadinstitute.org/morpheus/, accessed on 13 January 2022). Statistical analyses were performed using a repeated-measures ANOVA test (analysis of variance). The declared level of significance is $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). The principal component analysis (PCA) was performed to assess variations in the impact of drought stress on analyzed parameters. All data prior to PCA analysis were centered and log transformed.

2.6. DNA Extraction, Amplicon Sequencing and Bioinformatics Analyses

All analyses were performed by Eurofins Genomics (Constance, Germany). The DNA was quantified fluorometrically (Qubit 2.0), and the quality was assessed spectrophotometrically (NanoDrop 2000, Thermo Scientific, USA). The V1–V3 and V3–V4 regions of the bacterial 16S rRNA genes were amplified using primers 5′-AGAGTTTGATCATGGCTCAG-3′ [31], 5′-GTATTACCGCGGCTGCTG-3′ [32], and 5′-TACGGGAGGCAGCAG-3′ [33], respectively. The ITS regions of eukaryotic ribosomal DNA were amplified with the ITS1 and ITS2 primers [34]. The PCR products were sequenced using the Illumina sequencing platforms with a paired end-run type, as per the instructions provided in the manufacturer's manual. A standard genomic library using UDI (unique dual indexing) was performed.

After determining DNA integrity and quality, DNA was prepared and sequenced at Eurofins Genomics (Constance, Germany) using an INVIEW Metagenome (eurofinsgenomics.eu) product. This included fragmentation, end-repair and dA-tailing, adapter ligation, size selection and library amplification. The prepared libraries were then quality-checked, pooled, and sequenced on an Illumina platform (Illumina NovaSeq6000, PE150 mode). Briefly, raw sequencing data were processed using fastp [35] software to remove poor-quality bases (below Phred Quality 20). The sequences were trimmed with a quality score threshold of ≤30, and those shorter than 250 bp were discarded. Manipulation of the FASTA/Q file was performed using a cross-platform and ultrafast toolkit SeqKit [36]. Taxonomic profiling was performed using MetaPhlAn [37] and the NCBI database for bacterial and fungal genomes. Unclassified reads were subjected to KrakenUniq [38] software, which performs confident and fast metagenomics classification, using unique k-mer counts. Kraken [39] classifies the reads by breaking each read into overlapping k-mers. A Vegan bioconductor package [40] was used to collect and normalize the read counts and compare species richness from all samples in the analysis run. Alpha-diversity (Shannon diversity index) was calculated at the genus level to show the relative bacterial and fungal diversity.

3. Results

3.1. Chemical Properties of Soil Samples

The chemical properties of four agricultural soil samples are shown in Table 1. The texture of the investigated soils varies from haplic luvisol in Suchatówka (S), which was most sandy, to stagnic luvisol in Lulkowo (L), fluvisol in Wielka Nieszawka (N), and gleyic luvisol (or luvic gleyic) Phaeozem in Gniewkowo (G), which were richer in clay. In addition, soil samples from the G location were rich in humus. The texture of the soils was as follows: 91–94% sand (2–0.05 mm), respectively; 5–7% silt (0.05–0.002 mm), respectively; and 1–2% clay (<0.002 mm), respectively. The total carbon and nitrogen content were not significantly affected in studied soil samples between the sampling day and the end of the prolonged drought stress (8 weeks), as given in Table 1.

The highest moisture content, observed on sampling day (T0), was reduced significantly after the 1st week (T1) of drought conditions in all soil samples (Figure 2). The most intense decrease in moisture content was observed in the sandy soil from the S site also at the end of the experiment (8 weeks, T8). The less intense reduction in moisture content was noted in fluvisol soil from the N site. The average moisture content in soil samples ranged from 12.23% to 20.40% at the collection date and from 0.96% to 8.75% at the end of the experiment, indicating a reduction in water content by 2.3, 2.9, 3.1 and 21.2 times in N, G, L and S soil samples, respectively.

Table 1. Soil chemical parameters. T0, collection date, T8; 8 weeks of drought.

Location	Abbreviation	Total Organic Carbon Content (%) (mean ± SD)		Total Nitrogen Content (%) (mean ± SD)		pH (mean ± SD)	
		T0	T8	T0	T8	T0	T8
1. Gniewkowo	G	0.97 ± 0.010	0.89 ± 0.010 [b]	0.112 ± 0.002	0.110 ± 0.002	6.96 ± 0.006	7.49 ± 0.01 [c]
2. Lulkowo	L	0.77 ± 0.006	0.80 ± 0.012	0.092 ± 0.001	0.087 ± 0.002 [a]	6.52 ± 0.012	6.70 ± 0.006 [c]
3. Wielka Nieszawka	N	1.10 ± 0.015	0.99 ± 0.511	0.140 ± 0.001	0.135 ± 0.001 [a]	6.69 ± 0.01	6.66 ± 0.01
4. Suchatówka	S	0.62 ± 0.010	0.60 ± 0.015	0.057 ± 0.001	0.052 ± 0.001 [a]	6.17 ± 0.015	5.77 ± 0.012 [b]

[a] ($p < 0.05$), [b] ($p < 0.01$), [c] ($p < 0.001$); standard deviation (SD).

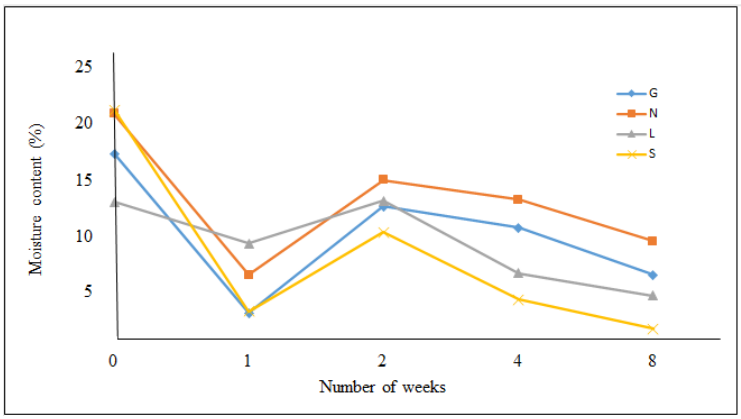


Figure 2. Soil moisture content under prolonged drought conditions in samples collected from Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S).

3.2. Influence of Prolonged Drought Stress on Number of Microorganisms

Generally, the number of bacteria was significantly higher than the number of fungi in all tested soil samples (Figure 3). The number of studied microorganisms, especially *Actinomycetota*, increased at the 1st week of induced drought stress. Further prolonged drought conditions decreased the number of bacteria, but not radically, and significantly increased the number of *Actinomycetota*, especially at the end of the experiment. The numbers of fungi during four weeks of the experiment were found to be like those at the sampling day, but in most cases, similarly to actinomycetes, they were the highest at the end of the experiment (Figure 3).

3.3. Effect of Drought Stress on Enzyme Activity

Overall, enzymatic activities varied during prolonged drought (Figure 4). Such conditions mostly negatively affected acid phosphatases activity in analyzed soil samples. The activity of acid and alkaline phosphatases was comparable in samples from the same location on the sampling day. In this study, the activity of alkaline phosphatase (ALP) was higher than that of acid phosphatases in corresponding soil samples. The ACP activity was strongly inhibited by drought in three soil samples, namely N, L and S sites. In turn, in the G soil sample, which was rich in organic matter content, the initial (1st week) drought conditions caused an increase in ACP activity and then a gradual decrease to be the lowest at the end of the experiment. Similarly, the ALP activity significantly increased together with a strong decrease in moisture content in the first week of drought stress and then decreased in all analyzed soil samples. A higher activity of dehydrogenases (DHs) was observed in soil samples with a higher amount of clay, especially those collected at G and N locations, which were less exposed to moisture loss than sandy soil from the S site. In the latter, the DH activity was significantly lower. Generally, DH activities in analyzed soil samples increased in the first month of induced drought and finally significantly decreased at the end of the experiment. The activity of urease (UR) varied in all analyses of

the soil samples, and its activity fluctuated during the drought period but was higher at the end of the experiment than on the sampling day (more prominent in the S soil sample).

Moreover, based on PCA analysis, we showed a positive correlation between the moisture and dehydrogenase activity (Figure 5), indicating the dehydrogenase enzyme as an indicator of drought compared to other biological parameters (Figure 5). The total variance explained by Axis 1 and Axis 2 was 85.82% (45.617% and 40.203%, respectively).

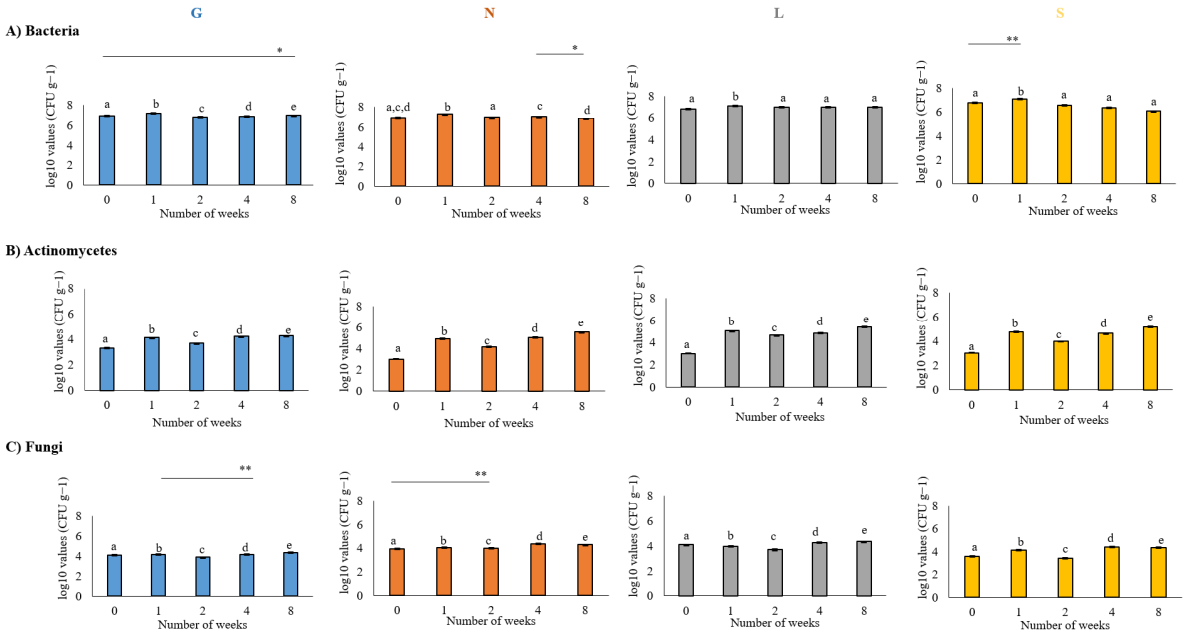
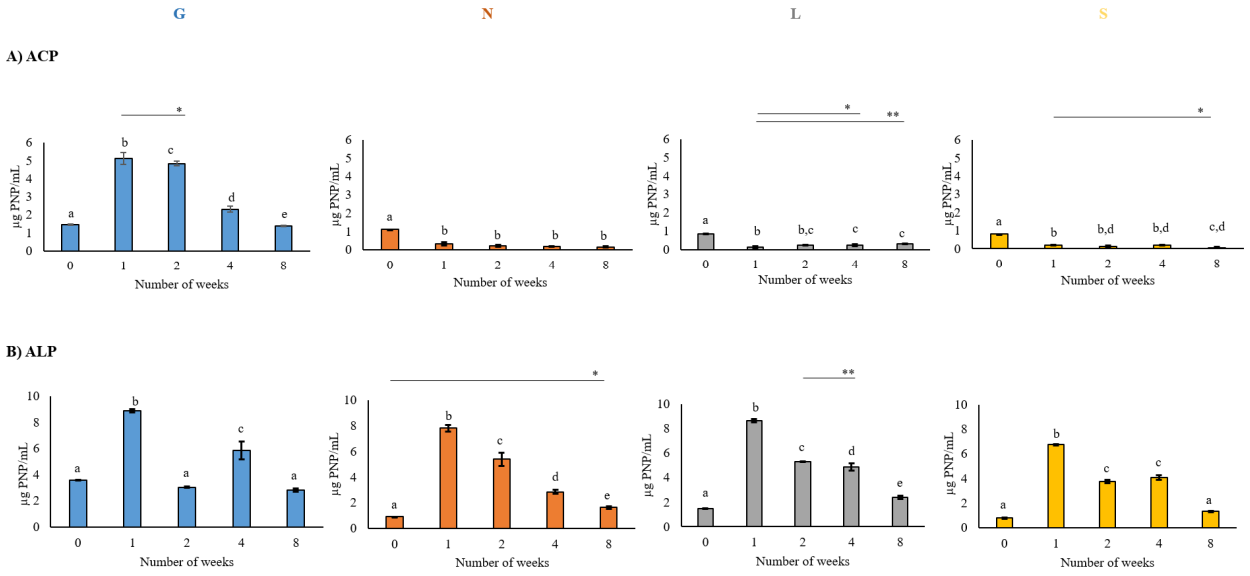


Figure 3. Changes in number of microorganisms under prolonged drought conditions in four agricultural soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). (**A**) Bacteria; (**B**) Actinomycetes; (**C**) Fungi. All analyses were performed in five replicates, and the data are presented as mean ± SD. All statistical analyses were carried out using repeated-measures ANOVA and Tukey test at $p < 0.001$ (* $p < 0.05$; ** $p < 0.01$). Mean values described with the same letters (e.g., aa, etc.) are not significantly different at $p < 0.001$. Error bars indicate standard errors of the mean (n = 5).



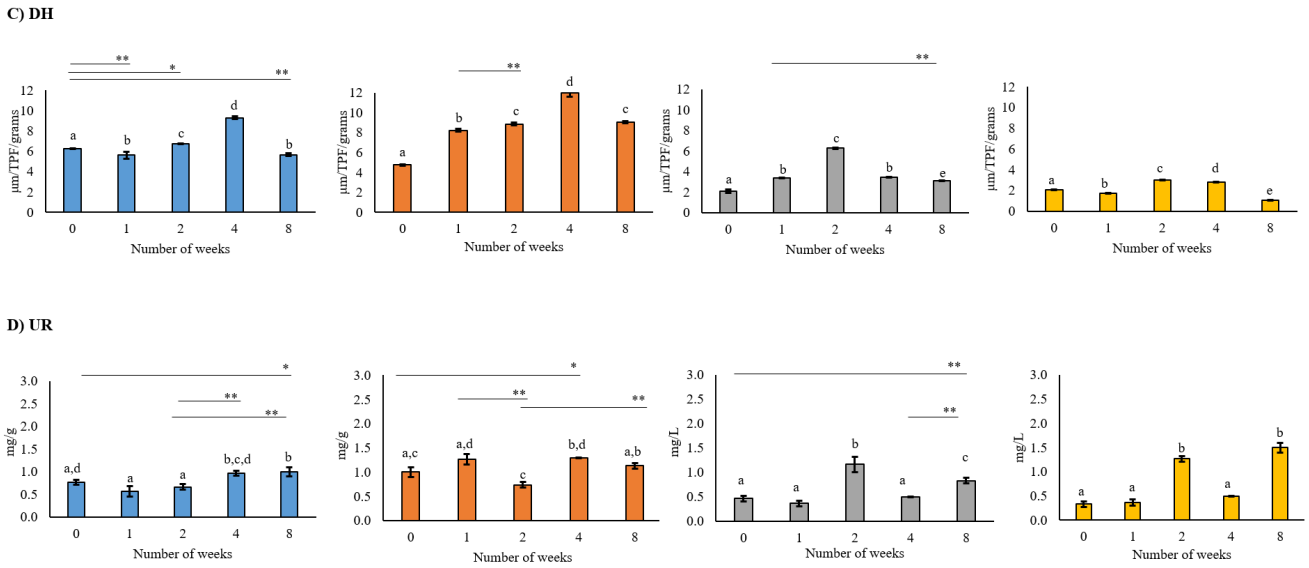


Figure 4. Enzyme activities under prolonged drought conditions in four types of agricultural soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). (**A**) Acid phosphatase (ACP); (**B**) Alkaline phosphatase (ALP); (**C**) Dehydrogenase (DH); (**D**) Urease (UR) enzyme activities. All analyses were performed in five replicates, and the data are presented as mean ± SD. All statistical analyses were carried out using repeated-measures ANOVA and a Tukey test at p <0.001 (* $p < 0.05$; ** $p < 0.01$). Mean values described with same letters (e.g., aa, etc.) are not significantly different at $p < 0.001$. Error bars indicate standard errors of the mean (n = 5).

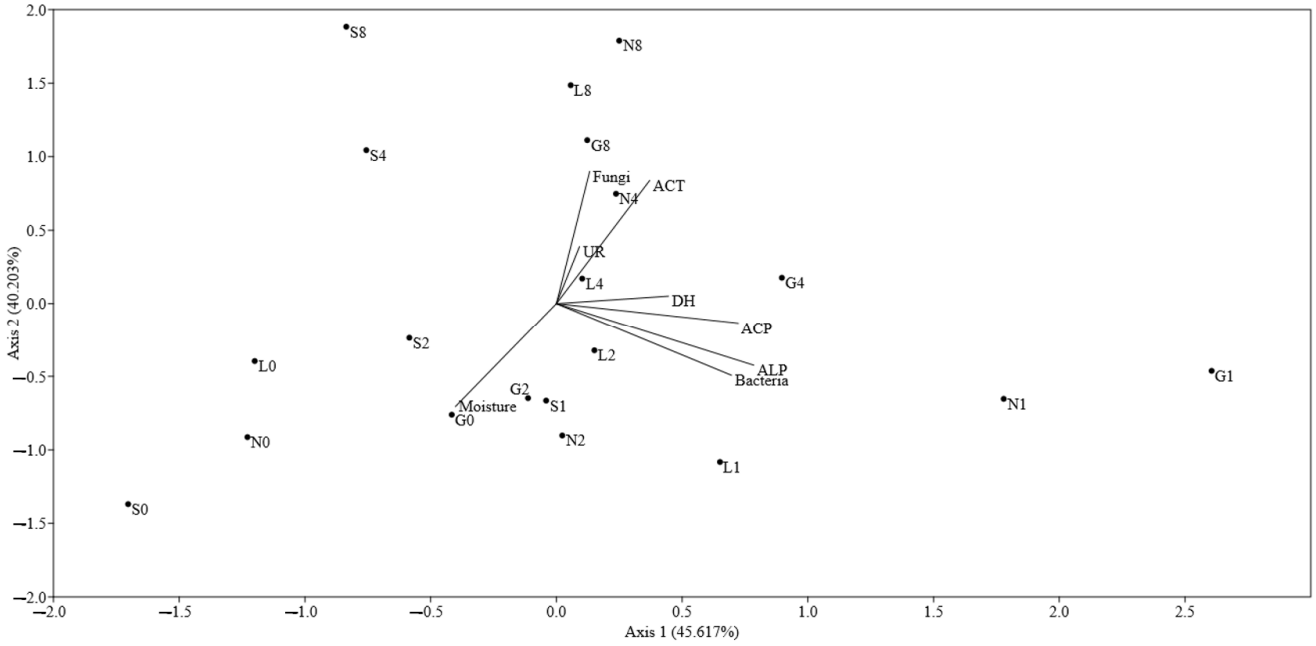


Figure 5. Principal component analysis (PCA) diagram indicating correlations between soil physicochemical and biological parameters in four soil samples at 0, 1, 2, 4 and 8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; ACP, Acid phosphatase; ALP, Alkaline phosphatase; ACT, Actinomycetes; DH, Dehydrogenase; UR, Urease; G0 (G at week 0); G1 (G at week 1); G2 (G at week 2); G4 (G at week 4); G8 (G at week 8) (likewise for L, N and S site).

3.4. Estimation of Community Level Physiological Profiling (CLPP) of Soil Samples under Drought Stress

In the present study, CLPP analysis revealed the different metabolic potential to substrate utilization by microorganisms in the soil samples under induced drought

conditions (Figures 6 and 7A–F). At prolonged water stress conditions, a decrease in the utilization of major carboxylic and acetic acids, amino acids, polymers, and amines, with the exception of one carbohydrate D-mannitol and one amino acid, L-asparagine, was observed.

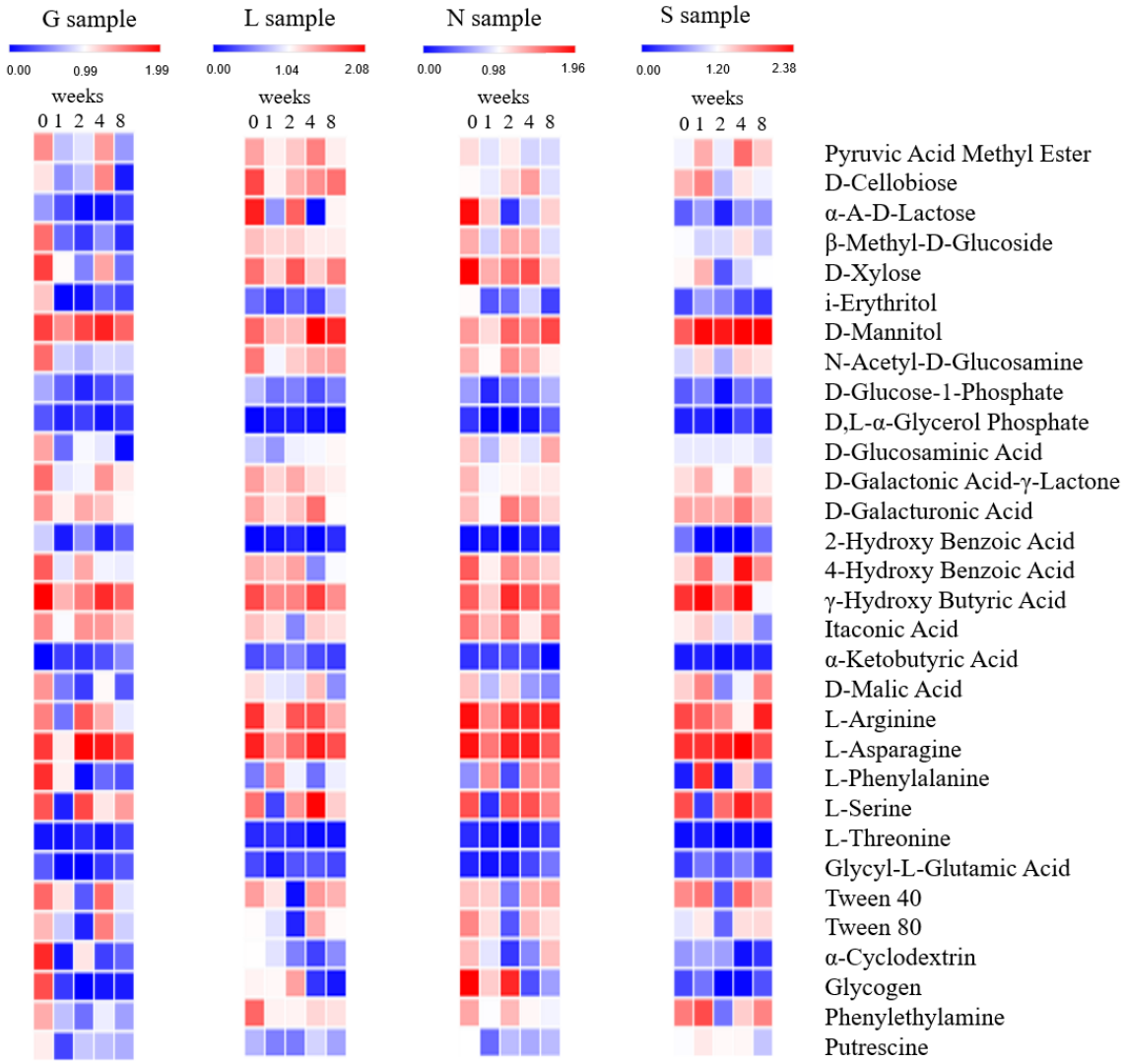


Figure 6. Heat map for community-level physiological profiles (CLPPs) in four types of agricultural soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)).

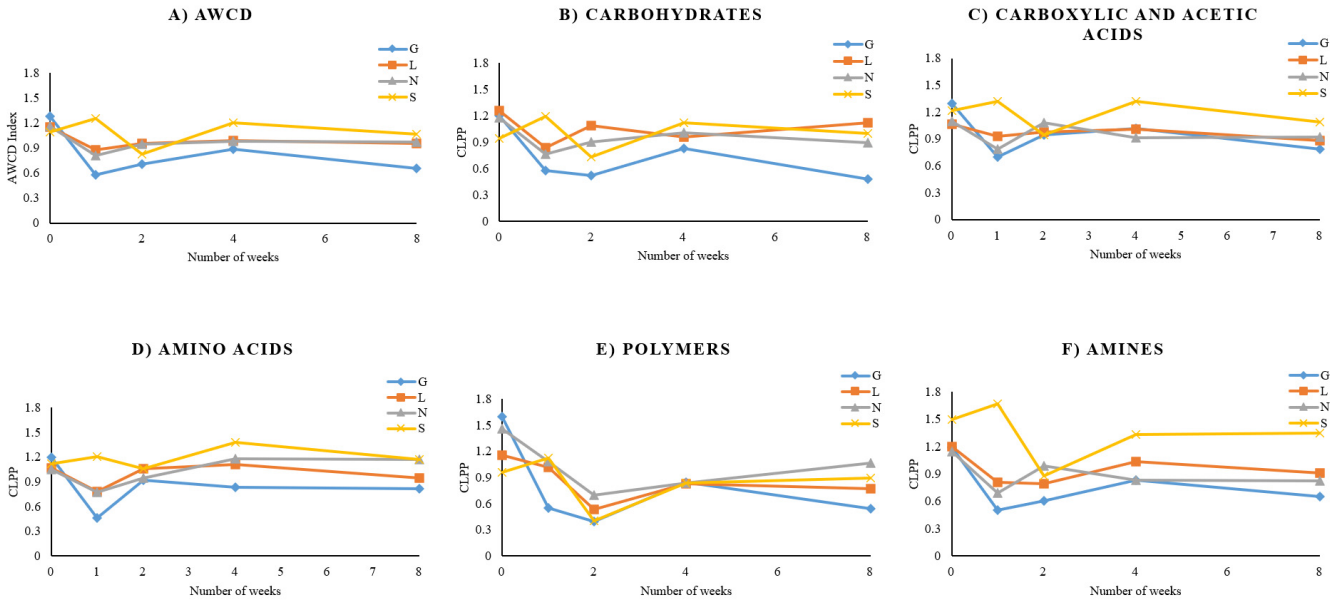


Figure 7. Absorbance values of Biolog Ecoplates in four types of agricultural soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) with carbon substrate utilization efficiency. (**A**) Average rate of the average well color development (AWCD) over the incubation time (ΔAWCD/weeks); Metabolism of (**B**) Carbohydrates; (**C**) Carboxylic and acetic acids; (**D**) Amino acids; (**E**) Polymers; (**F**) Amines.

In our experiment, CLPP analysis revealed the highest utilization of carbon substrates for the S site followed by N, L and G sites. Consequently, in the S sample, a decrease in the utilization of three carbohydrates (D-cellobiose, β-methyl-D-glucoside and D-xylose), two carboxylic and acetic acids (γ-hydroxy butyric and itaconic acids), a polymer (α-cyclodextrin) and an amine (putrescine) were recorded at the end of the experiment compared to the sampling day (Figures 6 and 7B–F). In turn, in the N sample, such a tendency was recorded for seven carbohydrates (pyruvic acid methyl ester, D-cellobiose, α-A-D-lactose, β-methyl-D-glucoside, D-xylose, i-erythritol and N-acetyl-D-glucosamine), three carboxylic and acetic acids (D-galactonic acid-γ-lactone, and 4-hydroxy benzoic and D-malic acids), an amino acid (L-serine), polymers (Tween 80 and glycogen), and amines. In case of the L sample, the weakness of the metabolism of five carbohydrates (pyruvic acid methyl ester, α-A-D-lactose, β-methyl-D-glucoside, N-acetyl-D-glucosamine and D-glucose-1-phosphate), six carboxylic and acetic acids (D-galactonic acid-γ-lactone, and D-galacturonic, 4-hydroxy benzoic, γ-hydroxy butyric, itaconic and D-malic acids), two amino acids (L-arginine and L-serine), polymers and amines was recorded at the end of the experiment compared to the sampling day. Finally, in the G sample, weak utilization was noted for eight carbohydrates (pyruvic acid methyl ester, D-cellobiose, α-A-D-lactose, β-methyl-D-glucoside, D-xylose, i-erythritol, N-acetyl-D-glucosamine and D-glucose-1-phosphate), eight carboxylic and acetic acids (D-glucosaminic acid, D-galactonic acid-γ-lactone, and D-galacturonic acid, 2-hydroxy benzoic acid, 4-hydroxy benzoic acid, γ-hydroxy butyric acid, itaconic acid, and D-malic acid), three amino acids (L-arginine, L-phenylalanine and L-serine), polymers and amines at the end of the experiment compared to the sampling day.

The AWCD values (representing carbon use intensities) were highest on the sampling day (T0) in all four soil samples and decreased with the decrease in water moisture levels at the 8th week. This can be correlated with a reduction in water content and bacterial community counts (Figure 7A). This analysis provides further evidence that prolonged induced drought led to an overall reduction in the metabolism of carbohydrates,

carboxylic and acetic acids, and amino acids, polymers, and amines in all soil samples at the 8th week, except for high-carbohydrate metabolism observed in the S soil sample (Figure 7B–F). Mid-water stress (after 4 weeks of drought) led to the reduction in the AWCD and weak utilization of substrates. Although microorganisms still utilized the carbon substrates under drought conditions, the patterns revealed slow degradation but not complete inhibition (Figure 7B–F).

3.5. Impact of Drought on Genetic Diversity of Bacteria and Fungi

A total of 1,120,019 to 1,657,588 and 1,344,712 to 1,931,231 bacterial 16S rRNA sequences were obtained through amplicon sequencing on the sampling day and after 2 months of drought conditions, respectively. The bacterial communities in the soil samples at the beginning (T0) and the end (T8) of the experiment were dominated by four phyla, namely *Actinomycetota*, *Bacteroidota*, *Bacillota* and *Pseudomonadota*, accounting for >95% of the total abundance of bacteria in all sites (Table 2A). *Pseudomonadota* and *Actinomycetota* were found to dominate in all analyzed soil samples constituting 57.65%, 63.84%, 59.08% and 60.83%, and 38.38%, 32.83%, 37.57% and 36.96% of bacterial population at the G, L, N and S sites, respectively. In contrast, *Bacteroidota* and *Bacillota* abundance was significantly lower, namely 1.14–3.14% and 0.83–1.21%, respectively, in all soil samples. The relative abundance of *Actinomycetota* and *Bacillota* increased, while that of *Pseudomonadota* and *Bacteroidota* decreased after prolonged drought conditions. The relative abundance of *Pseudomonadota* decreased at the end (8 weeks, T8) of our experiment in the corresponding samples by 12.74%, 17.13%, 6.7% and 12.46%, respectively. In contrast, the relative abundance of the phylum *Actinomycetota* increased after prolonged drought stress by 14.97%, 18.25%, 8.08% and 12.44%, respectively (Table 2A). The relative abundance of *Bacteroidota* was reduced in the analyzed soil samples after prolonged drought and was found to be in the range of 0.61–1.10%, while *Bacillota* increased in samples G, L and S (1.12–1.24%) and slightly decreased in the N sample, namely from 1.21% to 1.05% (Table 2A). On the other hand, the total bacterial sequences increased by 4.77% and 2.21% in the L and N sites, respectively, and decreased by 1.86% and 2.23% in the G and S sites, respectively.

Table 2. Relative abundance of bacterial and fungal taxa identified by 16S rRNA and ITS amplicon sequencing. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; 0; collection date; 8, 8 weeks of drought.

	Taxon	**Relative Abundance (%)**							
		G0	**G8**	**L0**	**L8**	**N0**	**N8**	**S0**	**S8**
A. Bacterial Phyla	*Pseudomonadota*	57.65	44.91	63.84	46.70	59.08	52.38	60.83	48.37
	Actinomycetota	38.38	53.36	32.83	51.09	37.57	45.65	36.96	49.40
	Bacteroidota	3.14	0.61	2.47	0.97	2.15	0.93	1.14	1.10
	Bacillota	0.83	1.13	0.85	1.24	1.21	1.05	1.06	1.12
Genera	*Bradyrhizobium*	26.70	28.16	29.93	33.61	48.51	46.87	36.97	24.63
	Streptomyces	17.33	19.38	12.18	20.66	12.25	16.56	12.14	17.42
	Sphingomonas	16.06	3.82	19.81	3.68	9.73	4.11	16.62	5.45
	Nocardioides	8.53	13.66	8.59	11.24	5.99	7.42	9.26	12.17
	Mycobacterium	6.22	8.22	7.16	8.91	6.73	8.01	4.42	15.46
	Micromonospora	4.44	7.07	2.72	4.28	3.03	3.75	3.58	4.56
	Lysobacter	6.67	2.18	7.48	1.74	5.01	1.74	5.38	2.58
	Solirubrobacter	3.11	4.96	2.82	4.38	3.18	4.07	2.69	3.44
	Actinoplanes	4.14	4.16	2.84	4.52	2.12	2.65	3.30	4.73
	Geodermatophilus	3.99	5.94	2.98	2.98	1.78	2.06	3.16	3.57
	Sorangium	2.80	2.44	3.49	4.01	1.67	2.77	2.47	6.00
B. Fungal Phyla	*Ascomycota*	83.48	82.58	83.46	83.22	83.27	85.23	81.54	83.76
	Basidiomycota	10.25	12.52	11.34	10.91	10.86	10.11	13.51	11.64

	Mucoromycota	3.57	2.82	2.79	3.95	3.31	2.18	2.11	2.86
	Apicomplexa	2.70	2.08	2.41	1.92	2.56	2.49	2.84	1.73
Genera	*Fusarium*	22.88	14.49	17.28	29.15	21.39	26.07	19.79	40.10
	Aspergillus	15.35	16.03	19.57	13.75	16.43	15.12	16.26	12.66
	Colletotrichum	5.99	8.61	5.76	6.16	7.36	5.76	8.39	5.02
	Trichoderma	3.88	4.85	5.19	5.02	14.09	12.49	3.37	2.40
	Penicillium	2.80	8.27	2.28	3.82	4.68	4.91	4.56	2.34
	Exophiala	4.73	4.85	2.68	6.67	2.74	3.99	3.65	3.14
	Ustilago	1.54	1.94	5.31	4.56	3.14	1.08	8.16	5.36
	Pseudogymnoascus	4.28	6.85	3.31	1.60	4.51	5.08	3.02	1.03
	Verticillium	3.42	2.62	11.87	3.02	2.05	1.43	2.85	1.94
	Chaetomium	7.24	2.68	1.60	1.31	1.65	2.00	2.80	1.43
	Lobosporangium	3.31	2.97	2.17	1.83	2.85	2.40	1.77	1.43
	Marssonina	2.17	3.54	2.34	1.88	2.28	2.00	2.91	0.80
	Metarhizium	1.25	3.02	1.48	1.77	1.54	1.71	5.19	1.48
	Anthracocystis	2.62	3.31	2.17	1.77	2.11	1.43	1.48	1.71
	Thermothelomyces	2.34	2.62	1.88	1.43	1.14	1.25	2.51	1.71
	Gaeumannomyces	1.71	1.83	1.77	1.65	1.08	1.48	1.83	3.08
	Rhizophagus	1.94	0.97	1.83	4.28	1.31	1.03	1.54	1.43
	Phycomyces	1.43	2.34	1.83	2.11	2.34	1.31	1.43	1.43
	Tilletiopsis	1.77	2.34	1.60	1.48	1.88	2.00	2.05	0.97
	Bipolaris	1.83	1.14	1.48	2.40	0.91	1.31	2.05	2.80
	Purpureocillium	4.45	2.00	0.63	0.97	1.77	1.88	1.48	0.74
	Pyricularia	2.05	1.94	1.54	1.48	1.60	1.65	2.34	1.14
	Alternaria	1.03	0.80	4.45	1.88	1.14	2.62	0.57	5.88

The investigated soil samples (G, L, N and S), at the beginning of the experiment, consisted of bacterial communities dominated by eleven genera, namely *Actinoplanes*, *Bradyrhizobium*, *Geodermatophilus*, *Lysobacter*, *Micromonospora*, *Mycobacterium*, *Nocardioides*, *Solirubrobacter*, *Sorangium*, *Sphingomonas* and *Streptomyces* (Table 2A). Genera *Bradyrhizobium*, *Streptomyces*, *Sphingomonas*, *Nocardioides* and *Mycobacterium* were the most abundant genera in the analyzed soil samples (34.4%, 16.0%, 9.9%, 9.6% and 8.1%, respectively), while others constituted < 4% of abundance (Table 2A). However, the abundance of these genera varied and showed different patterns depending on soil moisture fluctuations. The increase in abundance after prolonged drought was found for eight genera, namely *Mycobacterium* (by 8.22%, 8.91%, 8.01% and 15.46%), *Geodermatophilus* (by 5.94%, no significant difference in the L site, 2.06% and 3.57%), *Actinoplanes* (by 4.16%, 4.52%, 2.65% and 4.73%), *Micromonospora* (by 7.07%, 4.28%, 3.75% and 4.56%), *Nocardioides* (by 13.66%, 11.24%, 7.42% and 12.17%), *Streptomyces* (by 19.38%, 20.66%, 16.56% and 17.42%), *Solirubrobacter* (by 4.96%, 4.38%, 4.07% and 3.44%) and *Sorangium* (by 2.44%, 4.01%, 2.77% and 6%) in the G, L, N, and S sites, respectively. In contrast, the percentage of relative abundance of the genus *Sphingomonas* decreased at the 8th week of drought by 12.24%, 16.13%, 5.62% and 11.17%, whereas the genus *Lysobacter* decreased by 4.49%, 5.74%, 3.27% and 2.8% in the G, L, N, and S sites, respectively (Table 2A). Finally, the abundance of genus *Bradyrhizobium* decreased by 1.64% and 12.34% in the N and S sites at eight weeks, respectively, and increased by 1.46% and 3.69% in the G and L sites, respectively (Table 2A). The percentage of relative abundance of bacterial taxa living symbiotically with plant roots (*Bradyrhizobium*) in relation to the total number of bacterial taxa changed slightly during the study. The highest decrease in the relative abundance of symbiotic bacteria by 12% was observed in the S soil. In the remaining soil samples, the relative abundance of symbiotic bacteria was similar at the beginning and the end of the experiment (Table 2A). In case of alpha diversity, the drought treatment decreased the Shannon index of soil

bacterial communities significantly for only the L site ($p < 0.01$) but increased in the S site ($p < 0.001$) (Table 3). The result indicated that the distributions of bacterial alpha diversity (Shannon index values) were altered under drought conditions.

Table 3. Drought treatment effects on bacterial and fungal α-diversity measured as Shannon diversity indices between sites (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) at sampling day (0) and 8th week (8)).

	Sites							
	G0	**G8**	**L0**	**L8**	**N0**	**N8**	**S0**	**S8**
Bacteria	2.12	2.09	2.05	1.99 ^{b}	1.77	1.78	1.97	2.14 ^{c}
Fungi	2.72	2.81 ^{c}	2.68	2.60 ^{c}	2.62	2.57 ^{a}	2.73	2.33 ^{c}

^{a} ($p < 0.05$), ^{b} ($p < 0.01$), ^{c} ($p < 0.001$).

A total of 10,526 to 12,658 and 11,088 to 40,414 fungal ITS sequences were obtained through amplicon sequencing on the sampling day and after 2 months of drought conditions, respectively. The fungal communities in the soil samples on the sampling day were dominated by four phyla: *Ascomycota* (83.48% abundance), *Basidiomycota* (10.25% abundance), *Mucoromycota* (3.57% abundance) and *Apicomplexa* (2.70% abundance), as shown in Table 2B. In general, phylum *Ascomycota* was the most abundant in all sites, where their abundance slightly increased by 1.96% and 2.22% in the N and S sites, respectively, but decreased by 0.9% and 0.24% in the G and L sites, respectively, after 2 months of drought conditions (Table 2B). Phylum *Basidiomycota* was found to increase by 2.27% in the G site, but it decreased by 0.43%, 0.76% and 1.87% in the L, N and S sites, respectively, at the end of the experiment (Table 2B). Similarly, the relative abundance of phylum *Mucoromycota* increased by 1.16 and 0.76% in the N and S sites but decreased by 0.76% and 1.14% in the G and L sites, respectively, at the end of our experiment (Table 2B). The lowest abundance among recognized phyla was *Apicomplexa*, which showed a reduction under prolonged drought stress by 0.62%, 0.50%, 0.07% and 1.11% in the G, L, N and S sites, respectively (Table 2B). On the other hand, the total fungal ITS sequences increased by 23.41%, 1.72%, 3.42% in the G, L and N sites, respectively, and they decreased by 0.03% in the S site.

A total of 23 genera consisting of Fusarium, Aspergillus, Colletotrichum, Trichoderma, Penicillium, Exophiala, Ustilago, Pseudogymnoascus, Verticillium, Chaetomium, Lobosporangium, Alternaria, Marssonina, Metarhizium, Anthracocystis, Thermothelomyces, Gaeumannomyces, Rhizophagus, Phycomyces, Tilletiopsis, Bipolaris, Purpureocillium, and Pyricularia were noted (Table 2B). Among the fungal communities, the most dominated genera were Fusarium (23.89% abundance) and Aspergillus (15.64% abundance), whereas others constituted < 8.16% of fungal genera abundance (Table 2B). The relative abundance of the genus Fusarium increased by 11.87%, 4.68% and 20.31% in the L, N and S soil samples, but it decreased by 8.39% in the G soil sample, respectively, after 2 months of drought (Table 2B). In contrast, the relative abundance of the genus Aspergillus increased by 0.68% in the G site and declined by 5.82%, 1.31% and 3.59% in the L, N and S sites, respectively, after 2 months of drought (Table 2B). Overall, the higher reduction in the relative abundance of genera after 2 months of drought was observed in the S site (in case of Aspergillus, Exophiala, Chaetomium, Colletotrichum, Lobosporangium, Marssonina, Metarhizium, Penicillium, Phycomyces, Pseudogymnoascus, Purpureocillium, Pyricularia, Rhizophagus, Thermothelomyces, Tilletiopsis, Trichoderma, Ustilago and Verticillium) and the L site (in case of Alternaria, Anthracocystis, Aspergillus, Chaetomium, Gaeumannomyces, Lobosporangium, Marssonina, Pseudogymnoascus, Pyricularia, Thermothelomyces, Tilletiopsis, Trichoderma, Ustilago and Verticillium) when compared to the G site (for Alternaria, Bipolaris, Chaetomium, Fusarium, Lobosporangium, Purpureocillium, Pyricularia, Rhizophagus and Verticillium) and the N site (for Aspergillus, Anthracocystis, Colletotrichum, Lobosporangium, Marssonina, Phycomyces, Rhizophagus, Trichoderma, Ustilago and Verticillium), as shown in Table 2B. The average

percentage of relative abundance of mycorrhizal fungi (Rhizophagus) in relation to the other taxa participating in the decomposition of organic matter is less than 2%. The former abundance decreased by <1% in the G, N and S sites, but it increased by 2% in the L site at the end of the experiment. We did not observe significant changes between the sampling day and the end of the experiment (Table 2B). The drought treatment decreased the Shannon index of soil fungal communities significantly in three sites (L ($p < 0.001$), N ($p < 0.05$) and S ($p < 0.001$)) but increased in the G site ($p < 0.001$) (Table 3). The results indicate the alteration of fungal alpha diversity (Shannon index) when the soil was subjected to water stress conditions.

4. Discussion

Drought conditions alter the structure, abundance, and activity of microbial community in soils [4,6]. Under a low water amount, drought-sensitive microorganisms release substrates upon death into their surroundings, making it available for other drought-tolerant microbes [4,6]. However, under prolonged dry conditions, the abundance of active microorganisms may significantly decrease, leading to a reduction in soil enzyme activities and thereby lowering the nutrient mineralization (carbon, nitrogen, and phosphorus) and respiration [4,6]. Microbes either diminish or acquire a dormant phase when exposed to stressful environments, but the dormant ones regain their activity after the onset of favorable conditions. Therefore, understanding the impact of drought on microorganisms is critical for predicting the rates of decomposition and nutrient cycling in soils [41].

4.1. Soil Chemical Parameters

Soil chemical and nutrient properties can influence the overall biological structure of soil. Therefore, any changes in soil properties under drought conditions can have a significant impact on the ecosystem [42]. In this study, the chemical properties of the investigated agricultural soil samples largely varied under drought conditions. The soil moisture content was low at the end of drought conditions compared to the sampling day (Figure 2). However, between T1 (1st week) and T2 (2nd week), a significant rise in moisture levels in all the analyzed samples was observed (Figure 2). This could be related to rainfall and high humidity (>93%) on 12th June 2021 just before the sample collection at T2 (https://www.timeanddate.com/weather/poland/torun/historic?month=6&year=2021, accessed on 27 November 2022).

The soil sample from the S location, due to the highest sand and lowest organic carbon contents, was exposed to higher water loss. The total organic carbon and total nitrogen contents were slightly lower at the 8th week compared to the sampling day under drought stress conditions, but this decline was not significant (Table 1). Our results are in line with those by Zhang et al. [43] who observed no significant decrease in the organic carbon and total nitrogen contents after 2 years of drought stress in semi-humid forests, indicating that drought conditions slow down the transformations of carbon and nitrogen in soils. Such impacts may slowly decrease soil functionality, reducing the availability of soil nutrients to plants [41]. Interestingly, the highest increase in soil pH was observed in sandy soil and loamy soil with the highest organic matter content (Table 1). However, these changes were not considerable. Similar observations were reported by Siebielec et al. [6] in agriculture loamy, sandy, and sandy amended with compost soil samples under high drought conditions. The pH values of loamy soil were maintained at similar levels during prolonged drought up to 8 weeks, while in sandy soil, the pH slightly increased after the first period of drought (from 6.57 to 6.83) and then decreased at the 8th week of drought (6.43) (Table 1). In sandy soil amended with compost, a similar tendency (6.57, 6.73 and 6.67, respectively) was recorded [6].

4.2. Culture-Dependent and Culture-Independent Characterization of Microorganisms

The results of the present studies are in line with those previously published showing a negative influence of drought on the number of microorganisms in the soil environment and changes in the structure of cultivable microorganisms [6,44]. An increase in the abundance of spore-forming microorganisms, especially Gram-positive, spore-forming actinomycetes, unlike Gram-negative bacteria, in soil during drought conditions has been reported previously [6]. The high increase in the number of actinomycetes for two months' drought stress could balance the decrease in the remaining bacteria, and therefore, the decrease in the number of all cultivable bacteria was not significant (Figure 3). In fact, the total bacterial sequences were higher after 2 months of drought conditions compared to the sampling day (Table 2A), using the metagenomics approach [45,46].

Overall, a significant decrease in the abundance of bacteria during drought conditions in the Mediterranean forest was found based on culture-independent studies [47]. Similarly, a lower abundance of bacteria was noted in two types of agriculture soil in Poland during prolonged (8 weeks) induced heavy drought maintained under controlled conditions [6].

The biodiversity and relative abundance of specific taxa of bacteria obtained from culture-independent studies precisely indicated the influence of water availability on these microorganisms. Prolonged drought conditions (2 months) lowered the relative abundance of *Pseudomonadota* (dominant at the sampling day) and *Bacteroidota*, and they increased *Actinomycetota* and *Bacillota* compared to the sampling day (Table 2A). Thus, our results from culture-independent studies follow other findings showing an increase in the abundance of *Actinomycetota* and *Bacillota* in soils under drought conditions [6,45,46,48,49] and decrease in *Pseudomonadota* and *Bacteroidota* (Table 2A) [6,45]. Moreover, Siebielec et al. [6] showed a significant decrease in the relative abundance of *Verrucomicrobia* (now *Verrucomicrobiota*; https://lpsn.dsmz.de/phylum/verrucomicrobia-1, accessed on 17 May 2023) in such conditions.

The increase In the abundance of *Actinomycetota* and *Bacillota*in analyzed soil samples by the end of 2 months of induced drought stress could be due to the spore-forming ability present in the members of these taxa, thus making them more resistant to both desiccation and harsh environments. Among *Actinomycetota* communities, possibly the growth of desiccation-tolerant taxa was favored under soil water deficit conditions [48]. Similarly, Veach et al. [50] showed a higher abundance of phylum *Firmicutes* (now *Bacillota*) under drought stress in soils, which is in line with our results (Table 2A). Actinomycetes contain adaptive mechanisms toward drought such as the utilization of recalcitrant carbon sources in nutrient-poor soils and are present in great abundance in arid soils [51]. They can grow at a minimum osmotic potential with an increase in abundance in dry soils. This could be because their spores can generally withstand, grow, and dominate in dry environments. In addition, they contain genes for complex carbon degradation and are resistant to desiccation [46,51]. Other general mechanisms that help them to sustain growth under droughted conditions consist of sporulation and thick cell walls characteristic of Gram-positive taxa, biofilm formation, and the production of osmolytes (amino acids and carbohydrates) [10,52].

In addition, prolonged drought stress may affect bacterial abundance at the genus level [6]. In the present study, genera *Bradyrhizobium* followed by *Streptomyces* were found to be most abundant at the beginning of the experiment, and prolonged drought increased their richness in the analyzed soil samples (Table 2A). In contrast, the highest decrease in abundance was found to be in *Sphingomonas* and *Lysobacter* (both Gram-negative) (Table 2A). Furthermore, there is a lot of evidence of decline in bacterial and fungal richness under drought conditions [49,53,54]. This reduction in the total bacterial biomass under drought conditions could result from limited access to resources such as plant litter [15]. There are different reasons for the shifts in soil bacterial community composition under drought conditions that may verify their unique drought stress sensitivities. Gram-positive and Gram-negative bacteria harbor different substrate utilization and metabolic

potential. Therefore, the former is metabolically stable compared to the latter. In addition, Gram-positive bacteria can synthesize extracellular enzymes by utilizing inorganic nitrogen to break down complex organic compounds available in abundance in droughted soils, while Gram-negative bacteria prefer the utilization of labile carbon compounds and organic nitrogen from plant root exudates [55]. Under drought conditions, due to the very low availability of labile organic carbon in soil, it becomes difficult for the survival of Gram-negative bacteria [56]. Moreover, the lysis of sensitive bacterial cells results in the release of substrates that possibly act as an energy source for the drought-resistant microorganisms [57]. Therefore, due to microbial death and undergoing dormancy under drought soil conditions, the overall bacterial activity decreases [15].

Fungi are essential in the functioning of soil ecosystem, as they are one of the major contributors toward efficient biogeochemical cycles. They help in the decomposition or mineralization of organic matter from plant-available nutrients, thus contributing to the stability of soil organic carbon pools [58]. It should be highlighted that reports on the impact of prolonged drought stress on fungi in agricultural soils are limited (Figure 3). Although the numbers of fungi in analyzed soil samples varied during the experiment period, their increase in abundance was observed at the 8th week of prolonged drought stress compared to the sampling day (Figure 3). The resistance of fungal communities to drought stress was previously reported [59–61]. However, some studies are in contradiction to our findings, showing a negative influence of drought on soil fungi, as exemplified by studies of Ochoa-Hueso et al. [62] on mesic ecosystems and Hayden et al. [10] on grassland soil, and causing alterations in functional and compositional changes [63]. The variation of fungal response toward drought may develop based on the environmental structure, thus revealing the sensitivity of fungal communities toward harsh conditions in agricultural soil and mesic environments compared to other ecosystems [53]. During alterations in the soil moisture content, the dominant fungi adapted to previous moisture content may have reduced because the fungal community presents a specific composition depending on soil moisture [64]. Hence, it is difficult to predict the response of fungal communities under dry conditions, which makes this area of study lagging with incomplete understanding [65].

In this study, the fungal community was dominated by *Ascomycota* and *Basidiomycota* (Table 2B), as previously reported in most dryland, semi-arid grasslands, and agricultural soils [6,59,66,67]. Both phyla constituted 81.54–83.48% and 10.25–13.51% on the sampling day, and 82.58–85.23% and 10.11–12.52% during the 8th week of prolonged drought stress in four soil samples, respectively (Table 2B). Our results are in line with the findings of other authors who also reported a high relative abundance of *Ascomycota* and *Basidiomycota* in different soil habitats, such as grasslands (56% and 17%), mixed grasslands (54% and 25%), mixed woodlands (62% and 21%) and woodlands (58% and 24%) [66], agricultural soil 62–89% (*Ascomycota*) [59], drylands (89.3–93.5% and 2.6–6.3%) [68], and semi-arid ecosystem (91.88% and 7.27%) [67], respectively. Their ability to form extensive networks of hyphae makes them tolerant to low moisture environments via accessing nutrients and water from long distances [69]. However, a disturbance in nutrient diffusion in low water conditions can promote the expansion of soil hyphal networks [11]. It is known that fungi can preferentially live in large pores of the soil that are filled with high moisture levels but are void at water deficit conditions. Therefore, these large pores inhabit a reduced relative abundance of fungal taxa in dry conditions, while the abundance of other microbial populations could increase [70]. The occurrence of soil moisture shifts also results in a decline in the activity of dominant fungal communities that are previously adapted to moisture content, resulting in having a weak competitive ability against other fungal populations [71]. This explains the disproportion of fungal communities between the zero-sampling day and after the 2 month drought period in all sites. On the other hand, we found an overall highest relative abundance of the genus *Fusarium*, which includes numerous plant pathogens of important agricultural lands and are known to cause

dangerous lethal effects on crops. This genus is also responsible in the production of greenhouse gas nitrous oxide (N_2O) [11].

4.3. Impact of Drought on Soil Enzyme Activities

Generally, in water stress conditions, microbial enzyme activities slow down or completely decrease due to the lack of sufficient substrates, uneven diffusion of substrates, and accumulation of inhibitory osmolytes or ions toward enzymes. This leads to the alteration of their functioning and affects the decomposition of soil organic matter (SOM) [21]. DH is the most important soil enzyme which plays a vital role in the biological oxidation of SOM and the carbon (C) cycling in this biome [21]. DHs exist only in viable microbial cells; they do not accumulate in the soil, and therefore, they can be used as an indicator of the overall soil microbial activity, including the influence of abiotic stress on such activity [21]. Dehydrogenase activity is influenced by water content and decreases with the reduction in soil humidity [21]. Li et al. [72] found that an increase in aridity caused a decrease in DH activity in humid, mildly arid, and arid Mediterranean soil samples. However, DH activity is also positively correlated with the SOM content, which provides nutrients for microbial biomass and affects higher enzyme production [21]. Therefore, a higher dehydrogenase enzyme activity was found in loamy soils, especially in the G and N samples than in sandy soil samples (the S samples), even if the moisture content decreased for prolonged drought stress (Figure 4). Moreover, an increase or similar abundance of microorganisms was observed for the first month of drought stress (Figure 3). Prolonged 8-week stress finally decreased the abundance of bacteria and significantly decreased the DH activity (Figure 4). Similar observations were reported by Siebielec et al. [6] who showed an increase in DH activity in highly stressed loamy soil after one month and its significant decrease after two months of drought, while in sandy soil, this activity was similar during all periods of the experiment. Thus, soils exposed to drought or changes in soil water levels are critical in determining groups of physiologically active soil microorganisms [73].

Microorganisms are a prime source of soil phosphatases activity in the bulk soil [74]. Levels of soil phosphatase in the soil depend on various factors such as organic material content, microbial counts, tillage, organic and mineral fertilizers, and other agricultural-related practices [75]. Moreover, soil pH could determine phosphatase activity. Acid and alkaline phosphatase activity are higher in acid and alkaline soils, respectively [75]. As the pH of soil samples was slightly acidic and even increased after prolonged drought stress (Table 1), it was expected that the activity of acid phosphatases would be low. This situation was confirmed in three soil samples collected from the L, N and S soil samples (Figure 4). On the other hand, the G site showed higher ACP activity compared to the other sites during 2 months of induced drought stress (Figure 4). This could be due to the presence of high humic substances in the G site [76] that binds and protects the enzyme from heat degradation. In general, ALP activities are correlated with soil water content [20]. ALP activities due to propitious pH values of analyzed soils were higher than ACP activities (Figure 4). The increase in the alkaline phosphatase activities in the first week of drought stress, in which the highest decrease in moisture content was observed, could result from the increase in the number of microorganisms, especially actinomycetes (Figure 3). A subsequent decrease in this enzyme activity reflects the negative impact of prolonged drought on the activity of the microbial community and can affect the alteration of P cycles by water stress conditions [20]. The reduction in rhizosphere alkaline phosphatase activity due to water stress conditions was also proved by other researchers [77]. Reports on phosphatase activity in agriculture soils under drought stress are limited. Our results show similarity with other studies. Sardans and Peñuelas [20] confirmed that a reduction of 21% of soil moisture reduced acid phosphatase activity by 31–40% (pH 6.5) in the Mediterranean forest. Huang et al. [78] showed a decrease in acid phosphatases in the dry season ($p < 0.01$; 1.33 times lower) (4.06 (Masson pine forest), 3.82 (coniferous and broadleaved mixed forest) and 3.67 (monsoon evergreen broadleaved forest)) compared to the wet

season (4.14 (Masson pine forest), 3.95 (coniferous and broadleaved mixed forest) and 3.67 ($p < 0.05$; monsoon evergreen broadleaved forest)). According to Siebielec et al. [6], acid and alkaline phosphatases activities in loamy and sandy agriculture soils were negatively affected after one month under severe drought stress, and then its activities highly increased after two months of such stress conditions when compared with the sampling day. However, these activities were found to decrease when compared with control samples maintained under optimal conditions of 60% of field water-holding capacity. Under drought stress, the mineralization of P is affected due to the inactivation of microbial decomposers or accumulation of solutes or organic P on the upper soil layers [79]. Such water stress environments restrict the diffusion of the enzyme, substrates, and products, affecting the uptake of nutrients and results in a negative impact on soil microbial activity, microbial biomass, and plants [80]. Therefore, phosphatase serves as an indicator for the presence of inorganic phosphorus for microorganisms and plants [81].

The UR enzyme is the most important soil enzyme in the functioning of nitrogen (N) cycle in soil [20]. Although fluctuations in the UR activity were observed under the drought period, at the end of the experiment, its activity was found to increase when compared with the sampling day in all analyzed soil samples (Figure 4). Our results are in line with findings by Ng et al. [82] which showed that drought did not affect the soil urease activity. However, Sardans and Peñuelas [20] revealed that a reduction of 10% and 21% of soil moisture decreased UR activity by 10–67% and 42–60%, respectively, in Mediterranean forest soil, revealing the link between dry conditions and slower nutrient turnover. Although a drop in moisture content in the analyzed samples ranged from 12.23% to 20.40% in the analyzed soil samples (highest in the S soil sample) (Figure 2), the significant decreases in UR activity were not noticed (Figure 4). No effect of drought on the inhibition of urease activity could be due to the interaction of enzymes with clay and humic substances, helping the microorganisms retain functional levels of activity even under prolonged drought conditions [83]. Hence, the determination of UR activity can be useful in monitoring microbial metabolism, N cycling and soil fertility.

An overall decrease in the soil enzymes activities, which are responsible for regulating P, N and C nutrient cycles, in the stressed soils indicates that soil nutrients might be altered under such conditions, thus altering soil nutrient availability and reducing the nutrient supply to plants [20]. Our study reveals direct or indirect alterations in soil microbial abundances and communities, nutrient cycling, and enzymes under dry conditions ultimately hampering the soil quality and productivity.

4.4. CLPP Analysis under Drought Conditions

Biolog EcoPlates are useful in the evaluation of changes in microbial community structure and soil respiration [84]. This assay is helpful in revealing the functional profile of potential microbial communities in biological soil samples [84]. Biolog EcoPlates have been a rarely explored tool for studying the response of microbial communities to drought conditions. In our study, we observed a strong inhibition of microbial functions in two months of drought conditions. The highest metabolic diversity in soil samples was observed on the sampling day and decreased simultaneously up to 2 months under drought conditions (Figures 6 and 7A–F). The different substrate utilization potential of soil microbial communities under 2 months of drought conditions indicates their diverse metabolic capacity [85]. AWCD reveals variations in soil respiratory activity depending on the preference of substrate utilization by microbes [86]. The data show a decrease in the amount and rate of substrate utilization after 2 months compared to the sampling day under induced drought conditions (Figures 6 and 7B–F). In addition, a linear relationship between the concentration and physiological state of the microorganism and soil moisture levels with respect to AWCD rates was observed. It was noted that the metabolic activity of soil microbial species decreased with the decrease in soil water status [63].

This suggests that a change in the soil water availability is critical in discriminating the microbial abundance and physiologically active types of microbes [4,16,72,87].

According to Preece et al. [16], a great negative influence of drought on microbial community physiological profiles in soil was observed. It could be the result of a decrease in the number of microorganisms and microbial activity [4]. Our results are in line with the above findings, revealing a decrease in substrate utilization in response to drought in different soil ecosystems. Although our study shows different effects in CLPP between the soil samples, it confirms that a decrease in soil moisture status leads to a decrease in the overall soil respiration potential.

Although short-term drought had a significant effect on soil properties and microbial communities among the four sites, there may be other limitations in our study. At the time of sampling, there were no crops on all sampling sites, which was the effect of a very cold spring and delayed vegetation season in 2021 in Poland. All sites were not fertilized by organic fertilizers, and the last fertilization process was carried out in the autumn season in the previous year, and therefore, our samples were devoid of manure. Further contributing factors need to be also considered to understand the impact of water deficit conditions on agricultural soils. Some of such factors include the effect of plant-derived inputs (e.g., litter fall and root biomass), temperature, fertilizers, different crops, irrigation, and other agricultural practices.

5. Conclusions

This study showed the significant impact of prolonged induced drought stress on soil water content, microbial community, their enzymes, and metabolic diversity. Here, we provide evidence that the soil chemical properties, microbial abundance, dehydrogenase, phosphatase and urease enzyme activities, and overall metabolic diversity are sensitive to water stress conditions in the tested soil samples. Our results suggest that the reduction in soil enzyme activities can affect soil nutrient availability (i.e., phosphorus, nitrogen, and carbon), leading to obstruction of the nutrient supply to plants. After 2 months of induced drought conditions, we observed that the sandiest soil from Suchatówka (S) had the lowest moisture content and enzyme activities compared to soil samples from Lulkowo (L), Wielka Nieszawka (N) and Gniewkowo (G) that were richer in clay. Overall, an increase in the number of actinomycetes and fungi, with no significant changes in total bacterial numbers, was observed in all sites at the end of our experiment compared to the sampling day but with fluctuations in one month of induced drought stress. For enzyme activity, the decrease in overall phosphatase activity (acid and alkaline), dehydrogenases activity, and increase in urease activity was observed at the end of the experiment compared to the sampling day. The acid phosphatase activity was the most sensitive to drought conditions compared to other analyzed enzymes, with the overall lowest enzyme activities in site S. In case of metabolic diversity analysis, a decrease in the average well color development (AWCD) values was observed with a decrease in soil moisture content and overall reduction in the utilization of carbon sources, apart from D-mannitol and L-asparagine, in all sites. This may indicate substantial shifts both in the microbial community composition and metabolic diversity in our investigated soil samples. Our study found differences in the soil microbial community composition on the sampling day and after 2 months of induced drought conditions in agricultural soils by evaluating both fungal and bacterial taxa via amplicon sequencing. In general, we found a reduction in the abundance of *Pseudomonadota*, greater abundance of drought-resistant bacteria (*Actinomycetota* and *Bacillota*), lower abundance of *Basidiomycota* and *Apicomplexa* and high abundance of *Ascomycota*. This pattern suggests that microbial communities may respond differently to drought along moisture gradients, and fungal populations were more sensitive to drought in these agricultural lands compared to bacteria. The genetic diversity of bacteria and fungi reflects the importance of soil moisture levels in improving the ability of microbes to access nutrients and enhance their motility. Moreover, climate change scenarios can create new insights into the response of microbial communities under drought conditions. Thus, knowledge about their response to global climate change is of fundamental importance and should be used in building mitigation techniques.

Our understanding of the links between the direct effects of climate change (drought) on microbial community changes, their enzymes and metabolic diversity are vital to mitigate negative impact on agricultural soils. The limited knowledge on extreme weather events makes it difficult to deal with their harmful effects on ecosystems. Perhaps the implementation of various strategies to escape from drought conditions needs to be initiated in drought-sensitive regions. For example, water ponds and tank-fed watersheds, integrated in situ soil and water conservation practices, groundwater recharge, decrease in greenhouse gases at the field level, afforestation, mulching, optimum fertilizers and manures application, and many other management practices could be a remedy in several arid and semi-arid regions. Hence, this comprehensive study on the impact of prolonged water stress generates new insights about the modification of the soil microbiome, their enzymes and the metabolic potential in an ecosystem. Future research is important to precisely understand the shift of microbiological-derived soil functioning at the genetic diversity level under drought conditions. This knowledge maybe possibly be utilized to build a potential bridge between current issues and mitigation processes resulting from drought conditions.

Author Contributions: M.W. conceptualized the study. M.W. and P.G. supervised the study. K.A.B. performed all studies related to microbial isolation, enumeration and activity and analyzed the data. K.A.B. and P.S. were responsible for soil parameters analyses. K.A.B. and P.G. were responsible for the original draft preparation. P.G., M.W. and A.B.-B. reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

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PUBLICATION III

Effect of changes in soil moisture on agriculture soils: response of microbial community, enzymatic and physiological diversity

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Effect of changes in soil moisture on agriculture soils: response of microbial community, enzymatic and physiological diversity

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Abstract: Global warming-induced drought stress and the duration of changes in soil moisture content may reshape or complicate these ecological relations. Biological activity could be affected severely by the impact of drought on agricultural ecosystems. In this study, 4 agricultural different soils were collected, and analyzed at each time gradient (T0, T1, T2, T4, T8th week) to determine the physicochemical parameters, microbial abundance, enzyme activities (dehydrogenases (DH), phosphatases (acid ACP and alkaline ALP) and urease (UR)), and physiological diversity. These four types of soil were selected based on differences in their texture and bonitation classification (gleyic luvisol Phaeozem in Gniewkowo (G) (rich in clay and humus, 1st class), stagnic luvisol in Lulkowo (L) (3rd class), fluvisol in Wielka Nieszawka (N) (3rd class) and haplic luvisol in Suchatówka (S) (sandy, 5th class). This study showed that soil physicochemical properties fluctuated within the time gradient in all sites, but significantly decreased in total organic carbon (TOC), available phosphorus (P_2O_5 Olsena), nitrate (NO_3^-), ammonium (NH_4^+) (except for S site) and calcium carbonate ($CaCO_3$) content (except for L site). Also, an overall decrease in the number of bacteria and Actinomycetota, but not in the case of fungi was observed. Based on the genetic diversity of bacteria (16S rRNA region) and fungi (ITS region), significant changes were observed at T0 compared to T8. Overall, ALP activity changed over time compared to ACP activity. The DH activity was highest at T0 (high moisture content) in G and N sites, and at 2nd week for L and S sites, but significantly decreased at the end of the experiment. The UR activity decreased significantly in G, L, and N sites but increased in S site at the end of our experiment compared to the T0. Overall, the physiological diversity of the microbial community was strongly affected by water stress in the utilization of carbohydrates, carboxylic and acetic acids, amino acids, polymers, and amines, in all sites. This study highlights drought stress (8 weeks) had a significant influence on soil biological activity. This may improve the understanding of the impact of soil moisture changes on soil nutrient cycling and biological activities in agricultural ecosystems.

Keywords: agricultural soil, microbial abundance, biological activity, drought, physiological diversity.

1. Introduction

Global warming has modified the patterns and proportion of precipitation in the 21st century in different parts of the world, leading to protracted droughts [Hao et al., 2018; Xiao et al., 2023]. Drought, a consequence of climate change, is one of the catastrophes that result in significant agricultural losses and is defined as a water scarcity in an area/region [Xiao et al., 2023]. The report from the Intergovernmental Panel on Climate Change (IPCC), highlights the effect of drought on global agricultural production and soil quality, which are vital for agricultural sustainability [IPCC, 2021]. On the other hand, Eastern and Central Europe is mainly affected by drought, and it is predicted that in the future, Europe will be experiencing further drought events [Bogati et al., 2022].

In particular, a healthy soil consists of a complex dynamic ecosystem containing wide microbial communities, organic matter, minerals, and other nutrients [Lehmann et al., 2020]. Any alteration in the diversity and activity of microbial abundance has been employed as an indicator of soil health [Bogati et al., 2023]. Generally, microorganisms are sensitive to variation in environmental conditions, that provides insights about soil deterioration or enhancement [Saleem et al., 2019]. Soil microorganisms are proximate agents in soil biogeochemical nutrient cycling and decomposition of organic matter via secretion of enzymes and ultimately produce soluble substrates for biological assimilation [Klinerová and Dostál, 2020]. Many reports suggest a decline in microbial abundance and their activities under drought conditions [Santos-Medellín et al., 2017; Xu et al., 2018; Bogati et al., 2022]. Moreover, different groups of bacteria, *Actinomycetota* and fungi are sensitive to soil moisture change [Bogati et al., 2023]. Therefore, it is challenging to predict the impact of drought on numerous agricultural areas over time [Bogati et al., 2022]. Extracellular enzymes play a critical role in the cycling of soil nutrients, and they are mainly produced by soil microbes [Alster et al., 2013]. The presence of these enzymes in the soil indicates healthy microbial function and significantly react to environmental changes [Bogati et al., 2022].

Physiological diversity analysis of microbial communities is widely used in environmental research [Grządziel et al., 2018]. The method used to analyze microbial physiological diversity is the physiological community level physiological profiling (CLPP), with the help of 96-well Biolog® Ecoplate™ (Biolog Inc., Hayward, CA, USA). It is based on the evaluation of functional status of a soil microbial community as well as assessment of the ability of soil microbial communities to metabolize a range of organic carbon substrates [Bogati et al., 2023]. The enzymatic and respiratory activity of soil-dwelling microorganisms vary greatly with respect to soil type and depth, and they are sensitive to environmental changes. It entails taking measurements of a variety of physiological indicators, including biomass, enzyme activity, and respiration rate, which reflect the activity and health of the soil's microbial population [Bogati et al., 2023]. A soil microbial catabolic profile describes the types and quantity of organic substances that it can decompose and use as a source of energy. The catabolic profile will also be impacted by the microbial composition and their capacity to adapt to these

unfavorable conditions in the soil [Apostolakis et al., 2022]. This method can generate a substantial amount of data that is excellent for identifying impact of site-specific soil moisture variations in microorganisms, in turn assessing the link between biodiversity and site conditions [Grządziel et al., 2018]. Few studies have been conducted to determine whether differences in soil CLPP are caused by changes in a microbial community's ability to rapidly metabolize structurally complicated substrates [Tahtamouni et al., 2023].

The aim of this research was to investigate the influence of 8 weeks drop in soil moisture on soil physicochemical parameters, soil microbial abundance, their enzymes and physiological diversity in four agricultural regions collected in Poland during autumn season. The main hypothesis of this study includes: the lack of soil moisture should lead to significant changes in soil biological activity and composition of the soil microbiome. The results of this study will help to better understand soil nutrient conditions and microbial metabolic constraints under drought conditions in context of future global warming conditions and promote restoration and soil quality improvement in agricultural ecosystems.

2. Materials and Methods

2.1 Sample collection and physicochemical analyses of soil samples

Four agriculture soil samples (0-20 cm in depth from the soil surface; n = 5 per site) were collected, on 4th October 2022 (autumn season) from four sites: Gniewkowo (G; 52.902300°N, 18.433274°E), Lulkowo (L; 53.090675°N, 18.580300°E), Wielka Nieszawka (N; 53.005717°N, 18.467974°E) and Suchatówka (S; 52.907635°N, 18.466824°E) near Toruń, Poland (Figure 1). These four types of soil were selected based on differences in their texture and bonitation classification (gleyic luvisol Phaeozem in Gniewkowo (G) (rich in clay and humus, 1st class), stagnic luvisol in Lulkowo (L) (3rd class), fluvisol in Wielka Nieszawka (N) (3rd class) and haplic luvisol in Suchatówka (S) (sandy, 5th class). They were placed into plastic containers (high = 23 cm and Ø = 28 cm). For each site, consists of total five plastic containers filled with soil at T0, T1, T2, T4 and T8 week treatments. Altogether in total 20 containers were exposed to drought conditions by placing outside under the canopy for up to T8 weeks. Therefore, soil samples were protected against rainfall at ambient temperature, but were not maintained in strictly controlled conditions of humidity. At each treatment time, soil samples were collected from corresponding container (in five replicates) into the plastic bag, mixed well and subjected to further analysis in the laboratory.

Soil samples for the study of soil physicochemical, microbial abundance, their enzyme activities and physiological diversity analysis were conducted at time (T) intervals (T0, T1, T2, T4 and T8 weeks, where "T0" is sampling day (fresh soil)). For this purpose, a stainless-steel soil sampler probe (Ø 50 mm) was used to collect the soil samples (0-20 cm depth) from the containers after T0, T1, T2, T4 and T8 weeks, placed into the plastic bags and analyzed immediately, as described below. The mean soil moisture at corresponding time

intervals was determined in five replicates, by calculating the difference in soil mass between collected sample and dried sample (100 °C for 4 days). The soil pH was measured in five replicates using distilled water at the ratio of 1:2.5 using a pH meter CP-401 (ELMETRON, Zabrze, Poland). Total organic carbon (TOC) and total nitrogen (TN) were determined using organic elemental analyzer Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). The graining of the soil and its texture were determined according to Bouyoucos areometric method, modified by Warzyński et al., 2018, and the sieve method [Bednarek et al. 2004].

2.2 Determination of the number of bacteria, Actinomycetota and fungi in soil samples

Bacteria, actinomycetes (Actinomycetota) and fungi were enumerated using a standard ten-fold dilution plate procedure for the four sites. One mL of serial dilutions (10^{-4} - 10^{-6} and 10^{-2} -10^{-4}) of each soil sample were placed into sterile Petri plates and poured with Plate Count agar (PCA, Biomaxima, Lublin, Poland) for bacteria enumeration and Rose Bengal agar (Biomaxima) for fungal enumeration, respectively. 0.1 mL aliquots of serial dilutions (10^{-3} - 10^{-5}) of soil samples were spread over the surface of Actinomycete Isolation agar (Becton Dickinson, Franklin Lakes, NJ, United States). The media for isolation of bacteria and Actinomycetota were supplemented with cycloheximide (0.1 g L^{-1}), whereas chloramphenicol (0.1 g L^{-1}) for fungal isolation was used to prevent fungal and bacterial growth, respectively. The inoculated plates (3 replicates per dilution) were incubated at 28°C for 2 weeks. The number of colonies was counted using colony counter LKB 2002 (Pol-Eko, Wodzisław Śląski, Poland) after 7 and 14 days of incubation. The number of microorganisms were expressed as log10 of colony-forming unit (CFU) per gram of dry soil.

2.3 Soil enzymatic activities

Enzymatic activities were determined spectrophotometrically in five replicates for all four investigated soil samples. The acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were determined according to method described by Tabatabai, 1982 and modified by [Furtak et al., 2019] using sodium pnitrophenylphosphate (PNP). Absorbance at 410 nm was measured using spectrophotometer Marcel Pro Eko (Poland). The dehydrogenase (DH) activity was determined colorimetrically according to Furtak et al., 2019. Absorbance measurements of the triphenylformazan (TPF) at 490 nm were performed using spectrophotometer Marcel Pro Eko (Warsaw, Poland). The urease (UR) activity was determined using spectrophotometric technique according to Nakano et al., 1984, modified by Kandeler and Gerber, 1988. The absorbance at 420 nm was measured using spectrophotometer Marcel Pro Eko (Poland).

2.4 Physiological diversity of soil microbes- Biolog®EcoPlate

The impact of induced drought on microbial diversity was evaluated in the investigated soil samples, using physiological diversity profiling at T0, T1, T2, T4, and T8 weeks, respectively. This analysis is based on the ability of microorganisms to oxidize carbon substrates using 96-well Biolog® Ecoplate™ (Biolog Inc.,

Hayward, CA, USA), as described by [Siebielec et al., 2020]. Biolog® Ecoplate™ consisted of 31 carbon sources, including carbohydrates (10), carboxylic and acetic acids (9), amino acids (6), polymers (4), and amines (2), in triplicate [Siebielec et al., 2020]. Aliquots of 0.1 mL of soil suspension (dilution of 10^{-2}) were inoculated into each well and incubated for 4 days at 28°C. The absorbance was read after 4 days of incubation at 590 nm wavelength using Microplate reader Multiskan FC photometer (Thermo Fisher Scientific, Waltham, MA, USA) in triplicate. The changes in color from colorless to purple resulted from reduction of water-soluble triphenyl tetrazolium chloride to triphenyl formazan, thus indicating degradation of carbon sources. The average well color development (AWCD) was determined after incubation time for individual plates using the method described by [Garland and Mills, 1991], as a mean of the optical densities (OD_{590}) from the 31 wells. In addition, optical densities (OD_{590}) = 0.25 was assumed as a threshold value, below which a substrate was considered as unmetabolized.

2.5 Characterisation of bacterial (16S) and fungal (ITS) diversity at T0 and T8

The soil bacterial and fungal diversity at T0 and T8 were determined at Novogenes (Cambridge, UK). Total genome DNA from samples was extracted using CTAB method and DNA concentration and purity was monitored on 1% agarose gels. The 16S rRNA/ITS1 genes of distinct regions (16S V3-V4/ITS1) were amplified using specific primer (e.g., 515F-806R/ITS1) with the barcode. All PCR reactions were carried out with 15 μL of Phusion® High -Fidelity PCR Master Mix (New England Biolabs); 2 μM of forward and reverse primers, and 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃ for 30 s and a final extension at 72℃ for 5 min. The quantification and qualification of PCR products was performed by mixing same volume of 1X loading buffer (contained SYB green) with PCR products and electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios and purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform, 250 bp paired-end reads were generated and amplicon sequence analysis at T0 and T8 were determined by Novogenes (Cambridge, UK).

2.7 Statistical analysis

All biological and chemical parameters were measured using five repetitions and statistically analyzed using repeated measures ANOVA test (Analysis of variance, at the 0.05 confidence level) and Tukey test. The

declared level of significance is $p<0.05$ (a), $p<0.01$ (b) and $p<0.001$ (c). The Biolog® Ecoplate™-derived metabolic diversity indices, AWCD, variations in the impact of drought stress on carbon source utilization and heatmaps were analyzed using Morpheus heatmap (https://software.broadinstitute.org/morpheus/, accessed between 11th-12th May 2023). The principal component analysis (PCA) was performed to assess variations in the impact of drought stress on analyzed parameters. For the PCA, raw data was standardized prior to the analysis.

3. Results

3.1 Chemical properties of soil samples

The texture of the investigated soils varies from haplic luvisol in Suchatówka (S) which was most sandy, to stagnic luvisol in Lulkowo (L), fluvisol in Wielka Nieszawka (N), and gleyic luvisol (or luvic gleyic) Phaeozem in Gniewkowo (G). The physicochemical properties of four agricultural soil samples are shown in Table 1. The texture of the soils was as follows: 50-91% sand (2-0.05 mm), respectively; 7-37% silt (0.050.002 mm), respectively; and 2-13% clay (<0.002 mm), respectively.

The available phosphorus (P_2O_5 Olsena), nitrate (NO_3^-), and ammonium (NH_4^+) content was observed with strong significant difference ($p<0.001$) in studied soil samples between T0 and end of the prolonged drought stress (T8), as given in Table 1. Patterns on changes in moisture content (Figure 2) had a significant impact on the bulk soil TOC and TN contents ($p<0.05$ or $p<0.01$). In case of phosphorus content, only N site had significant influence of $p<0.01$. For $CaCO_3$ content, showed significant difference of $p<0.01$ or $p<0.001$.

The highest moisture content was observed at T0 and decreased thereafter until T8 in all soil samples (Figure 2). The most intense decrease in moisture content was observed in the G and S soil sample, until the end of the experiment (T8). The average moisture content in soil samples ranged from 11.63-21.87% at the collection date and from 6.81-16.16% at the end of experiment, indicating significant reduction by 4.82, 5.80, 5.71, 4.76% in N, G, L and S soil samples, respectively. The pH of the soil was mainly alkaline (ranged 7.6-8.3) in all samples.

3.2 Influence of prolonged drought stress on number of microorganisms

Drought stress had significant influence on number of bacteria ($p<0.05$), Actinomycetota ($p<0.05$, $p<0.01$ or $p<0.001$) and fungi ($p<0.05$, or $p<0.001$). Generally, the number of bacteria was higher compared to fungi in all soil samples (Figure 3). This research showed strong decrease of bacterial abundance with respect to decrease in soil moisture content from T0 to T8 ($p<0.001$). In case of Actinomycetota, strong decrease significant difference was observed for G and L site ($p<0.01$), but not for other two sites. The fungal abundance was observed to strongly decrease significantly ($p<0.001$) in G site, whereas in other sites, although it fluctuated between the T0 and T8, there was no major significant differences observed ($p>0.05$). Prolonged drought conditions decreased the number of bacteria and Actinomycetota, especially at the end of the

experiment. On the other hand, the overall fungal abundance was observed to be lower compared to bacteria and Actinomycetota abundance in all sites (Figure 3).

3.3 Effect of drought stress on enzyme activity

Drought stress had a significant influence on soil ACP, ALP, DH, and UR activities ($p< 0.05$) (Figure 4). Overall alkaline phosphatase activity was higher compared to acid phosphatase activity by 53.58, 91.43, 93.80, and 25.44% in G, L, N and S site respectively, thereby indicating strong inhibition of ACP activity by drought stress. The pattern of changes in soil moisture content had significantly increased ACP activities only in S site ($p<0.001$) after 8 weeks compared to T0, whereas in other sites there was no significant differences, but a decreasing tendency was observed after 2 weeks. On the other hand, an increasing tendency in ALP activity after 8 weeks compared to T0, although some fluctuations were observed between T1, T2 and T4 weeks in all sites. Statistical analysis for ALP activity shows significant differences in L, N and S ($p<0.05$) sites with a strong decrease in soil moisture content.

The dehydrogenase activity was highest at T0 (high moisture content) in G and N sites, whereas it was highest on T2 for L and S sites (Figure 4). Statistical analysis for DH activity reveals strong decrease in G, L and N sites at the end of our experiment ($p<0.05$), except for S site its activity increased significantly ($p<0.001$). In general, the highest DH activity was observed in G and L site, but lowest in S site.

The activity of urease (UR) varied in all analyses soil samples and its activity fluctuated with respect to changes in moisture content (Figure 4). The UR activity decreased significantly in G, L and N ($p<0.05$) sites but increased in S site ($p<0.05$) at the end of this experiment compared to T0.

Moreover, based on PCA analysis, showed positive correlation of moisture with bacteria, Actinomycetota, fungi, DH activity and UR activity (Figure 5), indicating significant impact of drought stress on soil biological parameters (Figure 5). The total variance explained by Axis 1 and Axis 2 was 106.74% (83.79 and 22.95%, respectively).

3.4 Estimation of community level physiological profiling (CLPP) of soil samples under drought stress

The average of the AWCD index for all soil samples (Figure 6A) was the highest at T0 for G (1.31), L (1.28) and N (1.00) site, whereas for S (1.05) site the highest was at T4 of drought treatment, indicating highest metabolic potential. An overall dramatic decrease tendencies in utilization of major carbohydrates, carboxylic and acetic acids, amino acids, polymers, and amines with decrease in water moisture levels was observed at the end of this experiment. Out of 31 carbon substrates, four were not utilized in all sites, which includes 4-Hydroxy Benzoic Acid, α-Ketobutyric Acid, L-Phenylalanine and α-Cyclodextrin.

In the case of carbohydrates, there was a decrease in the substrate metabolism in all sites, mainly the highest decrease in G site (10.32%), followed by S (6.52%), L (2.82%) and N (1.75%) site. Similar decrease tendencies in utilization of carboxylic and acetic acids were also observed in G site (9.56%), followed by N

(5.66%), L (1.14%) and S (0.82%) site. For amino acids, the percentage of decrease in metabolism was highest in N site (4.11%), followed by G (0.53%) and L site (0.47%), but increased in S site by 0.87%. In the case of polymers, the highest decrease in metabolism was observed in N site (6.86%), followed by L (6.76%), G (6.06%) and S (2.32%) sites. In the case of amines, the highest decrease in metabolism was observed in N site (10.16%), followed by G (5.55%) and S (0.77%) sites, but increased in L site (2.39%). In this experiment, CLPP analysis revealed the highest percentage of carbon substrate utilization was observed in S site followed by L, N and G sites (Figure 6A-F and 7). The more detailed observations resulted from the analysis of the metabolism of the individual substrates presented in Figure 7.

This can be correlated with reduction in soil water content and microbial community counts (Figure 2 and 3). The physiological diversity analysis provides further evidence that changes in soil moisture can cause overall reduction in metabolism of carbohydrates, carboxylic and acetic acids, amino acids, polymers, and amines in all soil samples at T8, with exception of lowest percentage of reduction observed in soil sample from S (Figure 6A-F and 7). Although there was consistency of utilization of carbon substrates (slow) under drought conditions by microorganisms, the patterns revealed no complete inhibition (Figure 7).

To explore the variations in the soil microbial community composition, the following parameters were subjected to PCA (principal component analysis): calculated AWCD of the whole Biolog® EcoPlate™ of all four sites calculated from the substrate's utilization pattern, as well as moisture content at T0 and T8 of all four sites. The PCA (Axis 1 and Axis 2) explained 90.32% of variation among the factors and clearly indicates the effect of moisture on soil types and physiological diversity. For G and S sites, 12 carbon substrates were positively correlated, whereas for L site, 6 carbon substrates were positively correlated. A negative correlation was observed for 13 carbon substrates with respect to the moisture content in all sites (Figure 8). On the other hand, PCA analysis reveals positive correlation between DH and UR enzyme activities with all 5 groups of carbon substrates (mainly carbohydrates, carboxylic and acetic acids, amino acids, polymers and amines) (Figure 9). It explains 97.672% (Axis 1 and Axis 2) of variation among the factors and clearly reveals significant correlation between soil enzyme activities with physiological activity.

3.5 Effect of Drought stress on structural diversity of microorganisms (16S and ITS region) The bacterial communities in the soil samples at T0 were dominated by 5 phyla, namely Proteobacteria (17.02%), Acidobacteriota (12.60%), Actinobacteriota (10.98%), Bacteroidota (7.13%), and Firmicutes (5.10%), and T8 were dominated by 5 phyla, namely Proteobacteria (18.76%), Acidobacteriota (10.22%), Actinobacteriota (8.36%), Bacteroidota (7.81%) and Myxococcota (5.87%) (Table 2A). This study showed a strong significant decrease in relative abundance of Actinobacteriota, Bacteroidota, and Acidobacteriota in L (5.91%), G (6.28%), and S (5.19%) sites, respectively, while other phyla showed weak or no significant changes (Table 2A). At T0, bacterial communities were dominated by 6 genera, namely Acidobacteria genus *RB41* (2.00%), *Sphingomonas* (1.70%), *Bryobacter* (1.63%), Proteobacteria genus *MND1* (1.14%), *Haliangium* (0.93%), and *Blautia* (0.92%) and also at T8, bacterial communities were dominated by 6 genera,

namely Proteobacteria genus *MND1* (2.79%), *Haliangium* (2.35%), Acidobacteria genus *RB41* (2.27%), *Gaiella* (1.33%), *Hassallia* (1.06%) and *Sphingomonas* (1.00%), in all analysed soil samples (Table 2B). The relative abundance of *Bryobacter* decreased significantly in L (4.61%) site, while other genera showed weak or no significant changes (Table 2B).

The fungal communities in the soil samples at T0 were mainly dominated by 3 phyla, namely Ascomycota (58.13%), Basidiomycota (17.55%), and Mortierellomycota (3.63%), whereas at T8 were mainly dominated by 4 phyla, namely Ascomycota (67.71%), Basidiomycota (5.50%), Mortierellomycota (4.51%), and Chytridiomycota (2.18%) (Table 3A). The relative abundance of Ascomycota significantly decreased in G site by 21.6%, but significantly increased in S (26.07%) site followed by L (21.28%) and N (12.13%) sites. On the other hand, the relative abundance of Basidiomycota decreased significantly in N (33.94%) followed by L (11.14%) site, but others showed weak or no significant changes (Table 3A). At T8, fungal communities were mainly dominated by 13 genera, namely *Panaeolina* (9.15%), *Plectosphaerella* (5.70%), *Mortierella* (3.44%), *Fusarium* (3.42%), *Kazachstania* (3.22%), *Cladosporium* (2.20%), *Alternaria* (2.04%), *Microdochium* (1.78%), *Gibellulopsis* (1.51%), *Ramophialophora* (1.25%), *Metarhizium* (1.16%), *Meyerozyma* (1.14%), and *Pyrenochaetopsis* (1.03%) (Table 3B). On the other hand, T8 was mainly dominated by 14 genera, namely *Blumeria* (8.15%), *Mortierella* (4.14%), *Ramophialophora* (2.75%), *Fusarium* (2.49%), *Schizothecium* (2.33%), *Cladosporium* (1.79%), unidentified (1.62%), *Trichoderma* (1.56%), *Metarhizium* (1.45%), *Gibellulopsis* (1.41%), *Plectosphaerella* (1.23%), unidentified_*2* (1.11%), *Pyrenochaetopsis (1.06%), and* unidentified_Agaricomycotina (1.06%) (Table 3B). The relative abundance of *Panaeolina*, *Plectosphaerella*, and *Kazachstania* showed strong significant decrease in N (36.42%), G (19.09%) and L (12.88%) sites, respectively, and significantly increased of *Ramophialophora* in S (7.16%) site, whereas among others weak or no significant changes were noted (Table 3B).

4. Discussion

4.1 Soil chemical parameters

Knowledge on soil physicochemical properties is crucial for understanding soil water mobility and predicting soil parameters that impact agricultural environment in the area [Zhang et al., 2019]. Soil physiological properties can influence the overall biological structure of soil depending on soil moisture content [Chodak et al., 2015; Wang et al., 2020]. Our results showed that drought stress had a negative effect on the investigated soil physicochemical properties. The TOC, available phosphorus (P_2O_5 Olsena), nitrate (NO_3^-), ammonium (NH_4^+) (except for S site) and calcium carbonate ($CaCO_3$) (except for L site) content decreased significantly in all soil samples ($p<0.05$) with decrease in moisture content (Table 1 and Figure 2). The TN content decreased in G site and increased in N and S sites, but not significantly and this effect is difficult to explain. On the other hand, the total phosphorus content increased in all sites but was significant in N site ($p<0.001$). Interestingly, the soil pH was observed to be in alkaline range, suggesting that drought

had no clear effect on bulk soil pH (Table 1). These results are in line with study conducted by Ochoa-Hueso et al., 2018, who indicated deleterious effect of drought on soil physiochemical parameters including carbon, nitrogen and phosphorus nutrient cycling, and lower rate of the mineralization processes. Other studies found no significant increase or decrease in the TN contents in semi-humid forests under drought conditions [Zhang et al., 2019]. This indicates slow transformations of carbon and nitrogen in soils under drought conditions, which can be the effect of decrease in enzymes activity. It is also known that extreme drought events result in serious structural destabilization of the soil with major impact on the soil carbon and nitrogen cycles [Quintana et al., 2023]. Such impacts may slowly decrease soil functionality, reducing availability of soil nutrient to plants [Nguyen et al., 2018]. It is known that air-drying has negative impacts on soil biochemical characteristics. Since soil moisture is a fundamental element regulating the survival of microorganisms and their activity, drought sensitive microorganisms will die under these unfavorable conditions [Ochoa-Hueso et al., 2018].

4.2 Culture-dependent characterization of microorganisms

The result of the present study indicates high bacteria and Actinomycetota abundance compared to fungal counts in all sites. These observations are consistent with previously published reports showing the negative influence of drought on the number of microorganisms in soil environment [Hartmann et al., 2017; Siebielec et al., 2020; Fadiji et al., 2023]. The decreasing tendency of bacterial abundance in this study (Figure 3) could be due to limited access to plant litter and variation in their metabolic potential among Gram-positive and Gram-negative bacterial groups. In which, the former is metabolically stable compared Gram-negative bacteria [Balasooriya et al., 2014]. Abundance of Actinomycetota was significantly highest between T2 and T4 of drought stress in all sites ($p<0.001$) (Figure 3). In general, Actinomycetota can withstand drought conditions in arid soils due to their thick cell walls, resistant spores, complex carbon degradation genes, utilization of recalcitrant carbon sources, and production of osmolytes (amino acids and carbohydrates) [Bouskill et al., 2016; Mohammadipanah and Wink, 2016]. On the other hand, there are many reports concerning reduction in fungal richness under drought conditions [Hawkes et al., 2011; Naylor and ColemanDerr, 2018; Fahey et al., 2020]. In case of fungal abundance, only microorganisms from G site showed strong significant impact ($p<0.05$) at T8 compared to T0, whereas no significant difference in other sites ($p>0.05$) (Figure 3). Although their overall abundance was lower compared to bacteria and Actinomycetota, changes in soil moisture content did not significantly affect their growth. There is very few evidence on the impact of soil moisture content on fungi in agricultural soils, but their resistance to drought stress is previously reported [Yan et al., 2019; Carbone et al., 2021; Hanaka et al., 2021]. This resistance mechanism could be from previously adapted dominant fungal communities in the presence of altered soil moisture content, and ability of hyphal formation [Lennon et al., 2011; Oliveira et al., 2020; Fadiji et al., 2023]. This study reveals

direct or indirect alterations in soil microbial abundances under dry conditions ultimately hampering the soil quality and productivity.

4.3 Impact of drought on soil enzyme activities

Soil enzyme activities play an important role in nutrient cycling and are closely related to soil structure and function [Raiesi and Salek-Gilani, 2018; Tan et al., 2023]. In this study, the enzyme activities were significantly decreased under drought stress conditions. In general, high alkaline phosphatase activity was observed at T8 compared to T0. This could be related to alkaline soil pH and water content (Table 1) as it can determine the soil phosphatase activity [Bogati et al., 2022], but it can be effect of outflow of enzymes from death cells after long time of drought in soil. Acid and alkaline phosphatase activity are higher in acid and alkaline soils, respectively [Bogati et al., 2023]. On the other hand, ACP activity was low in all sites except in S site it increased at T8 (Figure 4). Huang et al., 2011 and Bogati et al., 2023 confirmed that reduction of soil moisture reduced soil acid phosphatase activity by $p< 0.05$, respectively. Under water deficit conditions, phosphorus mineralization process is affected due to inactivation of microbial decomposers, or accumulation of solutes or organic phosphorus [Suriyagoda et al., 2014]. Such water stress environments restrict diffusion of the enzyme and substrates, affecting nutrient cycling and results in negative impact on soil microbial activity [Menge and Field, 2007].

The dehydrogenase enzyme activity was strongly influenced by soil moisture content [Tan et al., 2023] and decreased (Figure 4) with decline in soil water content in all investigated sites of this study. Dehydrogenase enzyme exists in only live microbial cells and has been used as an indicator for soil microbial activity [Wolińska and Stępniewska, 2012]. Therefore, a significant decrease in DH activity with decrease in microbial abundance in all sites was observed. Siebielec et al. 2020 also first observed increase in DH activity, but then its significant decrease after 8^{th} weeks of drought. However, fluctuation in urease enzyme activity with respect to changes in moisture content was observed in this study (Figure 4). Its activity decreased when compared with T0 in all soil samples, except in S site it increased. Our results are similar to those presented by Deng et al. (2021), that observed significant decline in soil UR activity in forest and shrub ecosystems under drought conditions. In this study, an overall decline in soil enzymes activities, responsible for regulating nutrient cycling, indicates negative impact of changes in soil moisture content on agricultural soil. Thus, altering soil nutrient availability, and decreasing the nutrient supply to plants [Bogati et al., 2022].

4.4 Soil physiological diversity under drought conditions

The differences in the level of metabolism and utilization of substrates in soils may result from the decrease in microbial abundance with respect to decrease in soil moisture content (Figure 2 and Figure 6AF). The lowest level of metabolism can be seen for G site, followed by N and L sites, and was highest in S site. In the first group, that is, carbohydrates (Figure 6B), we can see undisputed division in which L site demonstrated

significantly lower utilization profiles of substrates at 8th week. Carboxylic and acetic acids, amino acids, and amines are significantly less (or slower) metabolized by microorganisms inhabiting in N site at 8th week (Figure 6C-E). Another group is the polymers group (Figure 6F), which suggests S site with lowest metabolism at the end of our experiment. Nevertheless, the differences here are much smaller between the groups. Polymers (Figure 6F) did not show such a significant trend, that is, their metabolism was lowest (or slow) compared to other groups of carbon substrates. An interesting result may be indicated by the almost complete lack of utilization of the four carbon substrates, mainly carboxylic and acetic acids (4-Hydroxy Benzoic Acid and α-Ketobutyric Acid), amino acids (L-Phenylalanine) and polymer (α-Cyclodextrin). This might indicate adverse conditions for the growth of microorganisms, and their enzymes responsible in metabolic pathway. These results indicated that microbial metabolic limitation changed from T0 with highest moisture content to 8th week with lowest moisture levels (Figure 2) during drought conditions.

In our study, we found a positive correlation between enzyme activities (DH and UR) with physiological activity of the soil (Figure 9). And also, an overall decrease in ACP, DH, and UR enzyme activities ($p<0.05$). Soil moisture is an indispensable component of the ecosystem and dry periods slow down or even inhibit microbial growth in soil [Bogati et al. 2022]. Extracellular enzymes released by soil microbes are the primary catalysts for the breakdown of complex organic matter and the mineralization of nutrients, which eventually results in the production of soluble substrates for microbial absorption [Xiao et al., 2023]. This indicates the important role or connection between soil moisture, microbial communities, and their enzymes. But we observed negative effect of changes in soil moisture content on microbial abundance (Figure 3), in turn affecting enzyme activities (Figure 4) and thus resulting in reduction in metabolism of carbon substrate (Figure 6 and 7). This clearly demonstrates the inability of this soil to sustain adequate organic matter cycling that led to the buildup of waste and toxic chemicals [Grządziel et al., 2018].

The ability of microbial populations to break down organic C substrates is influenced by variations in the type, quantity, and bioavailability of these substrates within soil organic matter pool [Tahtamouni et al., 2023]. A major concept of CLPP analysis reveals the ability of soil containing low diverse community can degrade wide spectrum of organic compounds (diverse structural complexity) at same rate in comparison with functional diverse microbial communities [Creamer et al., 2016]. Rapid breakdown of more complex organic compounds necessitates the use of a broader range of enzymes found in various microbial populations. The relationships between soil factors that regulate microbial community composition, substrate availability, and microbial nutrient requirement determine the differences in CLPPs [Tahtamouni et al., 2023]. The dominance of tolerant species can be favored by severe drought and high temperature disruptions, which can impede sensitive species. After restoration of pre-stress environmental conditions and soil conditions, fast-growing species can proliferate [Tahtamouni et al., 2023]. However, these reasons are not sufficient to understand the impact of environmental stresses and disturbances on soil ecosystem. Therefore, a need for comprehensive evaluation of functional traits of soil microbial community may help to understand these complex dynamics.

Based on the different substrate utilization potential of soil microbial communities under drought conditions, indicates their diverse metabolic capacity [Ros et al., 2006]. In our study, we observed a strong decrease in microbial carbon substrate metabolism in drought conditions. The highest metabolic diversity in soil samples was observed on T0 and decreased simultaneously up to 8 weeks (Figure 6A-E and 7). In addition, a linear relationship between the soil moisture and physiological diversity with respect to AWCD rates was observed. It was noted that metabolic activity of soil microorganisms decreased with decrease in soil water status [Manzoni et al., 2012]. These results are consistent with Hueso et al., 2012 and Preece et al., 2020 findings indicating negative impact of drought on microbial community physiological profiles in soil that could result from decline in microbial abundance. This suggests that change in soil water availability is critical in discriminating the microbial abundance and physiologically active types of microbes [Bogati et al., 2022; Preece et al., 2020].

4.5 The effect of drought on genetic diversity (16S and ITS region)

A detailed indication of the impact of water availability on genetic diversity of microorganisms was provided by obtaining the relative abundance of taxa using Amplicon sequence analysis. In this study, drought stress showed significantly decrease (in more than one site) in the relative abundance of Proteobacteria (L, N and S sites), Actinobacteriota (L, N and S sites), Bacteroidota (G, N and S sites), Verrucomicrobiota (G and L sites), and Chloroflexi (N and S sites) (Table 2A). But the relative abundance of Acidobacteriota and Gemmatimonadota decreased slightly in only L and N site, respectively, while a very weak decline in Firmicutes and Myxococcota abundance in G and S site, respectively (Table 2A). These findings were in line with Bogati et al., 2023 and Siebielec et al. 2020, in which they observed decrease in relative abundance of Proteobacteria and Verrucomicrobia, and resistance in case of Firmicutes under drought conditions. On the other hand, they observed an increase in Actinobacteriota, Bacteroidota and Acidobacteria abundance under water stress, but this study shows increase in their abundance only in G site. Thus, demonstrating their sensitivity to drought conditions in other 3 sites (L, N and S). In general, Actinomycetes can grow in dry soils because of their resistant spores, thick cell walls, biofilm production and complex carbon degradation genes [Bogati et al., 2023]. On the other hand, Chodak et al. 2015 observed negative effect of drought stress in the shares of Chloroflexi, Gemmatimonadetes and Verrucomicrobia, which is consistent in this experiment. In the present study, genera *Bryobacter* and *Sphingomonas* were found to significantly decrease in all sites (Table 2A). Only two sites showed decrease in *Blautia* (G and L sites) and Acidobacteria genus *RB41* (N and S sites). In addition, at genera level, L site showed a significant decrease in *Pseudolabrys*, *Candidatus_Solibacter*, *Bacillus* and *Rhodanobacter* abundance, whereas *Arthrobacter* abundance decreased in G sites, respectively (Table 2B). In this study, limited microbiome recovery constituting <1% of relative abundance was observed under drought conditions, indicating long-term persistence of drought-tolerant microorganisms (Table 2B). Microbes experience specific physiological load because of soil drying. Water films develop on soil particles

as soils dry, that concentrates components of aqueous water pore (dissolved nutrients, solutes, and toxins), preventing the diffusion of substrates and extracellular enzymes, and enhancing interactions between microbial communities [Mallik et al., 2022].

In case of fungal communities, at phyla level there was strong significant decrease in the relative abundance of Basidiomycota (>10% in L and N site, but only <2% in other sites) and Ascomycota (>10% in G site) (Table 3A). A <5% decrease was observed in Rozellomycota (N site), Mortierellomycota (S site) and Monoblepharomycota (S site) (Table 3A). At fungal genera taxa level, strong decrease in relative abundance of *Panaeolina*, *Plectosphaerella*, and *Kazachstania* (>10%) was observed in N, G, and L sites, respectively (Table 3B). A reduction of <5% relative abundance was noted in *Fusarium* in G, L and N sites, *Alternaria* in L and S sites, *Microdochium*, *Mortierella* and *Cladosporium* in S site, *Gibellulopsis* and *Ramophialophora* in G site, and *Meyerozyma* in L site (Table 3B). The fact that there are few findings on impact of drought stress on fungal communities in agricultural soils should be emphasized [Bogati et al., 2023]. In this study, the fungal communities also varied in investigated sites, that is, only the relative abundance of Ascomycota showed very strong significant increase (>10%) in 3 sites (L, N, and S) but Mortierellomycota showed slight increase (<4%) in G, L and N sites (Table 3A). At genera level, *Kazachstania* and *Ramophialophora* abundance increased slightly between 4-5% in G and S sites, respectively, whereas among others the abundance increased very slightly (<1%) (Table 3B). Our findings are consistent with those by Hayden et al. 2012 on grassland soil and Ochoa-Hueso et al. 2018 on mesic ecosystems, showing a negative influence of drought on soil fungi, leading to changes at functional and compositional levels. Also, Oliveira et al., 2020 found greater sensitivity of fungal communities in tropical grassland soils under drought conditions. One of the reasons behind the strong negative effect on some fungal communities in this study could be due to reduction of their dominant fungi previously adapted to soil water content [Lennon et al., 2011]. Therefore, it is challenging to forecast concerning response of fungal communities under water deficient conditions, which highlights the need of further research.

However, further research is needed to understand the role of environmental factors and moisture interactions on soil microbial community composition (functional, taxonomic, and/or phylogenetic diversity). In addition, the present study's findings were derived from laboratory pot experiments, which could not accurately represent and predict the results under field conditions. Therefore, quantifying their correlation to these simulated pot experiments is thought crucial for conducting in situ field studies.

5. Conclusions

In this study, the results of the drought stress had a significant influence on most soil microbial abundance, enzyme activities (ACP, ALP, DH, UR) and Biolog® Ecoplate™ approach, as well as physiochemical parameters analyzed. Furthermore, soil moisture and physicochemical parameters were the major factors which influenced variations in soil enzyme activities, whereas microbial genetic and

physiological diversity drove the changes in all sites under variation in soil moisture contents. Soil microbial communities were co-limited by TOC, P_2O_5, NO_3^-, NH_4^+ (except for S site) and $CaCO_3$ (except for L site) content and TN content (only G site) from the perspective of microbial metabolism and nutrient competition. Overall, a decrease in number of bacteria and Actinomycetota, with no significant changes in total fungal abundance was observed in all sites at the end of our experiment compared to T0. Drought stress aggravated soil microbial enzyme activities and physiological diversity in each site. A significant decrease in overall DH activity (except for S site), UR activity (except for S site) and lower ACP compared to ALP was observed at the end of the experiment compared to T0. In case of physiological diversity, decrease in AWCD values were observed with decrease in soil moisture content and overall reduction in utilization of carbon sources, except 4-Hydroxy Benzoic Acid, α-Ketobutyric Acid, L-Phenylalanine and α-Cyclodextrin, in all sites. This would suggest significant changes in the metabolic diversity and microbial community composition of the soil samples we examined. It can be concluded that the EcoPlate™ method can be used to study the variability of the community-level physiological profiling of microorganisms from different soil types, as significant results have been obtained. Thus, the findings of this study provide an indirect theoretical basis for a deeper understanding of soil nutrient cycling, microbial nutrient limitation in future induced drought conditions. However, further research is still needed on the effects of soil moisture interactions and global change scenarios on soil biological systems at molecular level in agricultural regions.

Author Contributions: MW conceptualized the study. MW supervised the study. KB performed all studies related to microbial isolation, enumeration and activity and analyzed the data. KB and PS were responsible for soil parameters analyses. KB was responsible for the original draft preparation. MW reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Table 1. Soil chemical parameters. T0, sampling day, T8; 8 weeks of drought.

Location	**Corg [%](mean ± SD)**		**Nt [%](mean ± SD)**		**Pt [%](mean ± SD)**		**P_2O_5 (Olsena) [mg kg^{-1}] (mean ± SD)**	
	T0	T8	T0	T8	T0	T8	T0	T8
1. Gniewkowo	1.07±0.02	0.98±0.03[b]	0.104±0.001	0.099±0.001[a]	354±1.57	357±2.00	29.2±0.52	24.1±0.54[c]
2. Lulkowo	1.68±0.02	1.65±0.01[a]	0.158±0.001	0.158±0.001	653±0.61	653±2.51	127.1±0.14	120.1±0.08[c]
3.Wielka Nieszawka	1.32±0.01	1.37±0.03[a]	0.152±0.001	0.156±0.001[a]	487±1.07	498±1.6[b]	37.2±0.16	30.1±0.1[c]
4. Suchatówka	0.66±0.01	0.62±0.02[b]	0.052±0.001	0.055±0.001[a]	527±1.16	528±1.58	44.3±0.16	28.9±0.16[c]

Location	**NO_3^- [mg kg^{-1}] (mean ± SD)**		**NH_4^+ [mg kg^{-1}] (mean ± SD)**		**pH (mean ± SD)**		**$CaCO_3$ [%](mean ± SD)**	
	T0	T8	T0	T8	T0	T8	T0	T8
1. Gniewkowo	57.2±0.25	39.9±0.17[c]	1.08±0.02	0.58±0.02[c]	8.0±0.02	8.1±0.05	1.3±0.02	1.0±0.05[b]
2. Lulkowo	56.3±0.17	43.2±0.08[c]	0.82±0.02	0.70±0.02[c]	7.4±0.06	7.6±0.08[a]	0.54±0.01	0.62±0.02[b]
3.Wielka Nieszawka	94.8±0.16	206±0.57[c]	0.64±0.01	0.58±0.01[c]	7.8±0.07	7.8±0.06	0.50±0.02	0.21±0.01[c]
4. Suchatówka	46.1±0.08	27.9±0.17[c]	0.66±0.02	0.73±0.01[c]	8.3±0.05	8.3±0.15	7.8±0.17	7.0±0.05[b]

a($p<0.05$), b($p<0.01$), c($p<0.001$); Standard deviation (SD); n=5.

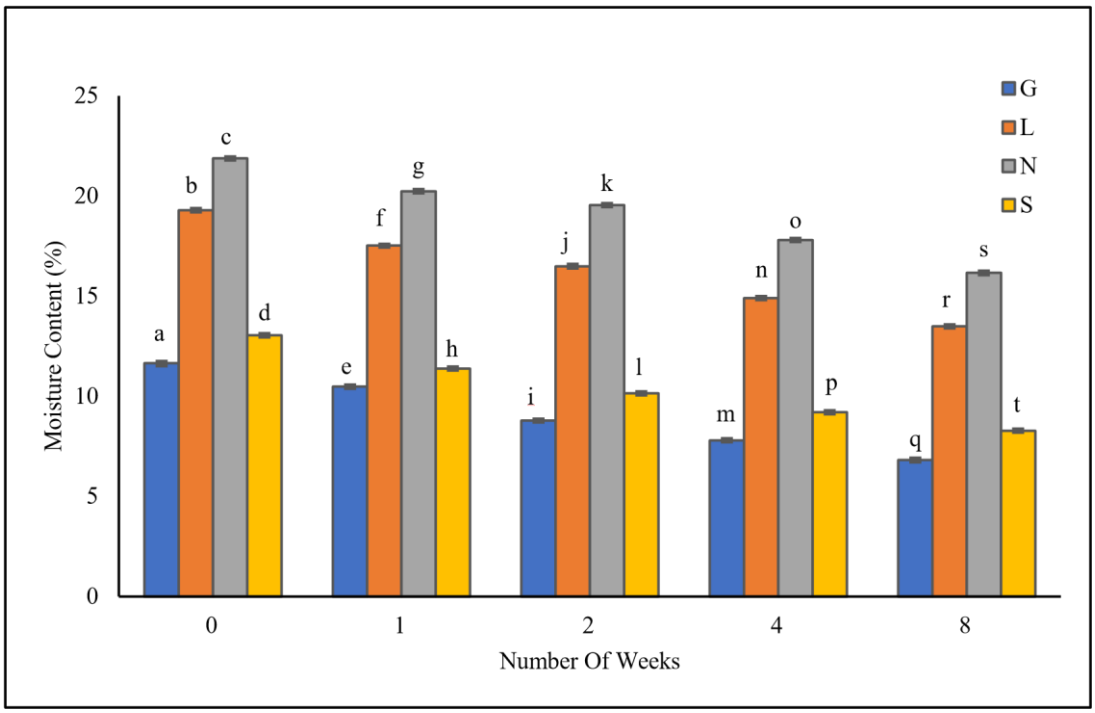

Figure 2. Soil moisture content under prolonged drought conditions in samples collected from Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S). Mean values described with the letters (e.g., a-t) are significantly different at $p < 0.05$. Error bars indicate standard errors of the mean (n = 5).

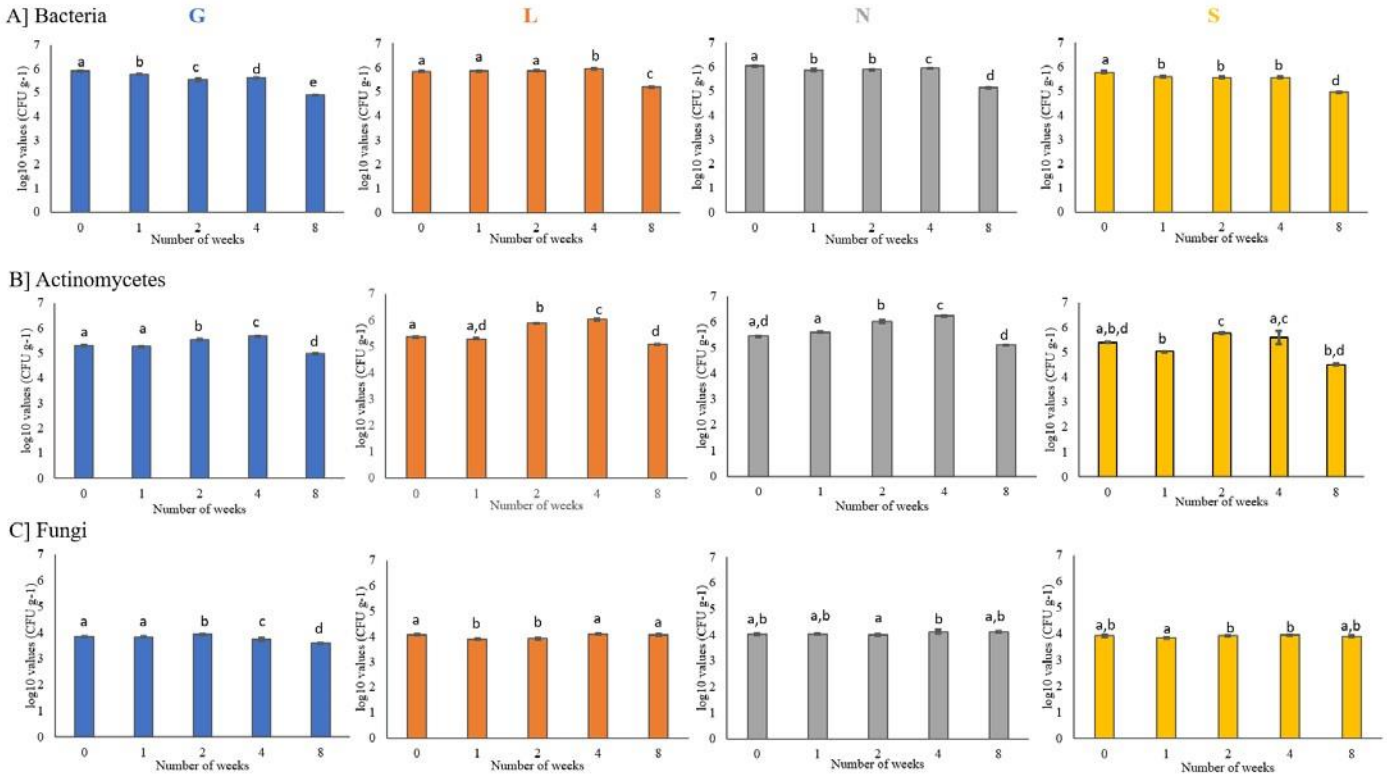

Figure 3. Changes in number of bacteria, actinomycetes and fungi under prolonged drought conditions in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). All analyses were performed in five replicates and the data are presented as mean ± SD. Mean values described with the same letters (e.g., aa, etc.) are not significantly different at $p < 0.05$. Error bars indicate standard errors of the mean ($n = 5$).

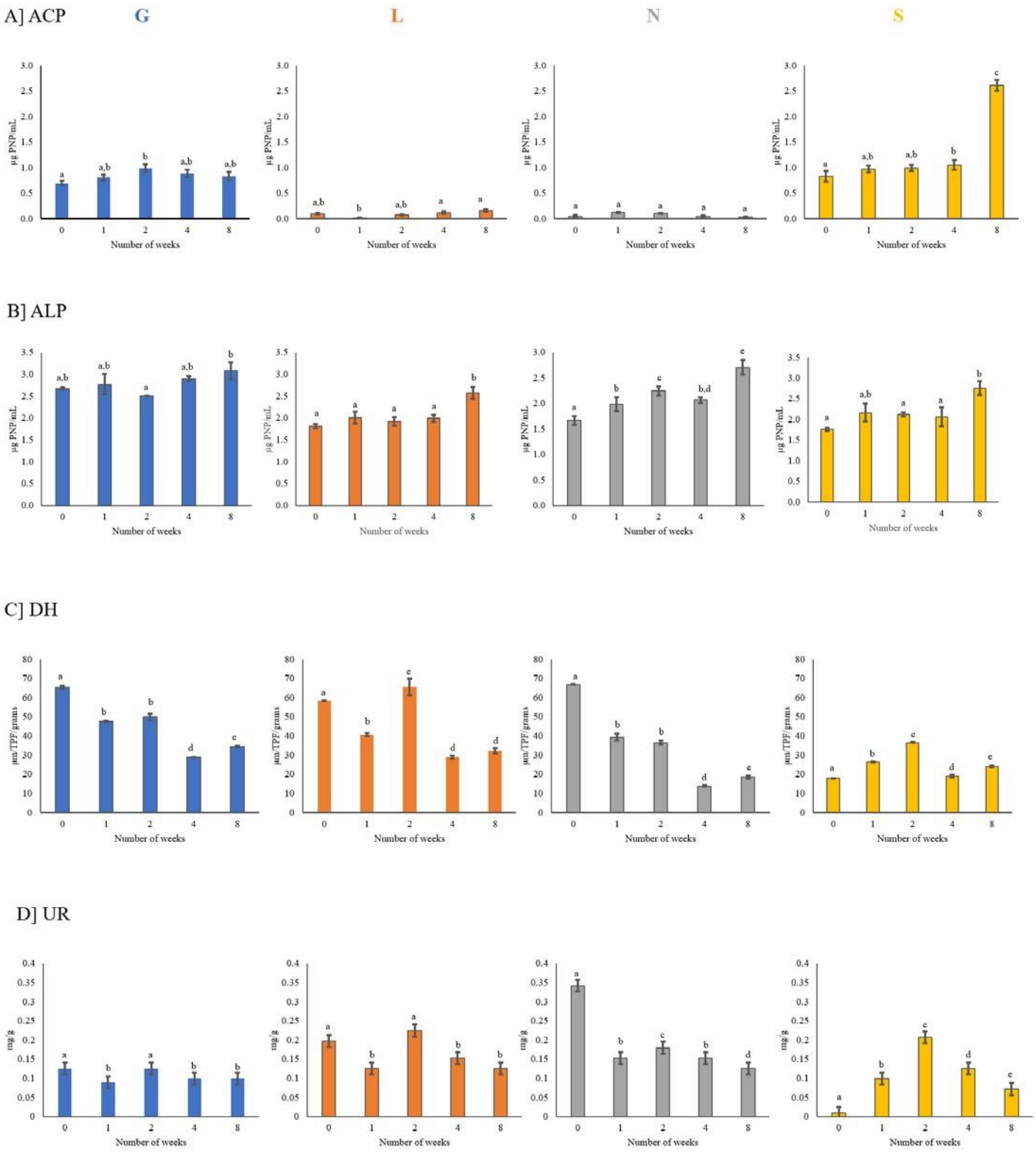

Figure 4. Enzyme activities under prolonged drought conditions in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). [A] Acid phosphatase (ACP); [B] Alkaline phosphatase (ALP); [C] Dehydrogenase (DH); [D] Urease (UR) enzyme activities. All analyses were performed in five replicates and the data are presented as mean ± SD. Mean values discribed with same letters (eg., aa, etc.) are not significantly different at $p < 0.001$.

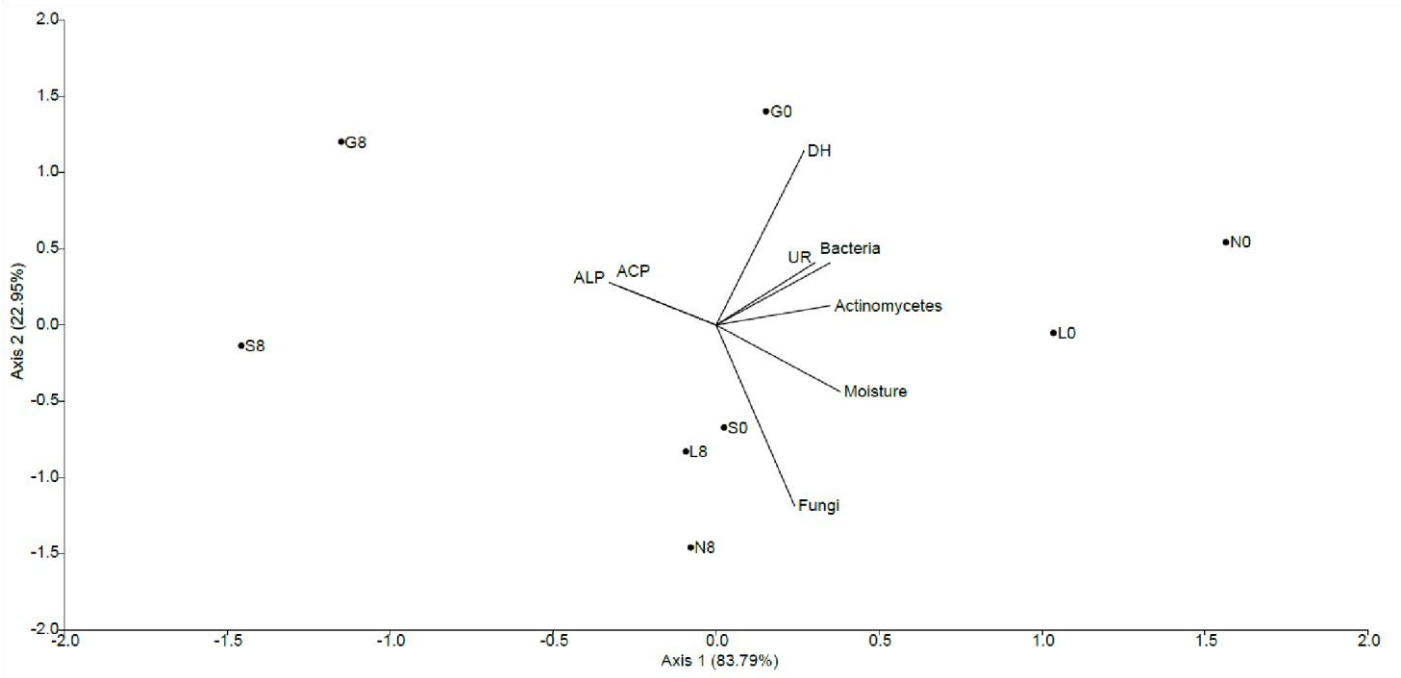

Figure 5. Principal component analysis (PCA) diagram indicating correlations between soil physicochemical, total bacterial, Actinomycetes and fungal abundance, and enzyme activities in four soil samples at T0, T1, T2, T4 and T8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; ACP, Acid phosphatase; ALP, Alkaline phosphatase; DH, Dehydrogenase; UR, Urease; G0 (G at week 0); G8 (G at week 8) (likewise for L, N and S sites).

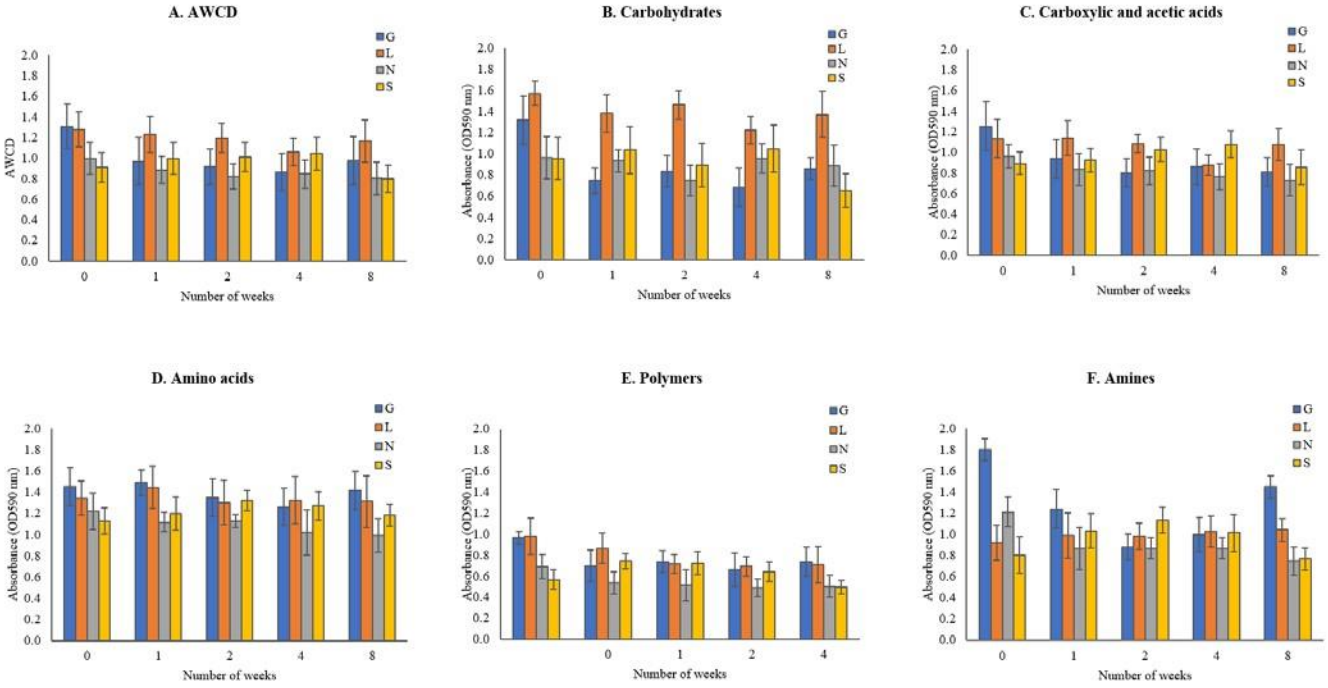

Figure 6. Absorbance values of Biolog-Ecoplates in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) with carbon substrate utilization efficiency. [A] Average rate of the average well colour development (AWCD) over the incubation time (ΔAWCD/weeks); Metabolism of [B] Carbohydrates; [C] Carboxylic and acetic acids; [D] Amino acids; [E] Polymers; [F] Amines.

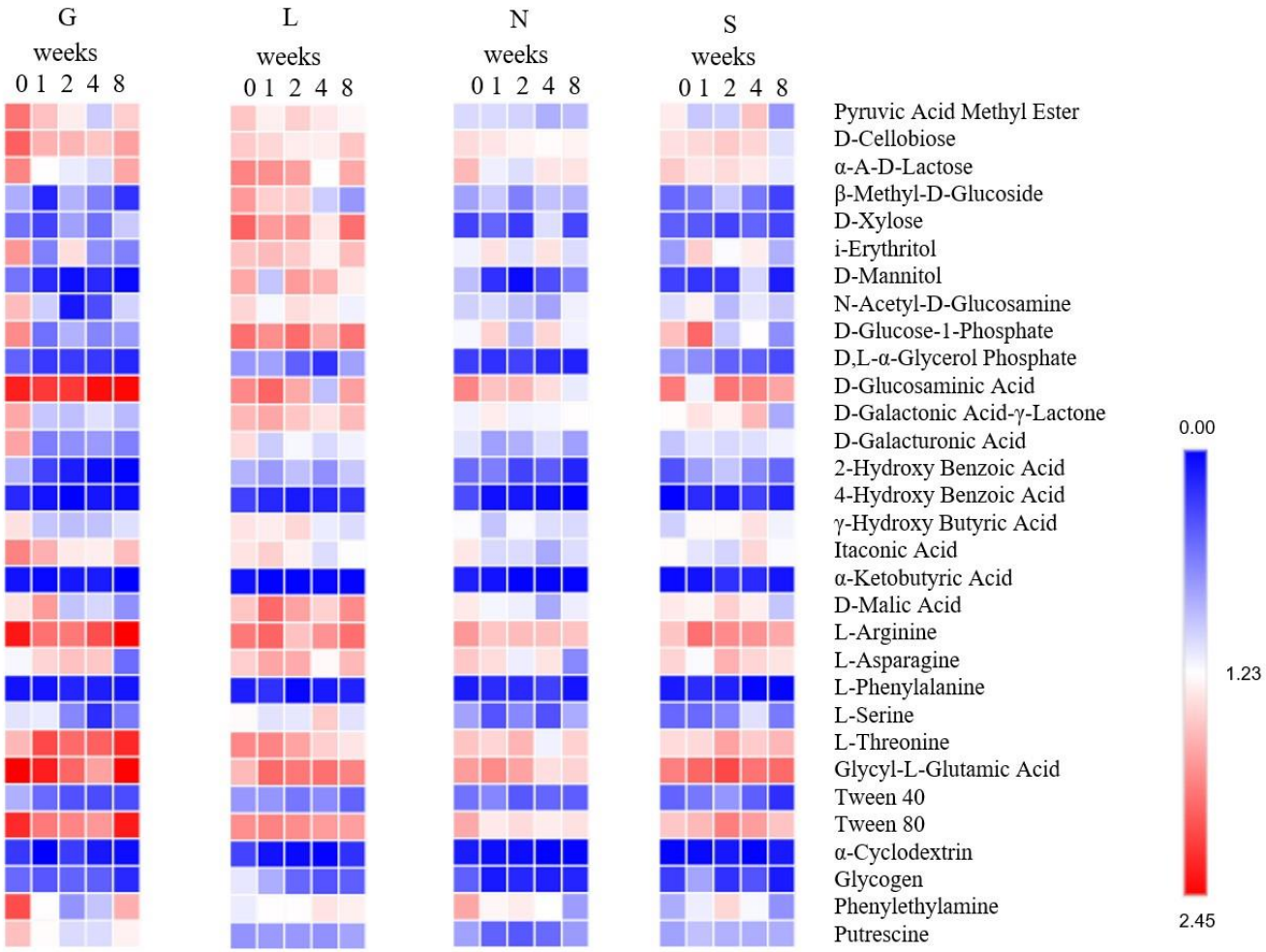

Figure 7. Heat map for community level physiological profiles (CLPPs) in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) with mean absorbance values (λ = 590 nm).

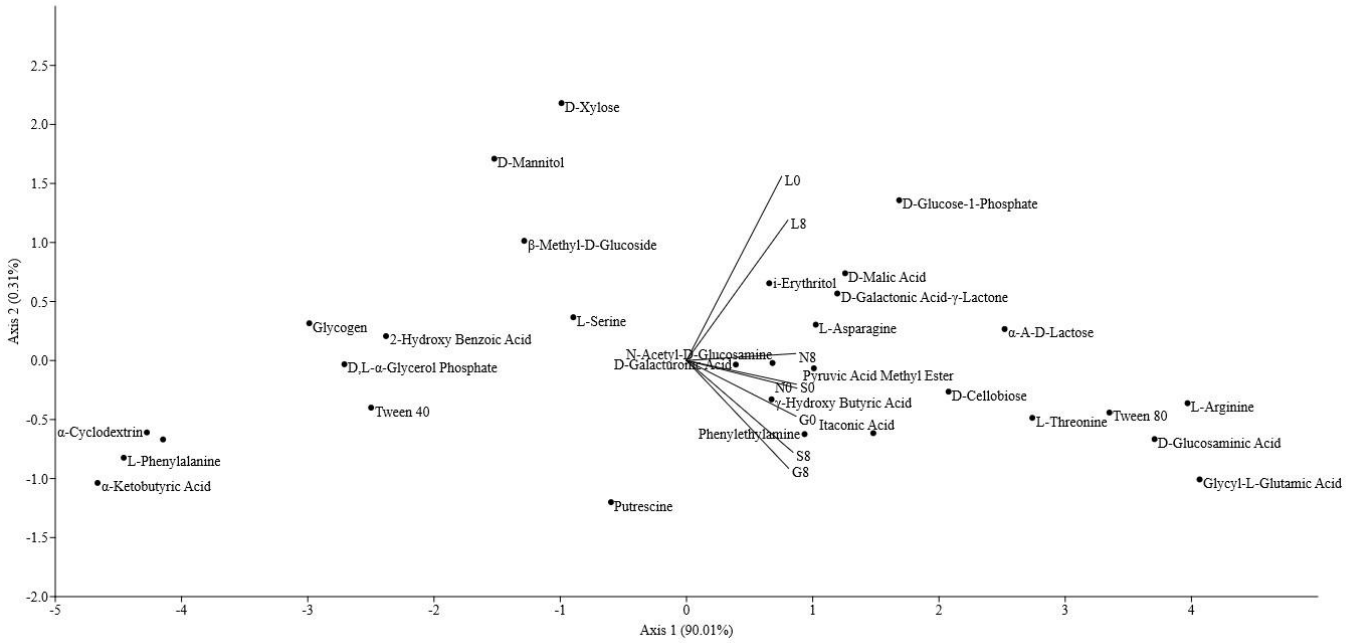

Figure 8. Principal component analysis (PCA). Average well color development (AWCD) index of 31 carbon substrates in four soil samples at T0 and T8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; G0 (G at week 0); G8, (G at week 8) (likewise for L, N and S site).

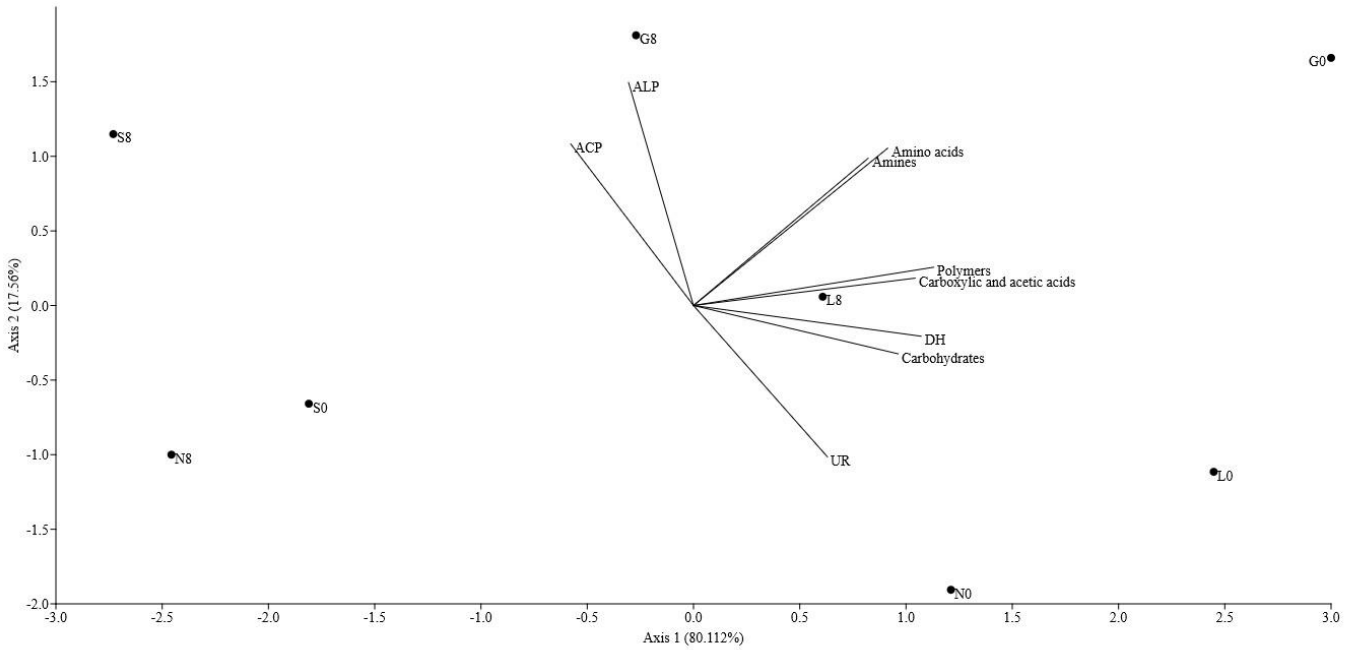

Figure 9. Principal component analysis (PCA) for Average well color development (AWCD) index of 5 groups of carbon substrates (carbohydrates, carboxylic and acetic acids, amino acids, polymers and amines) and enzyme activities (ACP, Acid phosphatase; ALP, Alkaline phosphatase; DH, Dehydrogenase; UR, Urease) in four soil samples at T0 and T8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; G0 (G at week 0); G8, (G at week 8) (likewise for L, N and S site).

Table 2. Relative abundance of bacterial taxa identified by 16S rRNA amplicon sequencing. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; T0; sampling day; T8, 8 weeks of drought.

Taxonomy	Relative abundance (%)							
A. Bacterial Phyla	**G0**	**G8**	**L0**	**L8**	**N0**	**N8**	**S0**	**S8**
unidentified_Bacteria	18.86	18.94	22.48	18.46	21.13	18.94	20.16	18.67
Proteobacteria	15.62	17.31	21.76	19.98	15.85	17.72	14.84	20.03
Acidobacteriota	9.13	9.78	15.40	9.49	11.99	10.49	13.87	11.09
Actinobacteriota	17.65	11.37	5.12	6.84	10.67	8.58	10.48	6.64
Bacteroidota	8.40	7.55	5.87	7.50	7.30	7.61	6.93	8.60
Firmicutes	5.29	4.49	8.03	7.02	3.21	3.92	3.87	4.30
Verrucomicrobiota	3.37	2.60	2.12	2.29	5.74	4.44	7.26	3.39
Chloroflexi	5.22	5.55	3.26	5.03	4.50	4.70	4.10	3.92
Myxococcota	3.28	4.75	2.18	5.93	3.04	6.32	2.73	6.48
Crenarchaeota	0.37	2.24	0.53	1.15	2.44	0.73	1.96	2.52
Gemmatimonadota	1.40	1.37	0.22	1.59	0.97	1.45	0.68	1.14
WPS-2	0.03	0.01	0.93	0.02	0.02	0.02	0.01	0.01
Gemmatimonadetes	0.46	0.37	0.91	0.62	0.19	0.65	0.13	0.45
Cyanobacteria	0.28	0.18	0.34	0.20	0.35	0.15	0.87	0.23
Nitrospirota	0.38	0.86	0.47	1.05	0.76	0.97	0.68	1.47
Planctomycetota	0.29	0.44	0.75	0.52	0.56	0.56	0.31	0.32
Bdellovibrionota	0.71	1.12	0.32	1.28	0.66	1.26	0.58	1.11
Armatimonadota	0.55	0.28	0.10	0.19	0.59	0.29	0.68	0.21
Latescibacterota	0.43	0.73	0.12	0.81	0.63	1.16	0.47	0.63
Desulfobacterota	0.38	0.25	0.52	1.08	0.63	0.41	0.25	0.31
Elusimicrobiota	0.23	0.59	0.11	0.41	0.25	0.70	0.35	0.56
B. Bacterial Genera								
Acidobacteria genus RB41	1.21	1.58	0.27	1.76	2.52	2.31	4.02	3.45
Bryobacter	0.44	0.43	4.99	0.38	0.45	0.38	0.64	0.56
Sphingomonas	1.46	0.84	2.40	1.10	1.48	1.17	1.47	0.87
Proteobacteria genus *MND1*	1.32	2.68	0.34	3.44	1.44	2.79	1.46	2.25
Haliangium	1.24	1.70	0.80	2.44	0.90	3.00	0.78	2.27
Blautia	1.33	0.69	1.17	0.91	0.58	0.66	0.60	0.92

Gaiella	1.43	1.65	0.20	1.25	1.26	1.73	0.64	0.69
Pseudolabrys	0.12	0.13	2.91	0.13	0.24	0.13	0.11	0.16
Candidatus_Solibacter	0.23	0.22	2.36	0.29	0.32	0.23	0.40	0.16
Rhodanobacter	0.05	0.04	2.82	0.05	0.04	0.04	0.03	0.04
Arthrobacter	1.75	0.03	0.22	0.01	0.33	0.00	0.55	0.01
Acidobacteriota genus *Subgroup 10*	0.79	0.63	0.11	0.54	0.93	0.81	0.86	0.86
Bacillus	0.21	0.19	1.83	0.32	0.24	0.21	0.34	0.09
Iamia	1.27	0.51	0.08	0.29	0.60	0.47	0.47	0.50
Nocardioides	1.06	0.64	0.17	0.38	0.58	0.42	0.59	0.39
Hassallia	0.50	0.80	0.10	0.67	0.77	0.95	0.68	1.80

Table 3. Relative abundance of fungal taxa identified by ITS amplicon sequencing. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; T0; sampling day; T8, 8 weeks of drought.

Taxonomy	**Relative abundance (%)**							
A.Fungal phyla	**G0**	**G8**	**L0**	**L8**	**N0**	**N8**	**S0**	**S8**
Ascomycota	75.45	54.28	56.87	78.15	42.11	54.24	58.10	84.17
Basidiomycota	6.17	4.16	15.58	4.44	41.93	7.99	6.52	5.41
Mortierellomycota	3.91	5.05	0.28	3.95	4.77	7.45	5.58	1.59
Monoblepharomycota	0.01	0.00	0.00	0.00	0.02	0.00	1.79	0.01
Rozellomycota	0.09	0.28	0.26	0.10	1.13	0.03	0.08	0.83
Glomeromycota	0.13	0.08	0.01	1.51	0.36	2.00	0.63	0.23
Chytridiomycota	0.31	2.90	0.49	2.03	0.45	3.53	0.48	0.25
Zoopagomycota	0.11	0.31	0.00	0.30	0.34	0.85	0.25	0.24
Olpidiomycota	0.02	0.05	0.00	0.01	0.05	0.09	0.19	0.03
Mucoromycota	0.08	0.06	0.01	0.00	0.06	0.02	0.08	0.03
Aphelidiomycota	0.02	0.02	0.00	0.20	0.00	0.00	0.01	0.04
Kickxellomycota	0.00	0.00	0.00	0.01	0.02	0.03	0.00	0.00
Blastocladiomycota	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
B.Fungal Genera								
Panaeolina	0.06	0.00	0.02	0.00	36.48	0.06	0.05	0.05

Plectosphaerella	22.31	3.23	0.02	0.27	0.19	1.25	0.27	0.15
Kazachstania	0.00	3.84	12.88	0.00	0.00	0.02	0.01	0.01
Microdochium	1.12	0.42	0.01	0.30	0.53	0.78	5.46	0.48
Mortierella	3.79	4.96	0.25	3.87	4.59	6.16	5.14	1.56
Fusarium	2.87	1.92	4.58	0.66	2.60	1.26	3.62	6.12
Alternaria	0.64	0.79	4.55	0.34	0.71	0.63	2.28	0.51
Gibellulopsis	4.54	2.50	0.06	1.93	1.14	1.04	0.30	0.17
Meyerozyma	0.00	0.02	4.52	0.00	0.01	0.00	0.04	0.00
Ramophialophora	4.48	1.32	0.00	0.66	0.44	1.76	0.09	7.25
Cladosporium	1.60	1.08	0.04	0.73	3.81	3.52	3.37	1.83
Pyrenochaetopsis	0.10	0.36	0.00	0.08	0.81	0.26	3.19	3.54
Schizothecium	0.16	0.50	0.00	1.45	2.55	5.78	0.56	1.59
Blumeria	0.04	0.39	0.01	0.03	0.09	0.11	0.65	32.08
Ascomycota_unidentified	0.01	0.31	0.00	0.34	0.02	5.71	1.79	0.14
Ascomycota_unidentified_2	0.03	0.06	0.00	0.14	0.58	0.14	0.05	4.09
Basidiomycota_unidentified	0.01	0.00	0.00	0.00	0.44	4.00	0.01	0.24
Metarhizium	1.43	4.34	0.03	0.26	2.11	1.18	1.07	0.04
Trichoderma	0.12	0.42	0.16	3.49	0.44	2.24	0.38	0.08
Psathyrella	0.00	0.04	0.00	3.08	0.03	0.04	0.01	0.33
Biappendiculispora	0.01	0.00	0.00	0.12	0.42	2.66	0.87	0.58
Natantispora	0.01	0.12	0.00	2.07	0.17	0.53	0.01	0.00
Exophiala	0.18	0.70	0.05	0.30	0.84	0.89	2.15	2.04

CONCLUSION

The functioning of agricultural soil ecosystems, including their microbiomes, will undoubtedly be profoundly and intricately impacted by global change, primarily by drought conditions. The health of the soil and its biological activity are significantly impacted by drought conditions. The current state of knowledge about the effects of drought on soil ecosystems certainly has limits. Soil types are one constraint, and there is little knowledge on the impact of drought on agricultural soil ecosystems and associated microbiomes, especially in the temperate climate zone . These information gaps should be filled through targeted research of microbiomes from undersampled regions. Clearly, experimental manipulations such as warming or simulated drought circumstances are valuable sources of knowledge on prospective future agricultural soil development trajectories. Thus, their formation in unexplored areas is an important requirement. Furthermore, the changes in soil biological activities and microbial community to modification over time demonstrate that the running experiments should be continued over sufficiently long-time intervals to capture the entire period of drought conditions. Because global change is an ongoing process, establishing a time series of agricultural soil microbiome data is critical, as trend changes must be distinguished from random fluctuation in microbiome composition.

We gained an even better respect for the significance of our living soil while writing this thesis, as these precious resources are threatened by the detrimental repercussions of climate change. Soil, which many people 'take for granted' and even neglect, is a non-renewable resource that is currently depleting faster than it is being generated. Although not within the subject of this thesis, loss of fertile soils is increasing because of unsustainable soil management techniques. Soil microbiomes provide essential ecosystem services such as maintaining soil nutrients for plant uptake, and the importance of soil microbiomes in sustaining a healthy soil for future generations cannot be emphasized. Hence, this comprehensive study on the impact of water stress generates new insights about the modification of the soil microbiome, their enzymes, metabolic potential, and genetic diversity in an ecosystem.

There is an urgent need to better understand the effects of climate change on biogeochemical processes carried out by soil microorganisms, to utilize these insights to mitigate climate impacts, and develop strategies to tackle future drought conditions and soil degradation. Given their potential importance as limiting determinants of agricultural output, this research thesis focuses on impact of drought on microbial communities, nutrient cycles (most notably carbon, nitrogen, and phosphorus), their enzymes, functional metabolic profile, and genetic diversity in agricultural soil ecosystem processes. This study showed the significant impact of induced drought stress on above processes and provides evidence about the sensitivity of soil biological activities to water stress conditions in the investigated soil samples.

Our results from spring season suggest that the lowest average moisture content and enzyme activities were observed in the sandiest soil from Suchatówka (S) site. In case of number of microorganisms in given conditions there were significant changes and their tendencies were higher for Actinomycetota and fungi, but no change in bacterial abundance was noted in all sites by 8^{th} week. Overall, significant fluctuation and ultimately decrease in overall enzyme activities by the end of this experiment was noted. The metabolic profile showed reduction in the average well color development (AWCD) index, indicating overall decline in the utilization of carbon groups mainly carbohydrates, carboxylic and acetic acids, polymers and amines, but amino acid utilisation fluctuated at the end of the experiment, in all sites. On the other hand, relative abundance of Pseudomonadota, Basidiomycota and Apicomplexa showed significant lower tendencies, but Actinomycetota, Bacillota and Ascomycota abundance were higher under prolonged drought conditions in all sites.

While the results from autumn season showed significant negative effects of given water deficit conditions on microbial abundance, mainly in bacteria and Actinomycetota, but no significant change in fungi. While overall enzyme activities declined after 8 weeks. At the end of our experiment, overall decrease tendencies in the AWCD index followed by utilization of carbohydrates, carboxylic and acetic acids, amino acids, polymers, and amines groups along with decrease tendencies in soil moisture content in all sites was recorded. The genetic diversity revealed strong significant reduction in relative abundance of Actinobacteriota, Bacteroidota,

Acidobacteriota, *Bryobacter*, and Basidiomycota. Only Ascomycota abundance showed strong significant decrease in G site but increased in other 3 sites.

The outcome from both seasons indicates a trend that highlights the response of microbial populations to drought can differ along moisture gradients. In general, this research work clearly demonstrates the negative effect of water defient conditions on agriclutural soils, via reduced microbial abundance, enzyme activitties, and metabolic potential. The taxonomic variety of bacteria and fungi demonstrates the importance of soil moisture levels in microbial access to nutrients and motility. Thus, the soil environment is unstable and susceptible to the drought conditions, leading to slow-down of nutrient cycling. These findings have the potential to shed light on the role of bacteria in soil processes in agricultural ecosystems as a result of global climate change. Furthermore, climate change events can provide novel insights into how microbial populations respond to dry conditions and how it will affect the cultivation of crops in the future. Thus, understanding their response to global climate change is critical and should be utilized in the development of mitigation methods.

As demonstrated throughout this study, soil is the most important component of agricultural ecosystems in terms of microbial activity, and top layers of soils are the most targeted components of microbiome investigations. However, deep soil microbiomes that contribute to mineral depletion and carbon immobilization in organo-mineral complexes, as well as microbial communities from other less investigated niches in soil ecosystems, should be evaluated. Understanding the relationships between the direct effects of climate change (drought) on microbial community alterations, enzymes, metabolic potential, and taxonomicc diversity is critical for mitigating the deleterious impact on agricultural soils. Unfortunately, we realised that the lack of knowledge about extreme weather events makes dealing with their adverse consequences on ecosystems difficult. Perhaps numerous drought-resistance methods should be implemented in drought-prone areas. Water conservation, reduction in greenhouse gases, afforestation, mulching, organic fertilizer/manure usage, implementation of special drought-resistant microorganisms classified as drought-resistant plant growth promotion rhizobacteria (DR-PGPR) , may contribute beneficial effect in several drought prone regions.

To increase the clarity of future predictions of changes in microbial structure and communities, future improvements to fully comprehend the alteration in microbiological-derived soil functioning at the taxonomic diversity level during drought conditions in dynamic monitoring are required. This knowledge could be used to bridge the gap between present concerns and drought-related mitigation processes. In this view, because microbiome diversity is positively related to ecosystem productivity and may be affected by drought in agricultural land, understanding how these microbiomes interact with global change will be critical for optimizing or maintaining primary output in agricultural ecosystems.

Although I used several analyses to understand the impact of drought on soil biological activities to aid future climate change mitigation, I do not believe that this will be adequate to offset the soil loss and greenhouse gas production that are already arising. To accomplish this, these results must link microbiome investigations to the various Earth system climate models.

SUMMARY

The presented doctoral dissertation focusses on the effect of drought on soil biological activity such as microbial diversity, ecophysiology, and enzymatic activity, on agricultural soils. The findings acquired during the execution of the research enabled the following final conclusions to be reached:

- Prolonged drought of 8 weeks has a deleterious effect on the growth and activity of the soil microbial abundance and taxonomic diversity.
- The soil water regime strongly modified the activities of enzymes, leading to a slowing-down and /or affecting the nutrient cycles. Clay-rich soil was more resistant to suppression of soil enzymatic activity during soil water deficiency, whereas sandy soil resulted in significant soil enzyme inhibition.
- Drought induced substantial shifts in the metabolic potential of microbial communities in investigated soils. The use of most of the carbon substrates were strongly inhibited by water deficit conditions.

This research will give a practical and theoretical foundation for other researchers to investigate soil water deficit issues, as well as benefit water and soil resource management and protection. It will also advance the application of fractal theory in soil science, which will aid the advancement of soil science. Currently, there is an increasing emphasis in metagenomic research on determining specific taxa that are influenced by water stress circumstances. A deeper understanding of the changes in soil microbial community structure and functions will provide insights into nutrient cycling under climate change using this approach.

CURRICULUM VITAE

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Research gate: https://www.researchgate.net/profile/Kalisa_Bogati

EDUCATION:

Bachelor studies	Master's Studies	PhD studies
B.Sc. Microbiology (2010-2013) St. Xavier's college, Mapusa-Goa India, affiliated to Goa University	M.Sc. Microbiology (2013-2015) PIMS, Bangalore-India, affiliated to Bangalore University	Since 2019: Department of Environmental Microbiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun-Poland.

MICROBIOLOGY AND OTHER RESEARCH RELATED INTERESTS:

- Evaluating Community Level Physiological Profiling (CLPP) using 96-well Biolog Eco plates and determining soil humidity and pH.
- Biochemical analysis - enzyme activity such as hydrolases, dehydrogenases, phosphatases (acid and alkaline) and urease.
- RStudio and Linux environment- Basic understanding on processing scientific data using specific pipelines (eg. QIIME2, DADA2, PANDAseq etc.) and statistics, including pairing of Illumina sequences, quality check and filtering of sequence data, denoising, chimera check, and binning of sequences to ASV or OTU, to obtain a table of relative abundances of ASV/OUT.
- Basics on latest technologies (eg, phenotyping Microarray Omnilog system), techniques and bioinformatics tools (software and platforms) associated with prokaryotic systematics (polyphasic taxonomy) and genomic of Actinomycetes.
- Molecular techniques and interpretation- Deoxyribonucleic acid (DNA) extraction from soil, plants, bacteria, and fungi; Polymerase Chain Reaction (PCR); Gel electrophoresis; DNA quantification with Nanodrop spectrophotometer; GelDoc; Sequencing; Amplicon sequencing (16S and ITS regions).
- Immuno assays (staining) for determination of immunolocalisation of Abscisic acid, pectin and control plant tissue samples grown under normal and drought conditions.
- Computer skills: Microsoft Office (Word, PowerPoint, Excel), email communication, data visualization, spreadsheets, data entry and analysis.

- Polyphasic taxonomic studies based on phenotypic studies: includes phenotypic analyses based on morphological and growth culture properties, chemotaxonomic markers related to diaminopimelic acid, whole cell hydrolysates and polar lipids using standards chromatographic procedures, enzymatic profile of the microbial strains and their ability to metabolise a wide range of carbon and nitrogen sources, to grow in the presence of several inhibitory compounds and their resistance to antibiotics using API ZYM and GENIII microplates in an OmniLog system, respectively.
- Phylogenetic studies based on 16S rRNA gene sequences: bioinformatics tools (databases, software, and programs) for phylogenetic analysis based on a single gene. Construction of phylogenetic trees using Neighbor joining (NJ), Maximum parsimony (MP) and Maximum Likelihood (ML) algorithms. Analysis of the phylogenetic relationship of the microbial strains with their close neighbours and within the evolutionary radiation of their genera.
- Genome analysis: annotation of the genomes of the studied strains and screening for biosynthetic gene clusters (BGCs) related to secondary metabolites (antibiotics).
- Evaluation of the antimicrobial potential of the microbial strains based on plug essay tests.
- Isolation of cultivable microorganisms using specific microbiological media from soil, water, and plant samples.
- Salt tolerance, inhibition assay and *In vitro* experiments with plant-microbe association.
- Curator- Database Analyst.

LANGUAGES:

- English- B2/C1 level.
- Polish as beginner.
- Nepali- Native and Fluent level.
- Hindi- Native and Fluent level.
- Konkani- Native and Fluent level.
- Marathi- Conversational level.
- Other limited proficiency languages include Punjabi and Bengali.

WORK EXPERIENCE:

1. **PhD studies (from November 2019):** Currently I am pursuing my **doctoral studies** on topic entitled "The impact of simulated drought on changes in microbial biodiversity and soil biological activity" at Nicolaus Copernicus University, Torun-Poland.

2. **CSIR-NIO Goa-India (April 2017-October 2019):** Designation- **Project Assistant**.

   With the area of specialization including taxonomic studies of zooplankton, total bacterial count (TBC) by DAPI method, estimation and extraction of chlorophyll, lipid extraction from seawater sample (phytoplankton) and filtered water sample (from filtered paper), studies on phytoplankton ciliate association from the

seawater samples, copepod ciliate association from the seawater samples and primary productivity using ^{14}C radioisotope experiment and bacterial productivity using thymidine experiment on Cruises (SSD_40, SSD_44 and SSD_55). I am a part of various offshore, coastal, and deep-sea projects including SSP-3070, SSP-3140, SSP-3134, SSP-3216, SSP-3164, SSP-3136, GAP3129, MLP-1702, GAP-2949, SSP-3115 and GAP3196. Through my work, I have gained expertise in Stereo microscope, epifluorescence microscope, Nikon Eclipse Ti2 Confocal microscope, manifold filtration unit, HT plankton net, vacuum evaporator and nitrogen purging cylinder.

3. **Molecular Connection Pvt. Ltd. Bangalore-India (July 2015-March 2017):** Designation– **Curator,** as Scientific Analyst (MNP Project) in Molecular Connection Pvt. Ltd. Bangalore for 1.8 years and the area of work was to build database (methods and protocols) from the respective research papers.

SCIENTIFIC INTERNATIONAL PUBLICATIONS:

1. **Bogati, K. A.**, Sewerniak, P., & Walczak, M. (2023). Effect of changes in soil moisture on agriculture soils: response of microbial community, enzymatic and physiological diversity. Ecological Questions, 34(3). doi: 10.12775/EQ.2023.0431. IF: 0.312.
2. **Bogati, K. A.**, Golińska, P., Sewerniak, P., Burkowska-But, A., & Walczak, M. (2023). Deciphering the Impact of Induced Drought in Agriculture Soils: Changes in Microbial Community Structure, Enzymatic and Metabolic Diversity. Agronomy, 13(5), 1417. doi: 10.3390/agronomy13051417. IF: 3.949.
3. **Bogati, K.,** & Walczak, M. (2022). Review- The impact of drought stress on soil microbial community, enzyme activities and plants. Agronomy, 12(1):189. doi: 10.3390/agronomy12010189. IF: 3.949.
4. Wilmowicz, E., Kućko, A., **Bogati, K.,** Wolska, M., Świdziński, M., Burkowska-But, A., & Walczak, M. (2022). *Glomus* sp. and *Bacillus* sp. strains mitigate the adverse effects of drought on maize (*Zea mays* L.). Frontiers in Plant Science, 13. doi: 10.3389/fpls.2022.958004. IF: 6.627.
5. Fernandes, V., & **Bogati, K.** (2022). Analysis of Bacteria–Phytoplankton relationships at three discrete locations in the Eastern Arabian Sea during winter. Continental Shelf Research, 243, 104751. doi: 10.1016/j.csr.2022.104751. IF: 2.629.
6. Chatterjee, T., Dovgal, I., Nanajkar, M., **Bogati, K.** (2019) Note on the genus *Lecanophryella* (Ciliophora: Suctorea) with description of a new species from west coast of India. Zootaxa, 4612 (4): 494-500. doi: 10.11646/zootaxa.4612.4.2. IF: 1.091.
7. Fernandes, V., & **Bogati, K.** (2019) Persistence of Fecal Indicator Bacteria associated with Zooplankton in a Tropical Estuary-West Coast of India. Environmental Monitoring and Assessment, 191:420. doi: 10.1007/s10661-019-7531-z. IF: 3.420.
8. Nanajkar, M., Fernandes, V., **Bogati**, K., Chatterjee, T. (2019) Gregarious growth of ciliate *Vorticella oceanica*, on a chain forming diatom *Chaetoceros coarctatus*: Indicating change in the function of association. Symbiosis, 1-9. doi: 10.1007/s13199-019-00640-4. IF: 3.109.

ACHIEVEMENTS, AWARDS AND GRANTS:

1. Received Erasmus+ funds (1770 €) for student mobility for traineeships at Julius Kühn – Institut, Federal Research Centre for Cultivated Plants (JKI) Institute for Epidemiology and Pathogen Diagnostics Messeweg 11-12, 38104 Braunschweig, Germany, from 12th January to 14th April 2023.
2. Received IDUB Excellence Initiative grant (15,400.00 PLN gross) - Mobility for doctoral students as part of the "Excellence Initiative - Research University" program, at Julius Kühn – Institut, Federal Research Centre for Cultivated Plants (JKI) Institute for Epidemiology and Pathogen Diagnostics Messeweg 11-12, 38104 Braunschweig, Germany, from 12th January to 10th February 2023.
3. Shortlisted as PROM Laureates (PROM project-II) on 2nd March 2022 and received funds (6,099.00 PLN) from Polish National Agency for Academic Exchange NAWA for active conference participation (miCROPe 2022 SYMPOSIUM "MICROBE-ASSISTED CROP PRODUCTION OPPORTUNITIES, CHALLENGES & NEEDS", held onsite from July 11th to 14th 2022, Vienna, Austria).
4. Received Erasmus+ funds (1220 €) for student mobility for traineeships at Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures, 38124 Braunschweig, Germany, from 6th May to 6th July 2022.
5. Received IDUB Excellence Initiative grant (26,944.00 PLN gross) - Mobility for doctoral students as part of the "Excellence Initiative - Research University" program, at Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, 38124 Braunschweig, Germany, from 6th May to 6th July 2022.
6. Shortlisted and received 5,000.00 PLN from Grants4NCUStudents grant (4th edition) under the "Excellence Initiative – Research University" programme on 22nd June 2022 for research scientific activities.
7. Participated on-board R.V. Sindhu Sadhana (Research expedition SSD-55) from 2nd - 31st August 2018 as a part of the project MLP1702 "Impact of the climate change on Physics, Biogeochemistry and Ecology of the North Indian Ocean (CliCNIO)".
8. Participated on-board R.V. Sindhu Sadhana (Research expedition SSD-44) from 21st November to 23rd
   December 2017 as a part of the project GAP2949 "Biogeochemical process and Paleooceanographic Studies of the Eastern Indian Ocean" funded by MoES, New Delhi.
9. Participated on-board R.V. Sindhu Sadhana (Research expedition SSD-40) from 3rd - 28th August to study "The impact of the climate change and rapid warming of the Arabian Sea on Indian Monsoon and bio resources" as a part of the project MLP-1702.
10. Participated in a National conference on "ENZYME RESEARCH IN AGRICULTURE, FOOD AND INDUSTRIAL BIOTECHNOLOGY (NCERAFIB-2015)" held at Maharani College for Women, Bangalore, India on 12th - 13th March 2015. Awarded 3rd position for M.Sc. dissertation oral presentation entitled "COMPARATATIVE ANALYSIS OF FECAL INDICATOR BACTERIA IN WATER AND ZOOPLANKTON IN ZUARI ESTUARY".

PARTICIPATION IN NATIONAL AND INTERNATIONAL CONFERENCES:

1. Participated and presented poster with flash talk entitled "The study on impact of drought stress on microbial communities and their enzyme activities in agricultural soil" in an international conference miCROPe 2022 SYMPOSIUM "MICROBE-ASSISTED CROP PRODUCTION OPPORTUNITIES, CHALLENGES & NEEDS", held onsite during July 11-14, 2022, Vienna, Austria.

2. Participated and presented poster entitled 'Immunocytochemical Assessment of Pectin Composition Remodeling in Maize Leaves in Response to Drought Stress' for the online conference Microscopy at the Frontiers of Science www.mfs2021 held from 29th September - 1st October 2021.
3. Participated and presented poster entitled 'Impact of drought stress on agricultural soil microbial communities and their enzyme activities' for the conference „54. KONFERENCJA IKROBIOLOGICZNA „MIKROORGANIZMY RÓŻNYCH ŚRODOWISK" Lublin, held on 20th - 21st September 2021 online.
4. Participated and presented poster entitled 'A study on the impact of soil moisture on microbiological diversity and their enzyme activity in agricultural soil' for the Virtual Conference „PLANT PRODUCTIVITY AND FOOD SAFETY: SOIL SCIENCE, MICROBIOLOGY, AGRICULTURAL GENETICS AND FOOD QUALITY" Nicolaus Copernicus University, Torun-Poland held on 15th – 17th September 2021.
5. Participated in the online conference Virtual Plant Biologicals Network Symposium on 12th November 2020 organized by Plant Biologicals Network Department of Plant and Environmental Sciences, University of Copenhagen.
6. Attended 'Silver Jubilee National conference on Mangrove ecosystem' on 26-27th July 2017, organised by Mangrove Society of India in association with CSIR-National Institute of Oceanography, Dona Paula, Goa-India.

PARTICIPATION IN WORKSHOPS AND OTHER WEBINARS:

1. Participated in online International Workshop on "Bioinformatics" on 8th May 2023 organised by the Department of Human Physiology, Holy Cross College, Agartala-India in collaboration with MycoAsia Journal of Modern Mycology, India.
2. Hands on online training on "Analysing microbial community structure using QIIME2 and data visualization" from 28th to 30th April 2023 organized by MYTOSCI-India.
3. Hands on online training on "Metagenomics: Basics of microbial community structure analysis using QIIME2" from 17th to 18th December 2022 organized by MYTOSCI-India.
4. Attended online seminar on "Tuesday with Science- prof Margaret Niznikiewicz" on 25th October 2022 organised by Faculty of Health Sciences Collegium Medium in Bydgoszcz.
5. Successfully completed online MycoAsia Workshop on "Advanced Phylogenetic Analysis using Workshop" on 9th October 2022, organized by MycoAsia Journal of Modern Mycology, India.
6. Participated in online webinar entitled "Next Generation Sequencing and its Clinical Applications" on 22nd September 2022 organized by Novogene.
7. Hands on online training on "Research Methodology and analysis using R programming" from 24-25th September 2022 organized by MYTOSCI-India.
8. Participated online session on "Metagenomics: Basics of microbial community structure analysis using QIIME2" on 4th September 2022 organized by MYTOSCI, India.
9. Successfully completed online MycoAsia Workshop on "Phylogenetic Analysis using MEGA software" on 11th June 2022, organized by MycoAsia Journal of Modern Mycology, India.
10. Participated online workshop on "Oral presentation online and offline" from November 15th - December 5th, 2021, offered by Natural Science careers, Benedendorpsweg 13, 6862 WB Oosterbeek, Netherlands.

11. Participated online workshop entitled "PhD! And, next? Career options, skills, and orientation for scientists" from September 27th - October 17th, 2021, offered by Natural Science careers, Benedendorpsweg 13, 6862 WB Oosterbeek, Netherlands.
12. Participated online National Training Workshop on "Research Article Writing and Plagiarism" organized by Zoology & Biotechnology Department and IQAC of Government Girl's P.G. College, Ujjain-India on 18th September 2021.
13. Participated online webinar entitled 'ORGANIC GROWING MEDIA-HOW CAN IT AFFECT THE HEALTH OF MY TRANSPLANTS?' on 2nd December 2020 organized by Oregon State University.
14. Special online event entitled 'Plantae Presents Creating Crops for the Future: Challenges, Technology and Sustainable Solutions' on 11th November 2020 hosted by Australian Research Council Centre of Excellence for Translational Photosynthesis and Australian Research Council Centre of Excellence for Plant Energy Biology.
15. Attended online seminar on POSTHARVEST 2020 WEBINAR SERIES organized by Plant and Food Research Rangahau Ahumāra Kai from 10th - 12th November 2020.
16. Attended online seminar entitled 'Opening agricultural research and data' organized by Centre for Agriculture and Bioscience International (CABI) on 11th November 2020.
17. Attended online seminar entitled "Highly specific multiplexed RNA imaging in tissues with split-FISH on 19th November 2020", "From Protein Structures to Drug Discovery: Novel therapeutic opportunities on 21st November 2020", and 'High-throughput CRISPR editing using the Onyx platform identifies essential residues in proteins' on 26th January 2021", organized by Labroots- the leading scientific social networking website and producer of educational virtual events and webinars.
18. Participated online workshop entitled 'The 6th Annual Nordic Plant Phenotyping Network Workshop 2020' on 27th November 2020 organized by The Nordic Plant Phenotyping Network Department of Plant and Environmental Sciences University of Copenhagen.
19. Participated on-site "Skill Training Workshop on Extremophilic Bioprocessing for Industrial Biotechnology" organised by CSIR-National Institute of Oceanography, Dona Paula, Goa during 7 - 9th August 2019.
20. Attended Hands-on Training Workshop on "TAXONOMIC IDENTIFICATION OF COASTAL AND OCEANIC COPEPODS (TICOC-2018)" during 25th – 28th, September 2018 sponsored by Ministry of Environment, Forest, and Climate Change (MoEF&CC), Govt. of India, under All India Co-ordinated Project on Taxonomy (AICOPTAX) organized by Marine Planktonology & Aquaculture Laboratory, Department of Marine Science, Bharathidasan University, Tiruchirappalli-24, Tamil Nadu.
21. Actively participated (oral presentation of M.Sc. project) in seminar entitled "POST- GENOMICS TECHNOLOGIES FOR BETTER LIFE" held on 19th March 2015 in Garden City College, Bangalore.