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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 p-nitrophenylphosphate (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 11^{th}-12^{th} 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 (0.05-0.002 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 Coleman-Derr, 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 6A-F). 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 8^{th} week. Carboxylic and acetic acids, amino acids, and amines are significantly less (or slower) metabolized by microorganisms inhabiting in N site at 8^{th} 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 8^{th} 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}

n = ?

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.

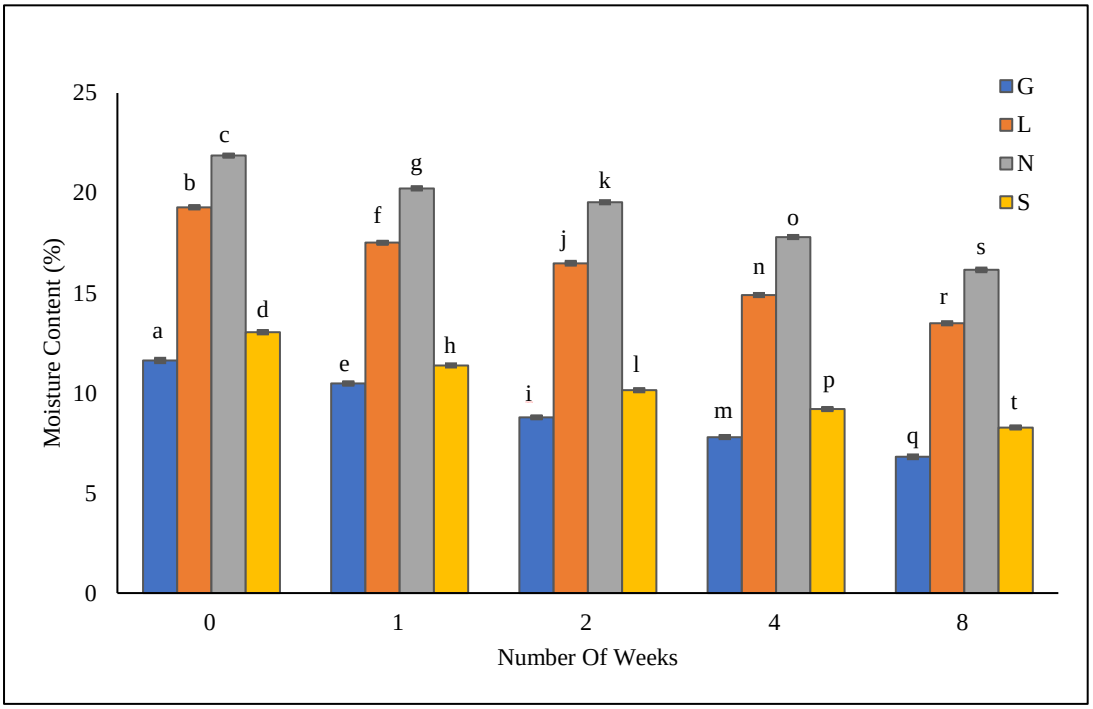

Figure 2. Soil moisture content under prolonged drought conditions in samples collected from Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S). Mean values described with the letters (e.g., a-t) are significantly different at $p < 0.05$. Error bars indicate standard errors of the mean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

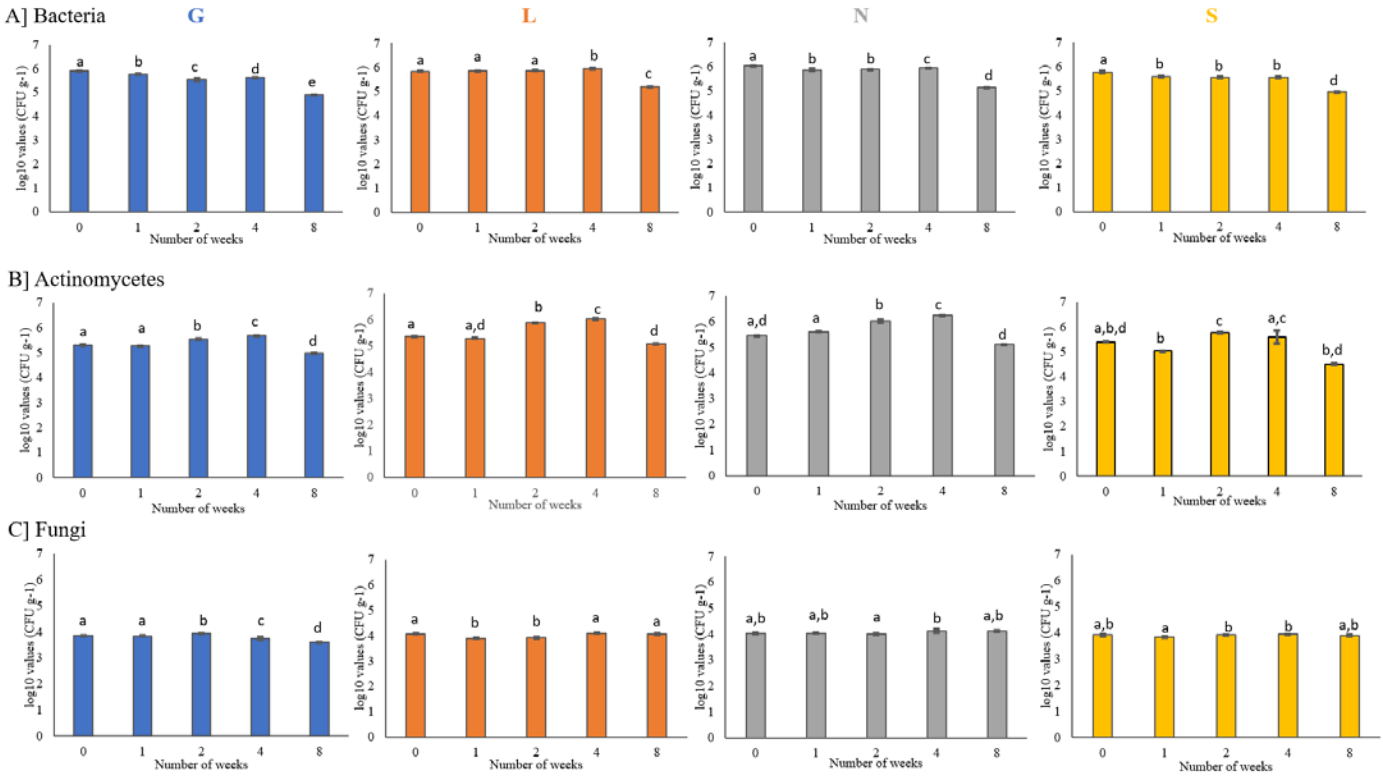

Figure 3. Changes in number of bacteria, actinomycetes and fungi under prolonged drought conditions in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). All analyses were performed in five replicates and the data are presented as mean ± SD. Mean values described 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).

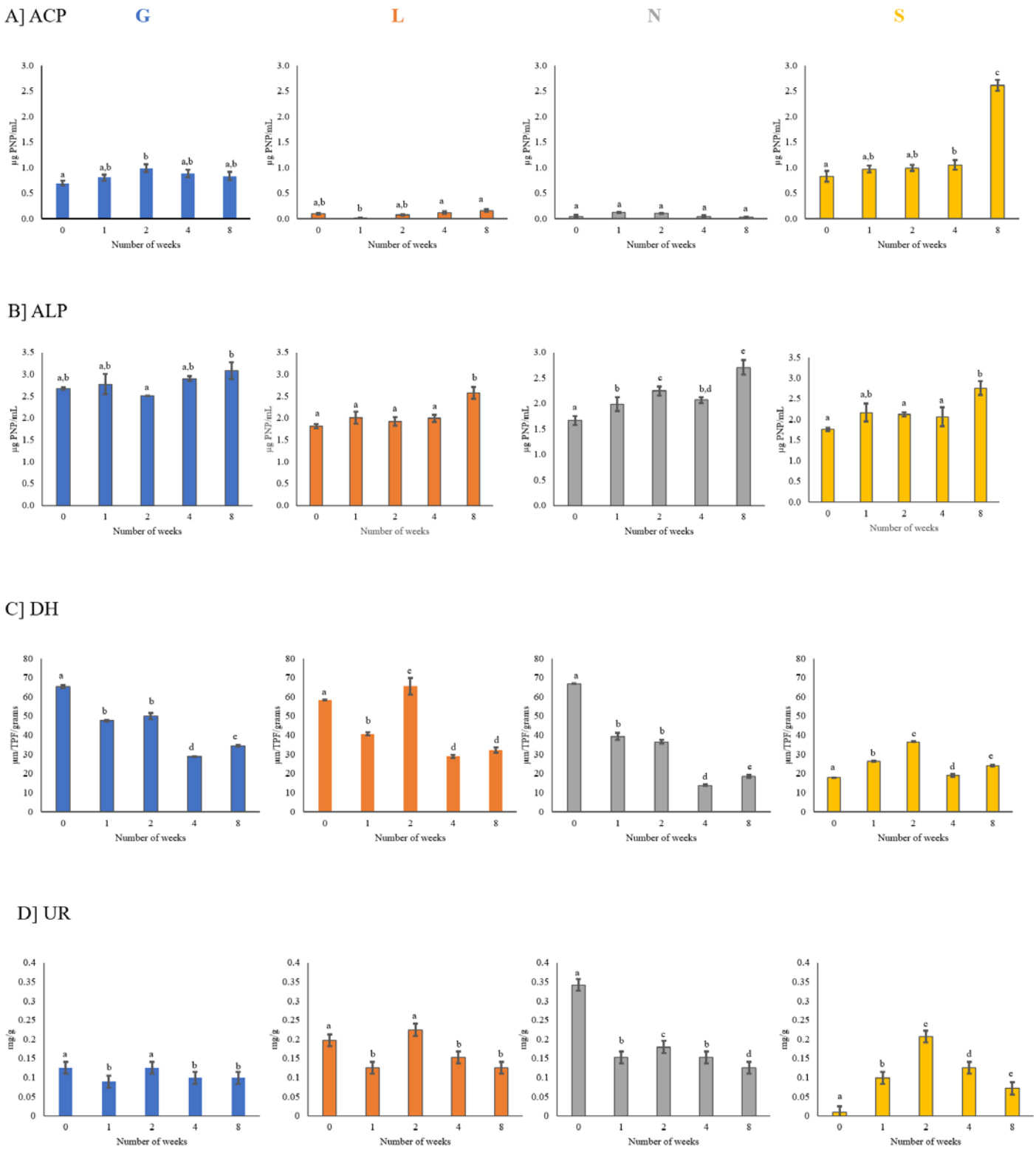

Figure 4. Enzyme activities under prolonged drought conditions in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)). [A] Acid phosphatase (ACP); [B] Alkaline phosphatase (ALP); [C] Dehydrogenase (DH); [D] Urease (UR) enzyme activities. All analyses were

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.

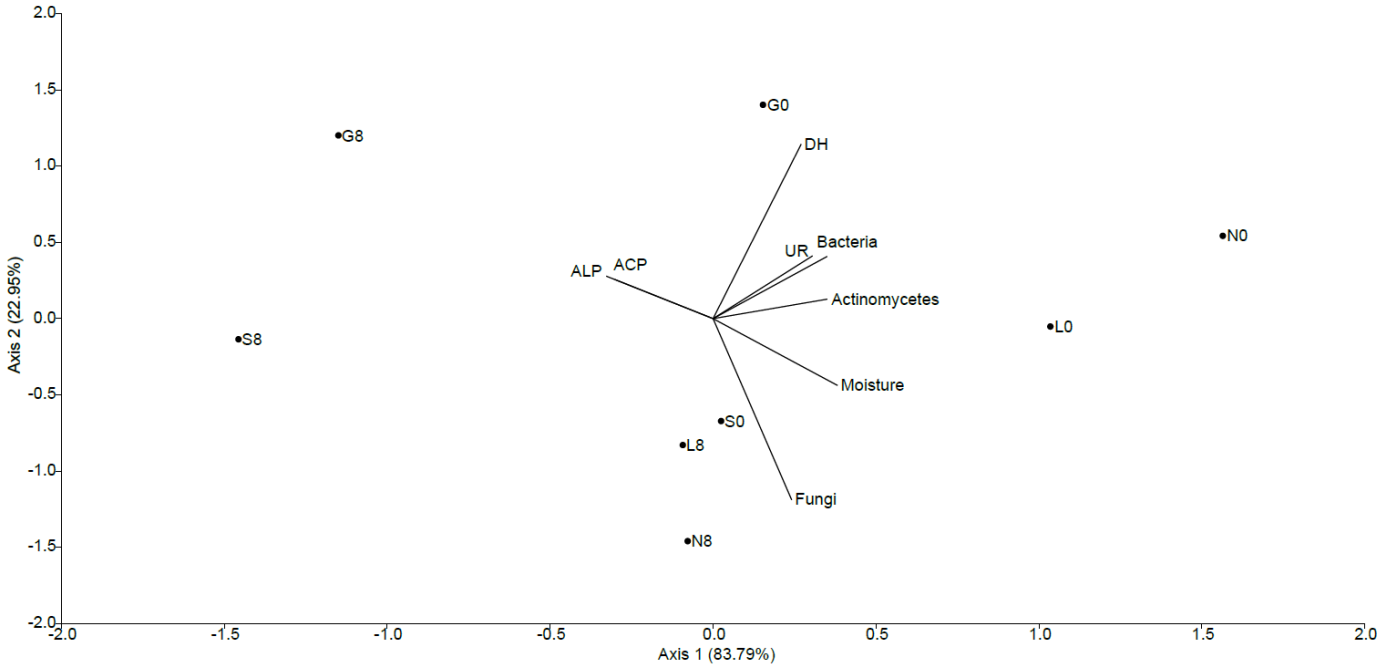

Figure 5. Principal component analysis (PCA) diagram indicating correlations between soil physicochemical, total bacterial, Actinomycetes and fungal abundance, and enzyme activities in four soil samples at T0, T1, T2, T4 and T8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; ACP, 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).

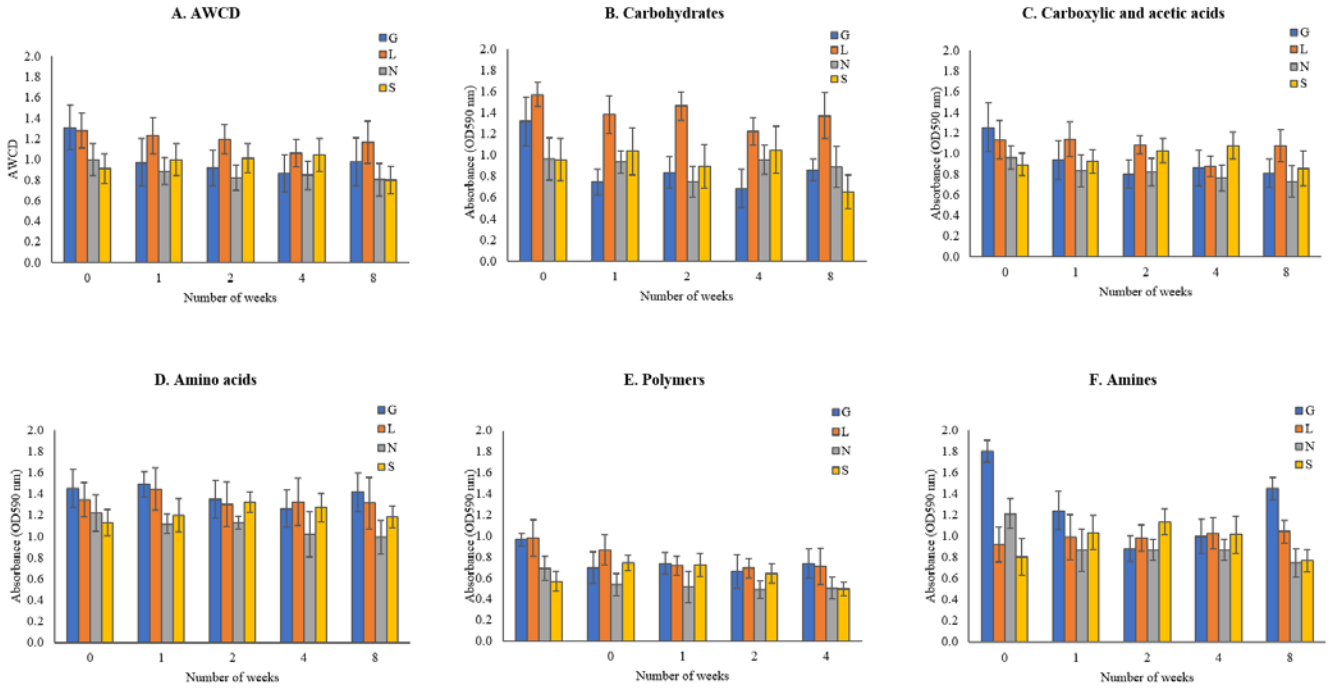

Figure 6. Absorbance values of Biolog-Ecoplates in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) with carbon substrate utilization efficiency. [A] Average rate of the average well colour development (AWCD) over the incubation time (ΔAWCD/weeks); Metabolism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

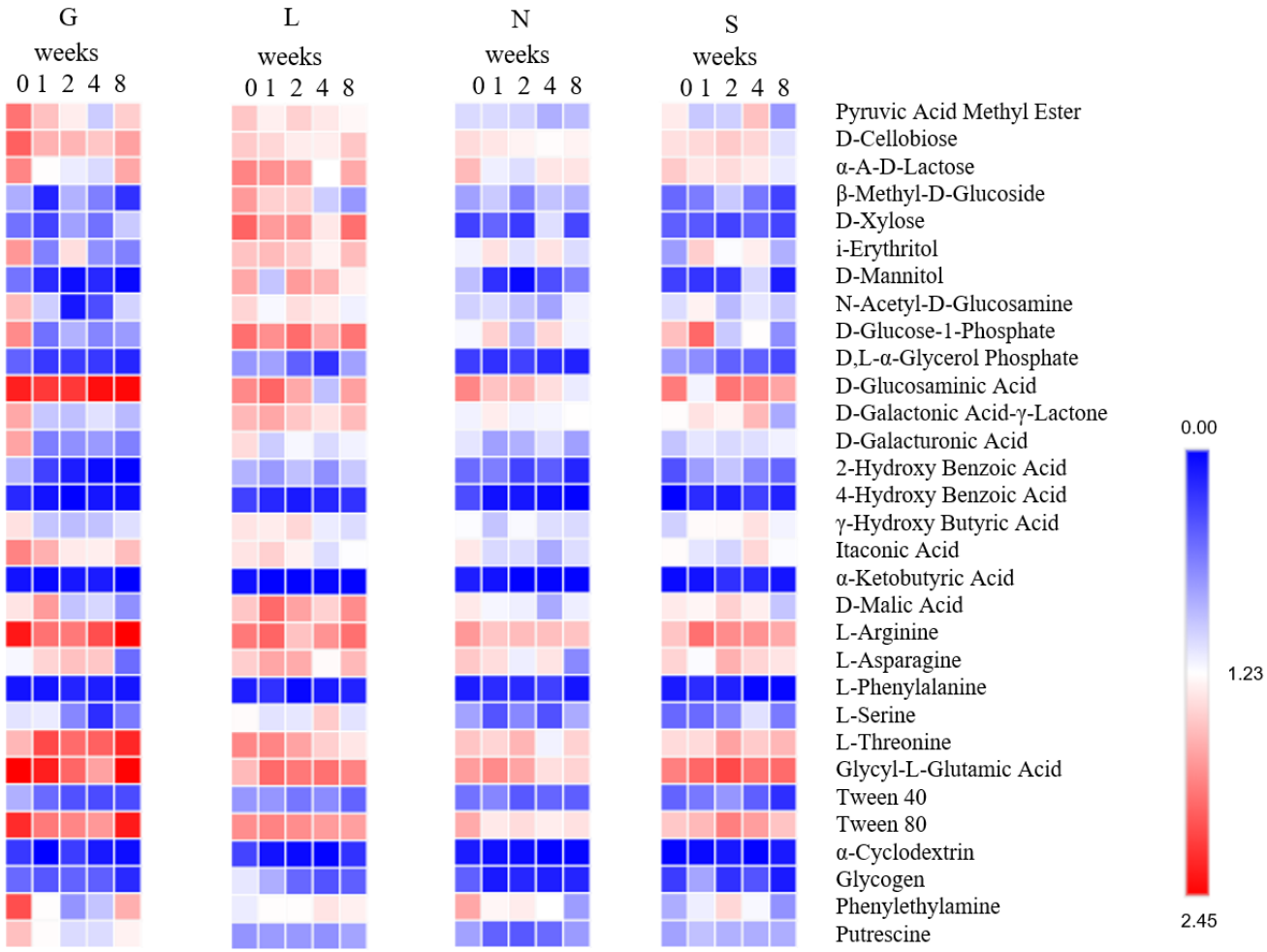

Figure 7. Heat map for community level physiological profiles (CLPPs) in investigated soil samples (Gniewkowo (G), Lulkowo (L), Wielka Nieszawka (N) and Suchatówka (S)) with mean absorbance values (λ = 590 nm).

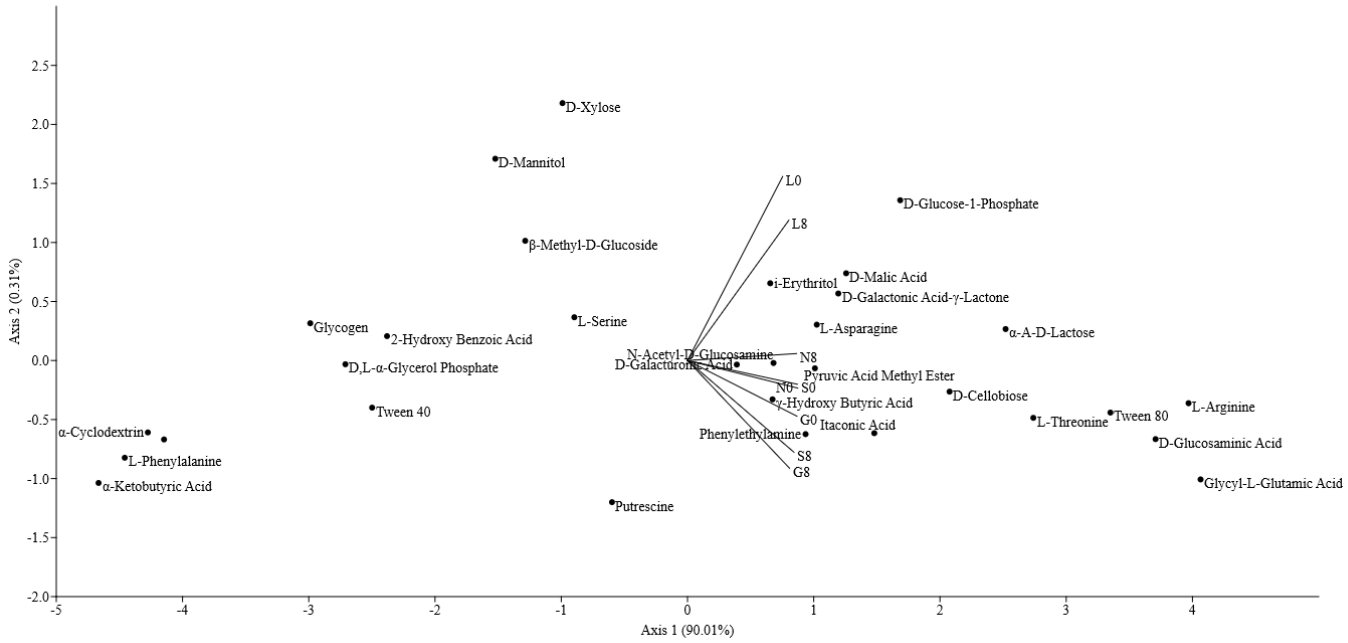

Figure 8. Principal component analysis (PCA). Average well color development (AWCD) index of 31 carbon substrates in four soil samples at T0 and T8 weeks. G, Gniewkowo; L, Lulkowo; N, Wielka Nieszawka; S, Suchatówka; G0 (G at week 0); G8, (G at week 8) (likewise for L, N and S site).

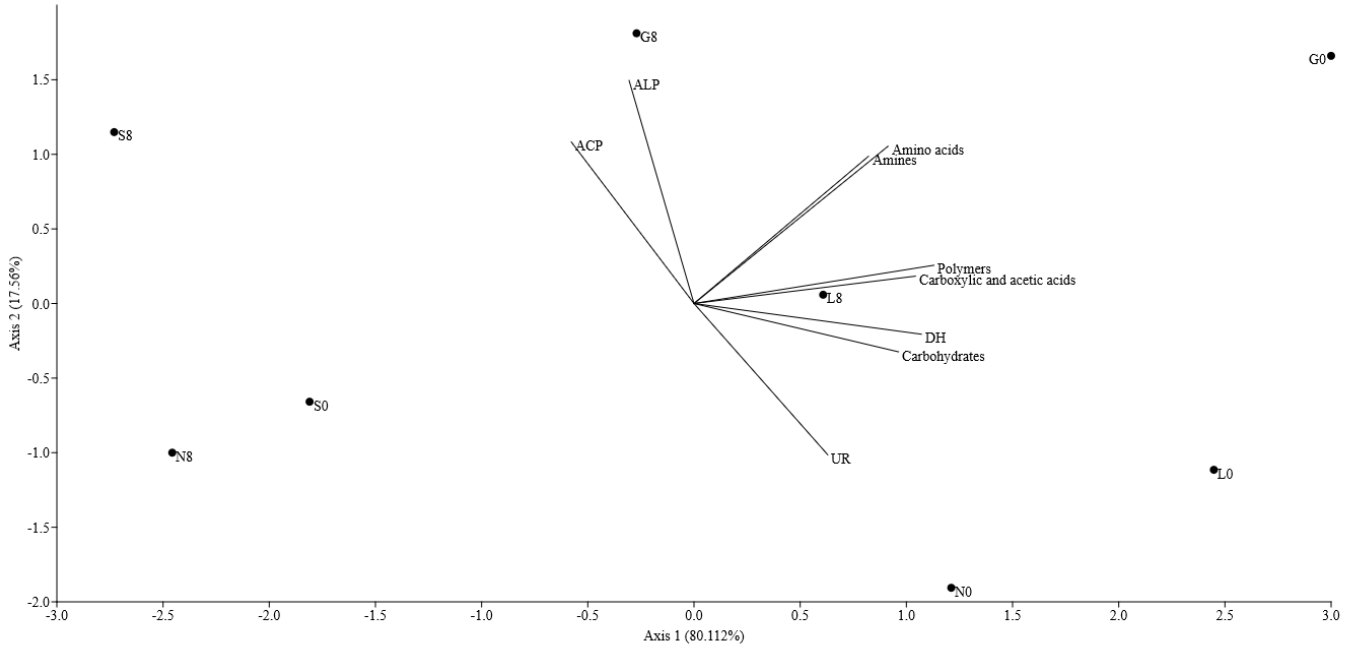

Figure 9. Principal component analysis (PCA) for Average well color development (AWCD) index of 5 groups of carbon substrates (carbohydrates, carboxylic and acetic acids, amino acids, polymers and amines) and enzyme activities (ACP, Acid phosphatase; ALP, Alkaline phosphatase; DH,

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