



Would fertilization history render the soil microbial communities and their activities more resistant to rainfall fluctuations?

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ABSTRACT

Water stress and nutrient supply are two of the most ubiquitous global changes that surely drive substantial variations not only in agricultural productivity but also extend to alert soil living organisms. The present study aims to understand the intrinsic changes in the composition of soil populations and their functions due to the interaction between long-term fertilization and rainfall fluctuations, seeing whether fertilization history would render the soil microbial communities and their activities more resistant to water stress or not. The experiment was established in 1988 on a typical meadow soil (Vertisols) as a rainfed maize monoculture receiving six elevated rates of NPK annually. The 30-year average annual precipitation of the growing season in this region is 345.1 mm. However, in 2010 rainfall was 106.1% greater than the average, while in 2011 it was 26.5% lower. The results show that long-term NPK fertilization has made the soil microbes more tolerant to changes in soil moisture content resulting from rainfall fluctuations. Soil microbes and their activities, however, did not follow a dose-response relationship of NPK as soil moisture content was the main driving factor. Numbers of total fungi, cellulose decomposing bacteria, and nitrifying bacteria increased as rainfall in 2010 increased. Moreover, microbial biomass carbon in 2010 was almost 2-fold higher than in 2009. Soil respiration in 2010 was 11 and 35% higher than in 2009 and 2011, respectively. Otherwise, high rainfall in 2010 significantly diminished soil NO_3^- content and nitrification rate. Soil enzyme activity showed a higher response to soil moisture than the rate of NPK. The highest activity of phosphatase, dehydrogenase, and saccharase was measured in the driest year (2011), while urease displayed its highest activity in 2010. High rates of NPK significantly reduced soil dehydrogenase activity. These results illustrate how important it is for fertilizer programs to be flexible to match expected climate change in order to improve productivity and reduce environmental pollution.

1. Introduction

Sustainability of crop production is an environmentally-friendly approach that cares about the health of the environment, biodiversity and agricultural crops. It must be adapted to ecological conditions and economic needs. In the same manner, water stress and nutrient supply are two of the most ubiquitous global changes that threaten sustainable crop production; also they surely drive substantial variations not only in the ecology of the aboveground of soil system but also extend to the living organisms in the soil. The composition of soil communities and their functions and services which mainly control the soil health and the efficiency of nutrient cycling heavily depend on these two drivers (Siebert et al., 2019).

Anthropogenic activities drive certain changes in our ecosystems that can hurt and disable the functions of these ecosystems. Soil ecosystem, including soil living organisms, is among the ecosystems that directly suffer from these changes (Steffen et al., 2006). Climate change is not expected to only increase drought events in some regions but also to increase the precipitation in other regions. Generally, climate change is predicted to mainly affect the frequency and quantity of rainfall (IPCC, 2007). Simultaneously, this will vary the response of soil microorganisms which are responsible for vital soil ecosystem functions such as mineralization of organic matter and turnover rates of nutrient cycling (Galloway et al., 2008).

However, only meager information is presented in the pieces of literature concerning the crucial role of soil biota under such global

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Fig. 1. Maize monoculture under long-term NPK fertilization and rainfed cultivation.

change. Consequently, comprehensive research on the dynamics of soil communities is a key step to understand and predict the behavior of soil ecosystem particularly in such a changing world (Eisenhauer et al., 2012). Both soil moisture and fertilizer contents affect the growth and activity of soil populations. Therefore, severe drought and/or soil logging (excess water) are likely to have a negative influence on their community structure and functions (Riutta et al., 2016). Low soil moisture content was reported to decline soil microbial biomass, soil respiration and diminish the microbial community structure (Hueso et al., 2012). Kardol et al. (2010) documented that soil bacteria and fungi behave oppositely under drought conditions as fungi are more dominant and function in a better way under this condition. Stevnbak et al. (2012) experienced that the lack of precipitation in the summer negatively affected the quantity of cellulose decomposing microorganisms and the quantity of microbial biomass.

Another key factor affecting the dynamics of soil populations is soil fertility. Nutrient fortification, directly and indirectly, influences soil microbial communities. Directly, it supplies the soil microorganisms by their required energy and carbon resources; while it alerts the physicochemical properties of soil which indirectly affect the structure of soil microbes as well as their function performance (Liu et al., 2010). Soil pH, texture, porosity, water holding capacity, organic carbon, and humus content are among the characters affecting soil microorganisms. Moreover, the availability of soil nutrients is affected essentially by the soil moisture content. The lack of a stable soil structure would result in

lower water retention capacity and water permeability, so a lower level of water absorption would be available for plants (Wall et al., 2012).

Nevertheless, several studies showed that the application of NPK negatively affects soil respiration due to a reduction in the activity of soil microorganisms and their structure (Pan et al., 2014; Ramirez et al., 2010; Kátai, 1999, 2006, 2014). Conversely, Li et al. (2014) stated that mineral fertilization enhanced the soil carbon pools and consequently improved soil biodiversity.

Kennedy and Gewin (1997) listed the factors that favor the maintenance of diversity in microbial populations, these are the agricultural management practices that conserve or increase the soil microbial biomass, enable niche environments to develop soil physical properties and provide a range of organic compounds on a regular base. In the long-term experiment, Mikanová et al. (2015) measured high values of saccharase and urease activities, total N content and microbial biomass carbon in treatment fertilized with farmyard manure combined with mineral fertilizers.

The present study aims to investigate the impact of NPK fertilization and fluctuation of soil moisture content due to a significant variation in precipitation amount on the soil chemical biochemical and microbiological characteristics of a 21-23-years old fertilization experiment. Also, the resistance of soil microbial communities and their functions to water stress due to long-term NPK fertilization was assessed. Soil samples were collected from control and five fertilized plots in the 21st, 22nd and 23rd years of the experiment (2009, 2010, and 2011). We

were curious about how the fertilization and the abnormal distribution of rainfall could affect the available nutrient and organic matter content of the soil, as well as the dynamics of microbial populations, the parameters of degradation processes, (net nitrification, CO₂-production, microbial biomass carbon and nitrogen, and different enzymes activities.

2. Materials and methods

2.1. Experimental location and soil sampling

A maize monoculture experiment was established in 1988 near to Görbeháza, Debrecen, Hungary (N47°81' E21°23'). The field is very near to the small river of Hortobágy. The field is cultivated by a rain-fed maize monoculture and fertilized continuously at different doses of NPK, i.e., control, (N₄₀P₂₅K₃₀), (N₈₀P₅₀K₆₀), (N₁₂₀P₇₅K₉₀), (N₁₆₀P₁₀₀K₁₂₀) and (N₂₀₀P₁₂₅K₁₅₀) kg ha⁻¹, in form of N, P₂O₅, and K₂O, respectively (Fig. 1). The type of the experimental soil is a typical meadow soil (Vertisols according to WRB). The soil texture is a clay-loam according to the fraction of clay-silt (50–56 %, respectively). The Arany-type plasticity index is 43–44. According to the Arany-types plasticity index, the soil texture was, also, a clay-loam. The physico-chemical properties of soil at start of the experiment in 1988 are not significantly different from today's control values (i.e., silt and clay: 50–56%; pH(H₂O) 7.5–8.0; organic carbon 19.0–21.0 g kg⁻¹; organic nitrogen 1.85–2.15 g kg⁻¹). The content of NPK in plant tissues the control does not also show significant but higher values than in 1988. In Hungary, maize cultivation is usually between 10 and 25 April when the soil temperature ranges between 8 and 10 °C. The optimal harvest time of maize usually starts from 15th September to 5th October depending on the plant variety, biological maturity, the moisture content of the maize kernels, and the weather conditions, mainly rainfall. Soil samples were collected from the upper 2–20 cm of soil affected by the root system of maize plants during 2009, 2010, and 2011. Soil sampling was conducted twice a year (in spring and autumn), making a total of six sampling times per treatment within the three years of the experiment with four replicates. However, we present only the annual average of spring and autumn samples since many results would have been presented. For chemical analysis, air-dried soil samples were ground using stainless steel crushing machine for homogeneity and sieved through a 2-mm mesh. For biological measurements, after removing plant debris and gravel, the fresh samples were sieved with a 2-mm mesh and kept in polyethylene pages below 4 °C for further analysis. The annual average precipitation is about 550–600 mm year⁻¹ in this region of the continental climate. The meteorological data during the experimental period from 2009 to 2011 presented in Table 1, interestingly, showed a significant difference in the annual average precipitation of the growing season: during these three years. It was 396.6 mm (in 2009), 711.2 mm (in 2010) and 253.6 mm (in 2011).

2.2. Determination of physicochemical properties of soil

Soil moisture content was measured gravimetrically by drying the soil at 105 °C for 24 h and expressed as m/m% (dry basis) of absolute

mass of dry soil. Soil pH was measured in 1:2.5 (w/w) soil: distilled water suspension (Buzás, 1988). The uptakeable AL-P₂O₅ and -K₂O of the soil was measured using 0.1 M ammonium-lactate-acetic acid (AL) solution (pH 3.7). The determination of phosphate was performed spectrophotometrically at 660 nm wavelength (Egnér et al., 1960), while potassium was measured with a digital flame photometer (Sherwood Model 410). The analysis of soil NO₃⁻ content was done using Na-salicylate as reagent; absorbance was measured with a spectrophotometer (UV-430) according to Felföldy (1987). Soil humus content was determined by Székely et al. (1960) and the organic carbon content was calculated from this result.

2.3. Analysis of soil microbial community

Regarding the population dynamics of microorganisms, the total number of bacteria and microscopic fungi was determined using plate dilution technique on bouillon and peptone-glucose agar medium, respectively. The aerobic cellulose decomposing and nitrifying bacteria were counted with the MPN (Most Probable Number) method in liquid culture media (Trolldenier, 1996). For determining the microbial activity in soil, the CO₂-production was measured after 10 days incubation with NaOH-trapping (Öhlinger, 1996), the microbial biomass carbon (MBC) was measured using the chloroform fumigation-extraction methods (Vance et al., 1987), net nitrification was measured after 14 days incubation with the Na-salicylate reagent (Felföldy, 1987). The quantitative determination of monosaccharides originated from the breakdown of saccharase was measured by the method of Frankenberger and Johanson (1983); saccharase activity was expressed as mg glucose 100 g⁻¹ soil 24 h⁻¹. Measurement of urease activity was done based on the quantitative determination of ammonia by the spectrophotometric method at 660 nm by Kemper's method (Filep, 1995). Phosphatase activity was measured by Öhlinger (1996) where the quantity of hydrolyzed phosphorus acid was expressed as mg P₂O₅ 100 g⁻¹ soil 2 h⁻¹. The dehydrogenase activity was measured by (Mersi, 1996) as the soil samples were mixed with INT-solution, incubated 2 h at 40 °C. The reduced iodonitro-tetrazolium formazan (INTF) was extracted with dimethyl-formamide and ethanol and measured photometrically at 464 nm.

2.4. Statistical analysis

Prior to the ANOVA test, Levene's Test for Equality of Variances is performed. The Levene's test for different variables at ten treatments was negative, *p* < 0.05, and then the variances showed homogeneity. The results of the experiments were subjected to one-way ANOVA by using Eisenhauer et al. (2012) and SPSS 13.0. Means were compared by Duncan's Multiple Range Test (Duncan, 1955) at *p* < 0.05.

3. Results

3.1. Maize yield

The precipitation during the growing season in 2010 was 106.1% higher over the 30-year average precipitation and in 2011 it was lower

Table 1

The precipitation (mm) during the experimental period (from April to September) in 2009, 2010, and 2011 at Görbeháza Experiment (Sárvári, 2011).

	30-year average	2009	Deviation from the average (%)	2010	Deviation from the average (%)	2011	Deviation from the average (%)
April	42.4	11.7	-72.4	79.3	+87.0	15.1	-64.4
May	58.8	72.5	+23.3	150.8	+155.1	19.8	-66.3
June	79.5	33.7	-57.6	157.9	+98.6	135.4	+70.3
July	65.7	233.6	+240.3	144.9	+120.2	27.8	-57.7
August	60.7	55.1	-9.2	85.2	+40.4	26.5	-56.3
September	38.0	0.0	-100.0	93.1	+145.0	29.0	-23.7
Total	345.1	396.6	+14.9	711.2	+106.1	253.6	-26.5

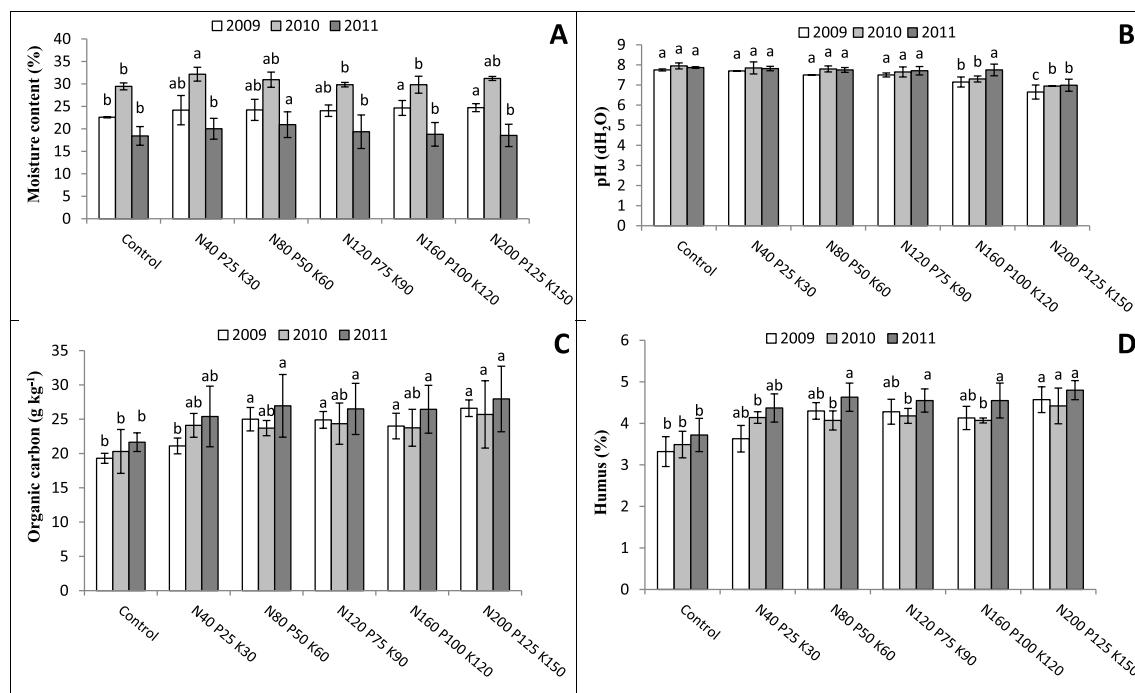


Fig. 2. Annual variations in soil moisture (A), pH (B), organic carbon (C) and humus (D) contents under long-term NPK fertilization of rainfed maize monoculture. Different letters above the same bars show significant differences at the level of $p < 0.05$.

by 26.5%. Rainfall fluctuation considerably affected the productivity of maize plants, which was 11.5, 8.8, and 12.5 t/ha in 2009, 2010, and 2011, respectively. The huge reduction in maize yield in 2010 (the雨iest year) may be attributed to the high moisture content of the experimental soil (Vertisol), which leads to poor aeration. Also, the average temperature in 2010, between germination and tassel vomiting, was lower than the optimal conditions Sárvári, 2011.

3.2. Consequences of precipitation and long-term NPK fertilization on soil physicochemical properties

Soil moisture content (%) displayed a significant response to the rainfall fluctuations, in our 3-year experiment, and to a lesser extent to long-term NPK fertilization (Fig. 2A). All treatments in 2010 including the control displayed higher soil moisture content than in 2009 and 2011 (Fig. 2A). The highest soil moisture content in 2009, 2010 and 2011 was 24.7, 32.2 and 20.9%, respectively. In addition to the precipitation, NPK rates significantly affected the soil moisture content. In 2009, all NPK rates resulted in similar soil moisture contents without significant differences except control. However, the response of soil moisture content to NPK treatments in 2010 and 2011 was different from that has been previously estimated in 2009. In 2010, the treatments of N₄₀P₂₅K₃₀, N₈₀P₅₀K₆₀, and N₂₀₀P₁₂₅K₁₅₀ displayed the highest soil moisture; while, in 2011, the treatment of N₈₀P₅₀K₆₀ recorded the highest soil moisture content. Overall, fertilization of maize plants at the rate of N₈₀P₅₀K₆₀ increased the soil moisture content in the three years of the experiment regardless of the precipitation rate.

Significantly, the addition of NPK reduced soil pH values compared to the control regardless of the application rate. Moreover, pH values gradually decreased with increasing the rate of applied NPK. Data presented in Fig. 2B showed that the treatment of N₂₀₀P₁₂₅K₁₅₀ resulted in the lowest pH values 6.65, 6.95 and 6.99 in years 2009, 2010 and 2011, respectively. Nevertheless, soil pH in 2009 was the lowest in comparison with pH measured in 2010 and 2011. In 2010, 2011, pH value was NPK dose-dependent as NPK doses below N₁₂₀P₇₅K₉₀ in 2010 showed higher pH values than in 2011; while an opposite response was noticed at the higher rates (Fig. 2B). Furthermore, the amount of

precipitation insignificantly influenced soil pH where similar pH values were measured in 2009, 2010 and 2011. However, we should not ignore the fact that this maize monoculture experiment is established in 1988; therefore, the change in pH values is not only due to the three years of the present study but this is an accumulative effect for more than 20 years.

The continuous NPK fertilization of maize monoculture significantly increased the soil organic carbon (SOC) content (Fig. 2C). Although the response of SOC content to the applied NPK rate was hesitant, the highest rate of NPK (N₂₀₀P₁₂₅K₁₅₀) resulted in the highest SOC content. The highest SOC content was estimated in 2011 (Fig. 2C). Likewise, NPK treatments increased the humus content compared to control in the three years of the experiment. However, the humus content in 2011 was the highest (Fig. 2D).

3.3. Effect of precipitation and long-term NPK fertilization on nutritional status of soil

Fig. 3 shows the content of soil N (in form of org-N and NO₃⁻), P (in form of P₂O₅) and K (in form of K₂O) after long-term NPK fertilized maize monoculture in three consecutive years (i.e., 2009, 2010 and 2011). The org-N content significantly increased in the fertilized plots, along the three years of the study, compared to the control plots. In 2011, higher org-N contents were measured in the fertilized plots than in 2009 and 2010. The wettest year (2010) had the lowest org-N content compared to the other two years except at the control and treatment of N₂₀₀P₁₂₅K₁₅₀, as org-N content in 2010 was higher than in 2009. The highest org-N content throughout the three years of the experiment was detected in the treatment of N₂₀₀P₁₂₅K₁₅₀ (Fig. 3A). The NO₃⁻ content in soil showed a significant response to the amount of precipitation. The lowest NO₃⁻ content was measured in the wettest year (2010) compared to 2009 and 2010. Also, the driest year (2011) showed lower NO₃⁻ content compared to 2009 except when maize plants were fertilized at the rate of N₁₆₀P₁₀₀K₁₂₀ (Fig. 3B). Egnér et al. (1960), NO₃⁻ content in 2009 and 2011 was higher in all treatments than control; only at the treatment of N₂₀₀P₁₂₅K₁₅₀ in 2010, the NO₃⁻ content was significantly higher than control. No significant differences

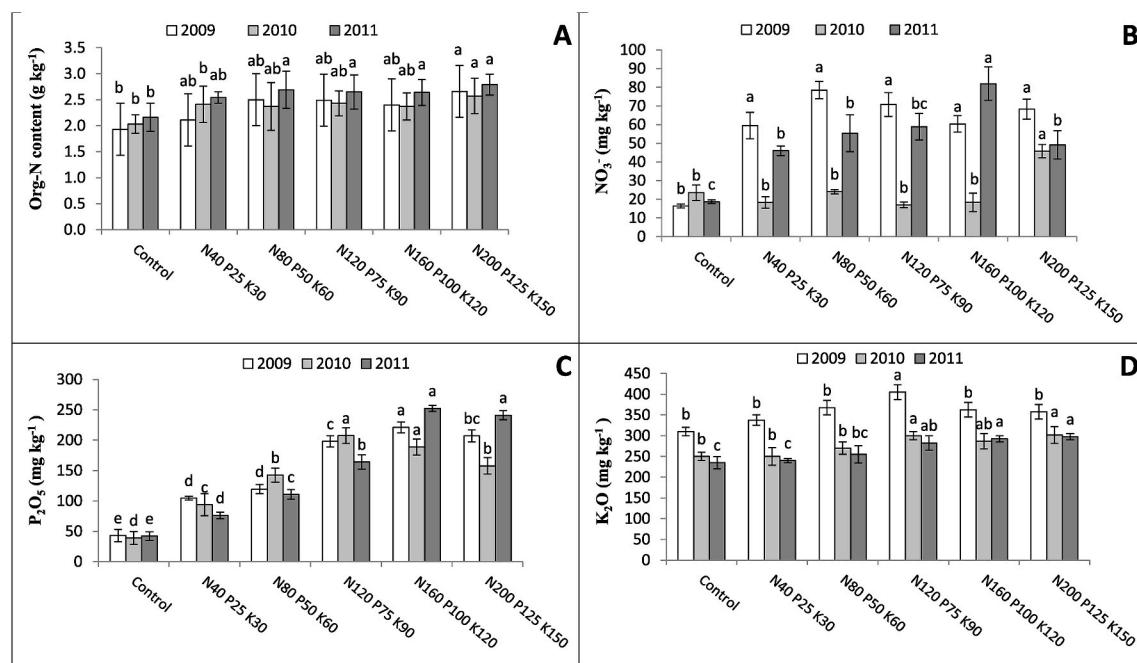


Fig. 3. Changes in org-N (A), NO_3^- -N (B), P_2O_5 (C) and K_2O (D) contents under long-term NPK fertilization of rainfed maize monoculture. Different letters above the same bars show significant differences at the level of $p < 0.05$.

in NO_3^- content between fertilized plots in 2009 were calculated. Increasing the rate of NPK in 2011 significantly increased the NO_3^- content to its highest measured value (81.9 mg kg^{-1}) at the treatment of $\text{N}_{160}\text{P}_{100}\text{K}_{120}$. All NPK treatments displayed higher uptakeable P content than control throughout the whole experimental period. Considerably, P content increased with increasing the rate of NPK up to $\text{N}_{160}\text{P}_{100}\text{K}_{120}$ ($221 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ as the highest detected content) in 2009 then slightly decreased to $207 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ when maize plants were fertilized with $\text{N}_{200}\text{P}_{125}\text{K}_{150}$. Likewise, in 2011 the content of available P in soil steadily increased to $252 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ when NPK rates increased up to $\text{N}_{160}\text{P}_{100}\text{K}_{120}$ then slightly declined to $241 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ at the highest NPK rate (Fig. 3C). The P content in 2010 increased regularly to $207 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ at the treatment of $\text{N}_{120}\text{P}_{75}\text{K}_{90}$ then started to drop down linearly. The P content was found to extremely rely on the application rate of NPK fertilizers and to a lesser extent on the amount of precipitation. Fig. 3D clearly showed that samples collected in 2009 had higher K content than 2010 and 2011. In 2010, K content was also higher than in 2011 except at the treatment of $\text{N}_{160}\text{P}_{100}\text{K}_{120}$. Nonetheless, NPK treatments showed were significant among them particularly at the high rates of NPK (Fig. 3D).

3.4. Annual variations in soil microbial populations

The total number of bacteria (Table 2) showed an increase in all NPK treatments, except the treatments $\text{N}_{120}\text{P}_{75}\text{K}_{90}$ and $\text{N}_{80}\text{P}_{50}\text{K}_{60}$ in 2009 and 2010, respectively, where significantly lower total bacterial counts than control were detected. In 2009, 2010, the highest rate of NPK ($\text{N}_{120}\text{P}_{75}\text{K}_{90}$) resulted in the highest total bacterial count (11.8 and $13.8 \times 10^6 \text{ g}^{-1}$ soil, respectively) among all treatments; while in 2011, the highest total bacterial count ($12.2 \times 10^6 \text{ g}^{-1}$ soil) was measured in maize plots fertilized at the rate of $\text{N}_{120}\text{P}_{75}\text{K}_{90}$. The highest total bacterial count was measured in 2010 at the treatment of $\text{N}_{200}\text{P}_{125}\text{K}_{150}$ compared to the other two years. NPK fertilization showed higher numbers of total fungi count than control regardless of the cultivation year. Statistically, in 2009 total fungi count responded linearly to the rate of NPK; the highest total fungi count ($47.6 \times 10^3 \text{ g}^{-1}$ soil) was detected under the highest rate of NPK ($\text{N}_{200}\text{P}_{125}\text{K}_{150}$) as shown in Table 2. Although total fungi count in 2010 did not display regular

increase, the total fungi count in all treatment was higher than those measured in 2009 and 2011. The highest total fungi count throughout the whole experimental period was $58.7 \times 10^3 \text{ g}^{-1}$ soil and detected at the highest rate of applied NPK. The number of cellulose decomposing bacteria was low, generally, in all treatments in 2009 while in 2010 and 2011 larger numbers were detected. It is especially true in the intermediate and high doses of NPK fertilizers. In 2009, 2010, a significant increase in the number of this physiological group of bacteria in all NPK treatments compared to control except treatment of $\text{N}_{40}\text{P}_{25}\text{K}_{30}$ (in 2009) in addition to $\text{N}_{80}\text{P}_{50}\text{K}_{60}$ (in 2010) was statistically proved (Table 2). The number of nitrifying bacteria was low in 2009 similar to cellulose decomposing bacteria; while in 2010 and 2011, a higher number of nitrifying bacteria was identified with some extremely high numbers. The measured values in 2011 demonstrated that the increased number of nitrifying bacteria was fertilization dose-independent. The number of this physiological group of bacteria increased in all fertilized plots significantly in 2011.

3.5. The activity of soil microbial communities

Results of soil respiration ($\text{mg CO}_2 100 \text{ g}^{-1} \text{ soil } 10 \text{ days}^{-1}$) were consistent with the total numbers of soil microorganisms. All NPK treatments in 2010 significantly increased CO₂ production compared to control recording higher values than in 2009 and 2011 (Fig. 4A). The lowest CO₂ production of was measured in 2011. In 2009, 2010, CO₂ production increased linearly with increasing NPK doses up to $\text{N}_{160}\text{P}_{100}\text{K}_{120}$ then slightly declined, while in 2011 the increase in CO₂ production was noticed when maize plants received high NPK rates up to $\text{N}_{120}\text{P}_{75}\text{K}_{90}$ afterward reduced regularly (Fig. 4A). In the case of the nitrate exploration (net nitrification), derogations were experienced among the three years. Generally, treatments of $\text{N}_{80}\text{P}_{50}\text{K}_{60}$, $\text{N}_{120}\text{P}_{75}\text{K}_{90}$, and $\text{N}_{160}\text{P}_{100}\text{K}_{120}$ resulted in a pronounced stimulating effect on this parameter in 2009; whereas in 2011, the highest net nitrification level corresponded to treatments of $\text{N}_{160}\text{P}_{100}\text{K}_{120}$ and $\text{N}_{200}\text{P}_{125}\text{K}_{150}$ (Fig. 4B). The lowest net nitrification level was measured in 2010 when the lowest NO_3^- -N content was detected too. Compared to the control, the net nitrification level increased significantly in almost all NPK treatments. It is remarkable that in the plots of the highest dose of NPK

Table 2

A 3-year change in the population dynamic of soil microorganisms under different NPK fertilization rates of rainfed maize monoculture.

Treatments	Total bacterial count (CFU)	Total fungi count	Cellulose decomposing bacteria	Nitrifying bacteria
	($\times 10^6 \text{ g}^{-1}$ soil) ^b	($\times 10^3 \text{ g}^{-1}$ soil)	($\times 10^3 \text{ g}^{-1}$ soil)	($\times 10^3 \text{ g}^{-1}$ soil)
2009				
Control ^a	8.4 ± 0.46 bc	25.0 ± 3.93 b	0.8 ± 0.19 c	0.3 ± 0.06 b
N ₄₀ P ₂₅ K ₃₀	9.0 ± 2.00 bc	25.0 ± 3.04 b	1.8 ± 0.95 c	3.1 ± 0.61 a
N ₈₀ P ₅₀ K ₆₀	9.6 ± 1.10 ab	27.8 ± 2.70 b	4.7 ± 1.14 ab	1.7 ± 0.38 ab
N ₁₂₀ P ₇₅ K ₉₀	6.1 ± 1.29 c	29.8 ± 7.12 b	2.2 ± 0.83 b	3.5 ± 0.25 a
N ₁₆₀ P ₁₀₀ K ₁₂₀	11.5 ± 1.65 a	47.5 ± 2.62 a	7.7 ± 0.89 a	2.3 ± 0.09 a
N ₂₀₀ P ₁₂₅ K ₁₅₀	11.8 ± 0.89 a	47.6 ± 6.59 a	7.8 ± 0.88 a	1.1 ± 0.69 ab
2010				
Control	7.8 ± 1.41 b	26.8 ± 3.59 c	1.1 ± 0.09 c	7.4 ± 1.95 b
N ₄₀ P ₂₅ K ₃₀	13.7 ± 2.63 a	32.0 ± 5.71 c	4.9 ± 0.21 c	9.5 ± 1.82 b
N ₈₀ P ₅₀ K ₆₀	7.7 ± 1.11 b	58.5 ± 1.37 a	8.2 ± 1.39 c	11.9 ± 0.06 b
N ₁₂₀ P ₇₅ K ₉₀	12.2 ± 0.95 ab	39.8 ± 12.25 bc	15.3 ± 1.09 b	10.8 ± 2.65 b
N ₁₆₀ P ₁₀₀ K ₁₂₀	7.9 ± 0.46 b	42.0 ± 12.77 bc	14.9 ± 2.80 b	23.3 ± 1.47 a
N ₂₀₀ P ₁₂₅ K ₁₅₀	13.8 ± 2.46 a	58.7 ± 5.82 a	21.7 ± 0.54 a	12.3 ± 0.05 b
2011				
Control	8.8 ± 0.86 b	19.9 ± 0.41 c	6.1 ± 5.55 c	4.2 ± 0.51 c
N ₄₀ P ₂₅ K ₃₀	11.5 ± 1.53 a	28.5 ± 0.50 bc	2.4 ± 0.60 c	22.7 ± 1.49 a
N ₈₀ P ₅₀ K ₆₀	9.2 ± 1.57 ab	49.3 ± 13.30 ab	5.1 ± 3.85 c	11.7 ± 1.05 b
N ₁₂₀ P ₇₅ K ₉₀	11.1 ± 2.50 a	41.2 ± 13.17 abc	26.2 ± 2.34 ab	24.2 ± 2.32 a
N ₁₆₀ P ₁₀₀ K ₁₂₀	9.6 ± 1.10 ab	35.8 ± 1.25 abc	14.6 ± 1.27 bc	11.1 ± 1.28 b
N ₂₀₀ P ₁₂₅ K ₁₅₀	9.4 ± 0.47 ab	52.6 ± 12.09 a	35.6 ± 8.91 a	11.1 ± 2.18 b

^a Control = no fertilizers were applied.^b All numbers are calculated based on 1 g dry soil. In the same column, different letters after means show significant differences at the level of $p < 0.05$.

the net nitrification level significantly increased in 2009 and 2011, but not in 2010. The content of microbial biomass carbon (MBC) has changed positively; it, significantly, increased by the application of NPK within all the three years of the experiment. The MBC in 2010 was 4–7 times higher in all NPK treatments than control. Particular attention has to be paid to the effects of intermediate and high rates of NPK because in these treatments outstanding contents of MBC were measured in 2010 and 2011.

3.6. Annual variations in soil enzymes activity

A very favorable effect of the high rates NPK was experienced on the activity of four soil enzymes, i.e., phosphatase, saccharase, urease, and dehydrogenase. Out of seventy-two average data, including four enzymes, six treatments of NPK fertilizers, and three years experiment, of enzymes activity only in three cases was measured slightly reduced activities of enzymes in the fertilized plots compared to control. In all other cases, the activity of the enzymes increased considerably except when maize plants were fertilized at the highest rates of NPK. In these soils, this increase was not significant for the activity of phosphatase, saccharase, and dehydrogenase enzymes, while the urease activity was lower than control (Fig. 5 A, B, C, and D). The activity of the phosphatase enzyme changed in a relatively small interval (Fig. 5A). The monotone character of results was interrupted by some outstanding results in some treatments. Except for the highest rates of NPK, other rates showed significant differences in the activity of phosphatase. Despite the highest rate of NPK (N₂₀₀P₁₂₅K₁₅₀) caused higher values of phosphatase activity than control, the increase was not significant. The activity of saccharase increased when maize plants were fertilized with N₂₀₀P₁₂₅K₁₅₀ compared to control in all the three years. The highest saccharase activity was measured in 2011; in this year the treatments of N₁₆₀P₁₀₀K₁₂₀ and N₂₀₀P₁₂₅K₁₅₀ significantly increased the enzyme activity. The lowest activity was detected in 2009, only the treatment of N₁₆₀P₁₀₀K₁₂₀ exhibited a significant increase. In 2010, a significant stimulating effect was determined regarding the activity of saccharase except for one treatment (N₂₀₀P₁₂₅K₁₅₀). The wettest year (2010) and the driest (2011) displayed higher activities of saccharase compared to 2009. The NPK fertilization improved the activity of the urease enzyme; it was statistically proved in the three investigation years except for one

treatment. The urease activity is well described in the following order: 2010 > 2009 > 2011. The urease activity reduced as the soil moisture content decreased (Fig. 5C). The dehydrogenase enzyme showed a considerable response to NPK treatments. The low and intermediate rates of NPK seemed to be the most favorable treatments in 2009 and 2011, while in 2010 intermediate rates only showed the highest activity of dehydrogenase. These results were very similar to the results of phosphatase enzyme.

3.7. Correlation between soil chemical, biochemical and microbiological parameters

Between the data of measured soil chemical, biochemical and microbiological traits correlation analyses were made to highlight the possible linkages among these parameters.

Regarding the soil moisture content, a close correlation was only measured in the driest year (2011). Soil moisture content correlated positively with organic carbon ($r = 0.707$), org-N (0.711), total bacterial count ($r = 0.680$); nitrifying bacteria ($r = 0.780$); ($r = 0.886$) and urease activity ($r = 0.910$); while a negative correlation with dehydrogenase activity ($r = -0.850$) was statistically calculated.

A positive correlation was reported between the biomass of fungi and soil moisture content by Baldock et al. (2012) when the soil moisture content increased. The same conclusion was not valid to the biomass of bacteria. In all the three years, a close correlation was measured among some parameters independent of the soil moisture content. The dehydrogenase activity showed close positive correlation to the total bacterial count ($r = 0.769$; 0.736; 0.753), nitrifying bacteria ($r = 0.782$; 0.647; 0.753) and negative correlation with urease activity ($r = -0.840$; 0.676; -0.637) as well as the number of nitrifying bacteria correlated positively with the urease activity ($r = 0.700$; 0.813; 0.846), respectively, in the three years of the experiment.

At least in two years, a close correlation was proved among several parameters. The total bacterial count was in close correlation with cellulose decomposing bacteria; nitrifying bacteria, soil respiration, net-nitrification, phosphatase, saccharase, urease, and dehydrogenase activities. Dehydrogenase activity was in close correlation with total fungi count, soil respiration, net-nitrification, phosphatase, and saccharase

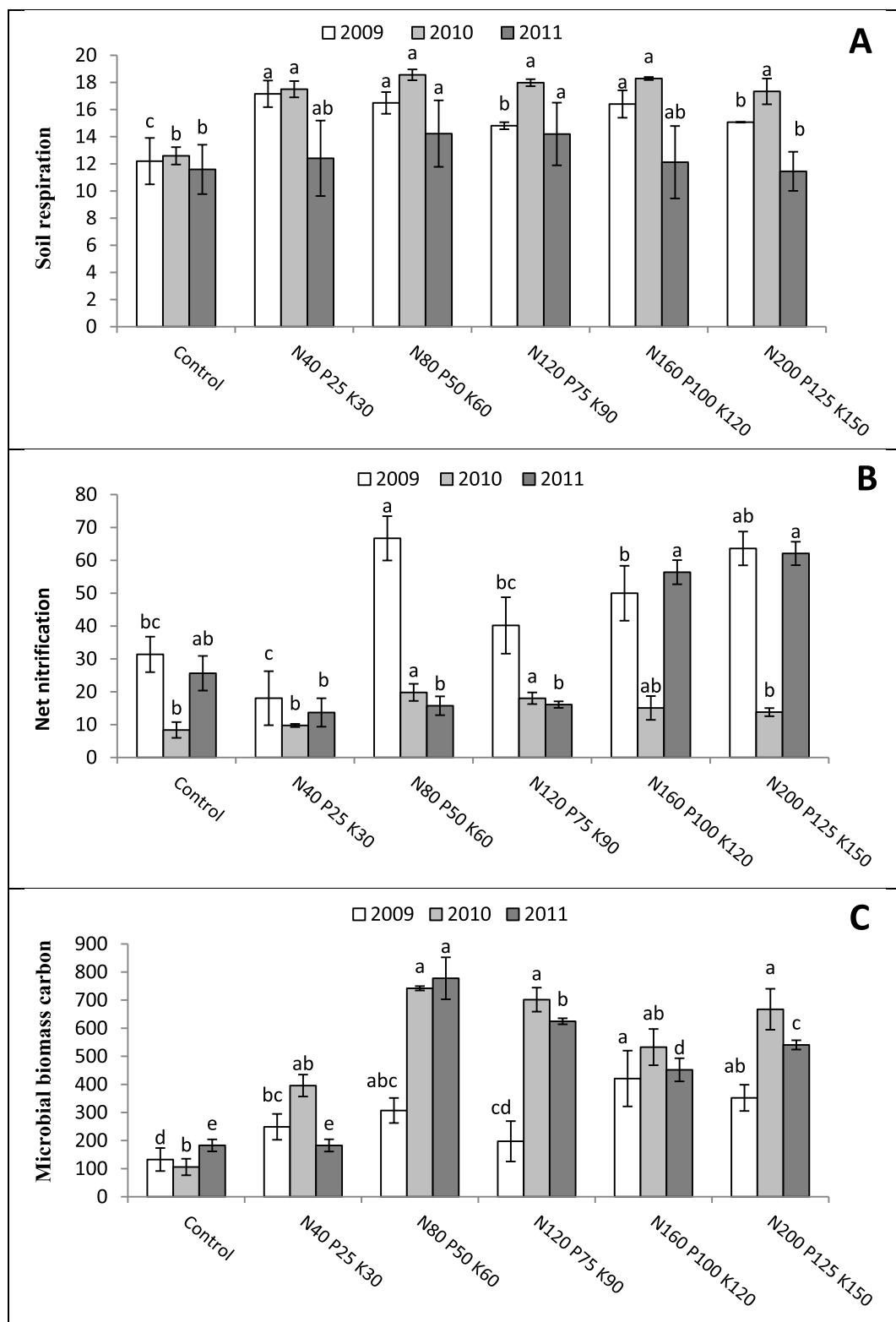


Fig. 4. A 3-year change in some soil biochemical processes under different NPK fertilization rates of rainfed maize monoculture. (A) Soil respiration ($\text{mg CO}_2 \text{ 100 g}^{-1} \text{ soil 10 days}^{-1}$); (B) net nitrification ($\text{mg kg}^{-1} \text{ soil 2 week}^{-1}$); (C) microbial biomass carbon ($\text{mg C g}^{-1} \text{ soil}$). Different letters above the same bars show significant differences at the level of $p < 0.05$.

activities. The soil respiration was in close correlation with available P, total bacterial and fungi count, the number of cellulose decomposing and nitrifying bacteria.

4. Discussion

Six increasing rates of NPK have been applied in a near 30-year long-term fertilization experiment in a typical meadow soil (Vertisol), according to the WRB, cultivated with rainfed maize monoculture. The

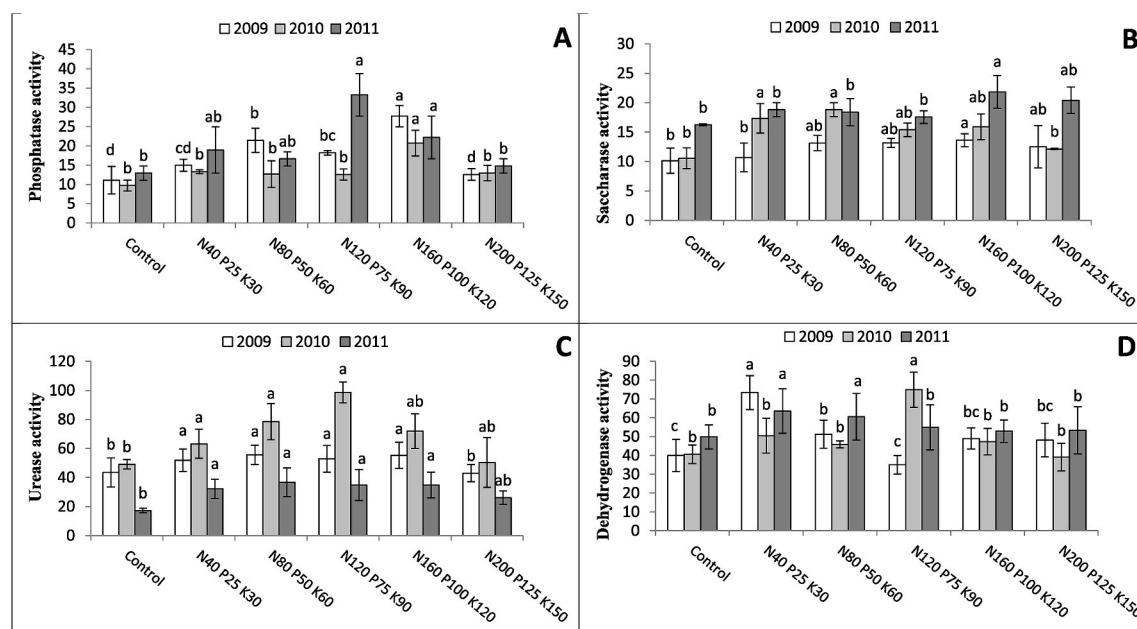


Fig. 5. A 3-year change in soil enzyme activities under different NPK fertilization rates of rainfed maize monoculture. (A) Phosphatase activity ($\text{P}_2\text{O}_5 \text{ 100 g}^{-1} \text{ dry soil } 2 \text{ h}^{-1}$); (B) saccharase activity ($\text{mg glucose} \text{ g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$); (C) urease activity ($\text{mg NH}_4^+ \text{-N g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$); (D) dehydrogenase activity ($\mu\text{g INTF g}^{-1} \text{ dry soil } 2 \text{ h}^{-1}$). Different letters above the same bars show significant differences at the level of $p < 0.05$.

investigated soil has clay-loam texture and is slightly alkaline due to the loess parent material. Subsequent and substantial changes were detected in the nutritional status of soil, i.e., mineral nutrients content and organic matter stock, and in the dynamics of transformation of the SOM by soil microorganisms due to NPK fertilization. However, fluctuations in rainfall during the experimental period, i.e., 2009, 2010 and 2011 had also a significant influence on all the measured soil properties.

4.1. Water retention in soil

Soil moisture content depends on the amount of precipitation in the rainfed cultivation. The annual expected precipitation in this region during the growth period is 345.1 mm. The highest soil moisture content was measured in 2010 (~30% larger), while the lowest was determined in 2011 (~30% lower); the lowest soil moisture content was about 59–65% of the highest soil moisture content due to the extreme quantity of precipitation fell in 2010 (Fig. 2A). This was 711.2 mm, which is 106.1% higher than the 30-year average precipitation of this region (Table 1). The soil moisture content in the wettest year was 10–12% higher than the driest year. The differences in soil moisture among treatments including the control are mainly due to the heterogeneity of soil than the fertilization treatments. However, Ge et al. (2018) reported a significant increase in soil moisture content as a result of long-term NPK application.

4.2. Chemical and biochemical soil properties as affected by rainfall fluctuation

The soil pH decreased in fertilized experimental plots compared to control. In the three experimental years, the highest NPK rate ($\text{N}_{200}\text{P}_{125}\text{K}_{150}$) caused a significant reduction in soil pH. Interestingly, the wettest year (2010) showed the highest soil pH when NPK were applied at rates below $\text{N}_{120}\text{P}_{75}\text{K}_{90}$; while high NPK rates caused a reduction in soil pH compared to the same treatments of 2011. Similar results were previously cited by Ge et al. (2018); they reported a reduction of 1.04 in soil pH value after long-term NPK application in an apple orchard. The differences among the pH values may be due to the

absorption of hydrogen ions on the surface of the colloids causing the hidden acidity of the soil.

The NO_3^- content varies from one year to the next since it is governed by many climatic and anthropogenic factors such as precipitation, soil texture, soil organic matter, temperature, previous plant species, fertilization of previous crop and management of plant residues (Tao et al., 2018). In the present study, NO_3^- content showed interesting findings; it was at its highest value (59.0 mg kg^{-1} as an average) in 2009, while increased rainfall in 2010 significantly declined it to 24.5 mg kg^{-1} (on average). The NO_3^- content increased again in 2011 to 51.7 mg kg^{-1} (on average) when the rainfall amount was 30% lower than the average annual precipitation in this region. These data clearly show that precipitation and temperature were the most influential on NO_3^- content; low temperature and high precipitation in 2010 reduced nitrification while accelerated denitrification. This caused an increase in NO_3^- loss in the form of N_2 gas due to anaerobic condition generated during high precipitation. Also, NO_3^- loss through leaching from the root zone cannot be neglected, especially in sandy soils with low organic matter, in rainy years or excess irrigation (Sahrawat, 2008; Tao et al., 2018). Also, high rates of NPK resulted in higher soil NO_3^- content; and it thus could be recommended that in years with expected high rainfall the applied amount of N should be decreased in order to avoid the excess leaching of soil NO_3^- which possibly can contaminate the groundwater and consequently threatens human health. Similar results were cited by Kátai (2006) from a fertilization experiment on a calcareous chernozem soil in mono-and triculture.

Results of SOC content obviously illustrated that, except for the highest rate of applied NPK, the activity of soil biota and vegetation cover and its related plant litter are the major two agents that control the variation in SOC (Boivin and Kohler-Milleret, 2011). The SOC content increased in 2011, which is the next season to the wettest year, as a result of the increase in numbers of organic matter decomposers, i.e., bacteria, fungi, and cellulose decomposing bacteria. Regardless of NPK rate, SOC content increased with the growing season achieving the highest content (25.8 mg kg^{-1}) in 2011; this emphasizes the hypothesis that vegetation cover and plant litter are among the main factors affecting the SOC content.

4.3. Functionality of soil microbes under rainfall fluctuation

Nitrification is the aerobic oxidation of NH_3 to NO_3^- and N_2O (Sahrawat, 2008). Accordingly, the wettest year (2010) displayed the lowest net nitrification rate compared to 2009 (highest rate) and 2011. This could be due to the leaching of NO_3^- from the soil profile. In addition, a huge amount of NO_3^- can be converted into N_2 gas via denitrification as a result of anaerobic condition generated by large amounts of water that soil received in 2010. This assumption is supported by findings reported earlier by Tan et al. (2018) who illustrated that gross net nitrification significantly decreased with increasing moisture in soil profile while denitrification increased.

The amount of produced CO_2 in the soil is an indicator for the soil health; CO_2 is generated during respiration of soil organisms, i.e., microbes, soil animals and plant roots (Bao et al., 2016). Interestingly, the wettest year (2010) displayed the highest soil respiration on average followed by 2009, while the driest year (2011) recorded the lowest soil respiration rate. To a lesser extent, the application rate of NPK affected soil respiration as all NPK treatments showed almost similar rates of soil respiration in all the three years. Similar results were previously documented by Bao et al. (2016); they studied changes in soil respiration under different temperatures and soil moisture. They stated that low soil moisture diminished soil respiration rate particularly at high temperatures (i.e., 25 °C). This supports the low respiration rate reported in this present study in 2011 (the driest year).

Results of MBC clearly proved that the growth of soil biota depends heavily on soil moisture content compared to the rate of NPK fertilizers. In 2010 (wettest year), MBC content was almost 2-fold higher than in 2009; it was also higher than in 2011. However, the higher MBC content measured in 2011 (driest year) in comparison with 2009 may be attributed to the extended effect of the wettest year. The results of MBC were consistent with those of the total numbers of different microbial groups in soil (Table 2). The results of the present study were consistent with those that have been previously cited by Luo et al. (2015); they examined the influence of long-term (33 years) fertilization on the dynamics of soil microbial biomass and changes in bacterial and fungal communities. Long-term fertilization substantially enhanced MBC; moreover, these parameters displayed a significant response to the addition of organic manure. Also, they reported a significant increase in the ribotype diversity of bacteria and fungi. Cerny et al. (2007) also documented the positive impact of organic fertilizers on the microbial biomass N and C-content in the long-term field experiment; while a negative effect was caused by the application of mineral nitrogen fertilizers in an experiment of maize.

4.4. Structure of soil biota under rainfall fluctuation

Regarding the dynamic of soil microorganisms in the present research, the total number of bacteria and fungi, as well as the cellulose decomposing and nitrifying physiological groups of bacteria showed significant positive responses to rainfall fluctuation and to a lesser extent to NPK fertilization. The total bacterial count showed the least response to rainfall fluctuations as it did not significantly change due to the different amounts of precipitation between 2009 and 2011. On the other hand, total fungi count displayed its highest number in the wettest year (2010) and this effect extended to the driest year (2011) recording a higher number than in 2009. Interestingly, numbers of both cellulose decomposing bacteria and nitrifying bacteria clearly was rainfall-dependent as their numbers ($\times 10^3 \text{ g}^{-1}$ soil) varied from 4 to 2 in 2009 to 11 and 13 in 2010, respectively; however, this influence continued in 2011 where higher numbers 15 and 14, respectively, were calculated. Control plots exhibited almost the same number of total bacteria and fungi counts in all the three years regardless of the rainfall fluctuations, except in 2011 total fungi count was lower than those recorded in 2009 and 2010. The total bacterial count showed a higher response to different applied rates of NPK fertilizers in the wettest year

(2010) compared to 2009 and 2011 recording higher numbers under most of the tested NPK rates. Likewise, NPK fertilizers had a better influence on total fungi counts in 2010 (wettest year) in comparison with 2009 and 2011 as the all rates of NPK displayed the high numbers of total fungi count except at the rate of $\text{N}_{160}\text{P}_{100}\text{K}_{120}$. Although in 2009 cellulose decomposing bacteria poorly responded to the applied rates of NPK except at the higher rates, in 2010 and 2011 they showed different behavior as higher numbers in 2010 (wettest) were measured and the high NPK rates increased their number. Moreover, this effect was even more obvious in 2011 (driest year) where cellulose decomposing bacteria showed their highest numbers particularly under high rates of NPK. Nitrifying bacteria displayed similar responses to cellulose decomposing bacteria as low numbers were detected in 2009 and increased with increasing rainfall and NPK rates in 2010; also, their numbers in 2011 were higher than in 2009 but high rates of NPK above $\text{N}_{120}\text{P}_{75}\text{K}_{90}$ diminished their number. These results clearly proved that the variation in the structure of soil biota mainly depends on soil moisture content and to a lesser extent to the rate of mineral fertilizers. These results are strongly supported by the findings of Ge et al. (2018), who documented that soil microbial structure did not significantly influence by long-term NPK application. Contrarily, Diallo-Diagne et al. (2016) presented different results; they analyzed the bacterial population by PCR-denaturing gel electrophoresis targeting the eubacterial 16 S rRNA gene. They reported that bacterial clusters significantly affected by both mineral and/or organic fertilizers. However, our results were in agreement with the conclusion of Haynes and Naidu (1998); they stated the primary effect of fertilization is a direct effect that could manifest in the change of soil fertility and in the increasing carbon stock. This change led to an increase in the total number and biomass of soil microorganisms, as well as the activity of enzymes in the proportion of the applied amount of nitrogen. Hopkins and Shiel (1996) studied changes in soil parameters in grassland plots under the long-term application of manure and inorganic fertilizers. In their experiment, farmyard manure with NPK fertilizers led to smaller biomass and lower biomass to organic C ratio, while single application of NPK resulted in greater glucose affinity was measured, but did not significantly affect the biomass C to organic C ratio or qCO_2 . According to Kátai et al. (2014) the organic carbon and org-N contents, MBC, MBN, net-nitrification, and soil respiration increased as a result of mineral fertilization.

4.5. Rainfall fluctuation and soil enzyme activities

The results obtained from the current experiment clearly illustrated that NPK fertilization had lesser consequences on the activity of the tested four soil enzymes compared to rainfall fluctuations. Nevertheless, fertilized plots with NPK displayed higher activities of soil enzymes compared to control. However, using NPK at the highest rate ($\text{N}_{200}\text{P}_{125}\text{K}_{150}$) caused a reduction in enzyme activity compared to lower rates. It can be assumed that the composition of soil biota was changed by the effect of fertilization, but the investigation was not carried out in this regard. According to Fog (1988), the variation in soil biota involves a change in soil enzyme activity and consequently, the nutrient cycling, i.e., carbon and nitrogen cycles will go through in a rapid change and cause nitrogen accumulation. This is primarily due to the degradation of the easily decomposable organic material; it is quickly followed by the accumulation of N and slow breakdown of the hardly decomposable organic material by microbes.

Soil phosphatases (i.e., acid and alkaline phosphomonoesterases) convert organic phosphorus to mineral phosphorus playing a crucial role in the phosphorus cycle. The effect of anthropogenic activities and/or biotic and abiotic environmental stressors cannot be adequately discussed because the current methods used for measuring phosphatase activity cannot distinguish between the sources of soil phosphatases either associated with living microbial cells or the extracellular phosphatases adhered to the surface of soil colloids (Gianfreda and

Ruggiero, 2006). The activity of soil phosphatase showed higher dependence on soil moisture content than application rates of NPK, where activities of soil phosphatase noticeably hesitated when NPK increased. The soil phosphatase activity significantly diminished in 2010 when soil received 106.1% rainfall larger than the 30-year average precipitation. Marklein and Houlton (2012) cited that N fertilization enhanced phosphatase activity while the addition of P fertilizer reduced the activity. On the other hand, a 31–40% reduction in phosphatase activity was attributed to the limited moisture content in the soil profile (Sardans and Peñuelas, 2004).

Saccharase (invertase) cleavages the glycosidic bond in disaccharide sucrose resulting in monosaccharides glucose and fructose. Frankenberger and Johanson (1983) documented that total N and organic C contents significantly enhanced the saccharase activity; whereas soil pH, cation exchange capacity and soil texture showed no effect. Moreover, they cited that saccharase activity reduced with soil depth. However, results obtained in the present study confirmed the effect of organic carbon on saccharase activity, while increasing the rate of N did not cause a similar increase. On the other hand, fluctuations in rainfall changed the activity of saccharase; in 2010, higher activity was measured compared to 2009 while the highest activity was determined in 2011. Kátai et al. (2014) reported a significant decline in the activity of saccharase in chernozem soil.

Soil dehydrogenases are intracellular enzymes and are not accumulated outside the living microbial cells in the soil. Therefore, dehydrogenases are important as an indicator of soil health and microbial activity (Salazar et al., 2011). Dehydrogenases are responsible for redox reactions in soil and the transformation of soil organic matter via the biological oxidation (Moeskops et al., 2010). However, the relationships between dehydrogenases and the total microbial activity are not always evident due to the complexity of soil and its heterogeneity (Salazar et al., 2011). Wolinska and Stepniewska (2012) listed the stimulatory factors of soil dehydrogenase activity as follows: soil moisture, soil aeration, soil organic matter, soil pH, temperature and season of the year. Controversy, they cited profile depth, mineral fertilization, pesticides and heavy metal pollution as inhibiting agents to dehydrogenase activity. Our results revealed that high rates of NPK fertilization had a significantly negative influence on dehydrogenase activity, where low rates of NPK exhibited higher activities of dehydrogenase. On the other side, seasonal and rainfall variations did not show a significant impact on soil dehydrogenase activity as similar activities were measured in 2009, 2010 and 2011. Also, it could be interpreted that higher activities measured in 2011 are due to high organic carbon content determined in this year. Luo et al. (2015) studied the changes in soil dehydrogenase activity after long-term (33 years) fertilization, and they reported a substantial enhancement in dehydrogenase activity.

Urease is one of the key soil enzymes; it is strongly associated with chemical changes of nitrogen forms and thus plays an important role in the nitrogen cycle (Liang et al., 2003). In the present study, urease displayed different responses to rainfall fluctuation compared to the other soil enzymes. Urease was the most sensitive enzyme to soil moisture content as its activity directly proportioned to rainfall. The highest activity of urease was measured in 2010 (the wettest year) followed by 2009 while the lowest activity was determined in 2011 (the driest year). Urease activity showed a lesser response to NPK fertilization, where high NPK rates displayed lower activity than low and intermediate rates. A decline in urease activity in chernozem soil was cited by Kátai et al. (2014).

It could be summarized that with limited nitrogen supply the microbes produce a phenol-oxidase enzyme, which can help to release carbon and nitrogen from the hardly biodegradable organic matter (Craine et al., 2007). In addition, at higher nitrogen content of the soil, the rapid transformation of organic matter takes place; where the cellulose decomposers and producers of cellulase enzyme (Manning et al., 2008) play an important role. In soils containing a limited or higher

quantity of nitrogen, the ratio between fungi and bacteria is very interesting, it can be stated that the fungi are more effective at the beginning of the decomposition, while the utilization of inorganic matter and simple organic compounds is much better for bacteria. Our results demonstrate that the number of fungi increased dynamically and their number was more rainfall-depended than the total number of bacteria.

5. Conclusion

In Hungary, in the Görbeháza long-term fertilization field experiment the NPK fertilizers - applied near 30-year in a typical meadow soil - had a positive effect on some soil chemical and microbiological properties. Fertilization caused significant changes in the mineral nutrients and organic matter stock of soil as well as in the dynamic of the transformation of the soil organic matter. The results show that while the phosphorus was greatly accumulated by the increasing doses of fertilizers, the accumulation of potassium had a much lower rate in the soil. Among the microbial parameters, the number of microscopic fungi and the amount of cellulose decomposing bacteria have increased significantly by rainfall fluctuation. The remarkable positive effect on the net nitrification and the CO₂-production was caused by the intermediate dose of fertilizer and mainly by precipitation. The content of MBC, the activity of phosphatase, saccharase, urease, and dehydrogenase enzymes have grown almost in all fertilized plots compared to control. However, a high dose of NPK fertilization generally lowered enzyme activity. The different climatic factors, especially the quantity and distribution of precipitation, have an emphasized influence on the physical, chemical and microbiological properties in the soil ecosystems due to global climate change. For sustainable agricultural production regular monitoring of soil physical, chemical and microbiological properties would be indispensable to control not only the agro-technical methods but also the climatic effects on soils.

Credit author statement

János Kátai and Ágnes Oláh Zsuposné: Conceptualization, Magdolna Tállai and Tarek Alshaal: Methodology, Software, Tarek Alshaal: Data curation, Writing- Original draft preparation. János Kátai and Ágnes Oláh Zsuposné: Visualization, Investigation. János Kátai: Supervision.: Magdolna Tállai: Software, Validation.: János Kátai and Tarek Alshaal: Writing- Reviewing and Editing.

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