

Leaching of Mineral Nitrogen and Phosphate from Rhizosphere Soil Stressed by Drought and Intensive Rainfall

J. Elbl, J. K. Friedel, J. Záhora, L. Plošek, A. Kintl, J. Přichystalová, J. Hynšt, L. Dostálová, K. Zákoutská

Abstract—This work presents the first results from the long-term experiment, which is focused on the impact of intensive rainfall and long period of drought on microbial activities in soil. Fifteen lysimeters were prepared in the area of our interest. This area is a protection zone of underground source of drinking water. These lysimeters were filled with topsoil and subsoil collected in this area and divided into two groups. These groups differ in fertilization and amount of water received during the growing season. Amount of microbial biomass and leaching of mineral nitrogen and phosphates were chosen as main indicators of microbial activities in soil. Content of mineral nitrogen and phosphates was measured in soil solution, which was collected from each lysimeters. Amount of microbial biomass was determined in soil samples that were taken from the lysimeters before and after the long period of drought and intensive rainfall.

Keywords—Mineral nitrogen, Phosphates, Microbial activities, Drought, Precipitation.

I. INTRODUCTION

In the recent years, we have witnessed weather fluctuations in the Czech Republic. Fig. 1 shows changes in air temperature in the area of our interest over the last 25 years. Percentages represent the difference from the long-term average. Changes of weather conditions were detected in all parts of our country. Precipitation totals are the same, but their layout has changed. There are long period of drought and intensive rainfall. These changes have a negative impact on microbial activity in the soil.

Changes of microbial activity have a direct effect on the availability of nutrients in the soil and soil hydrophobicity. Intensive rainfall and high temperatures accelerate the leaching of nutrients from soil organic mineral complex. This leads to loss of organic carbon (C_{org}) and nitrogen from the soil. Loss of these components has resulted in destruction of microbial activity in the soil [10], [12], [14], [16].

J. Elbl, L. Plošek, A. Kintl, J. Záhora, J. Hynšt, J. Přichystalová and L. Dostálová are with the Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic (e-mail: jakub.elbl@mendelu.cz).

J. K. Friedel is with the Division of Organic Farming, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences Vienna, Gregor-Mendel-Str. 33, A-1180 Vienna, Austria.

K. Zákoutská is with the Department of Applied and Landscape Ecology, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno 13, Czech Republic.

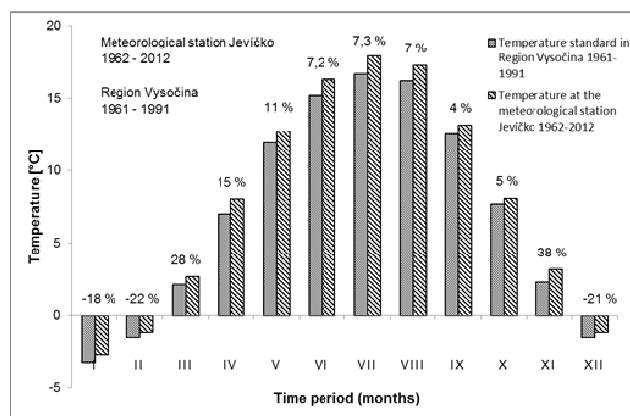


Fig. 1 Changes in air temperature on experimental site Březová nad Svitavou

These problems can be solved only by sustainable farming. Sustainable agriculture is not possible without sustainable (healthy) soil. The soil cannot be healthy without microbial activity. Because soil microorganisms are necessary for cycling of essential nutrients, soil aggregates and soil hydrophobicity are formed. Absence of microbial activity due to lack of SOM is the main reason for the susceptibility of soils in the Czech Republic (CZ) to listed problems [3]-[16].

Area of our interest is the protection zone of underground source of drinking water “Březová nad Svitavou” (further protection zone). This protection zone is located in the northern part of the Czech-Moravian highland and it is responsible for protection of underground source of drinking water against contamination by pollutants. Unfortunately, the function of this zone is ineffective which is indicated by increasing mineral nitrogen concentrations in the drinking water from this area.

Leaching of mineral nitrogen (consisting of NH_4^+-N and $NO_3^- -N$) from arable land is a major threat to the quality of drinking water from underground reservoirs in the CZ [4]. The area is situated on the Bohemian Cretaceous basin and consists of a system of soil isolators and collectors. The isolators are made by impermeable soil. Conversely, collectors are filled with light soils. These soils allow infiltration of precipitation and water transfer. Unfortunately, most collectors are under agricultural land. Therefore, N_{min} from arable land can quickly contaminate underground sources of drinking water there.

In the soil, the microbial activity is the key to stop the

leaching of mineral nitrogen. Soil microorganisms have the ability to immobilize the mineral nitrogen in their bodies. Moreover, microorganisms help to restore SOM, if organic carbon is added to the soil. SOM has a direct impact on the capacity of the soil for retaining mineral nitrogen and other nutrients [16]-[20].

There is a hypothesis that availability of water for plant has a positive impact on microbial growth in rhizosphere. Increasing microbial biomass helps to retain nutrients in soil. Subsequently, nitrogen and phosphorus cannot be leached out of the soil.

II. MATERIALS AND METHODS

A. Experimental Design

Influence of drought and intensive rainfall on microbial activities in rhizosphere soil was tested by pot experiment. Fifteen lysimeters have been used as experimental containers and located in the area. The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou, where annual climatic averages (1962-2012) are 588.47mm of precipitation and 7.9°C mean of annual air temperature. The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25kg of subsoil and 25kg of topsoil (arable soil). See Fig. 2.

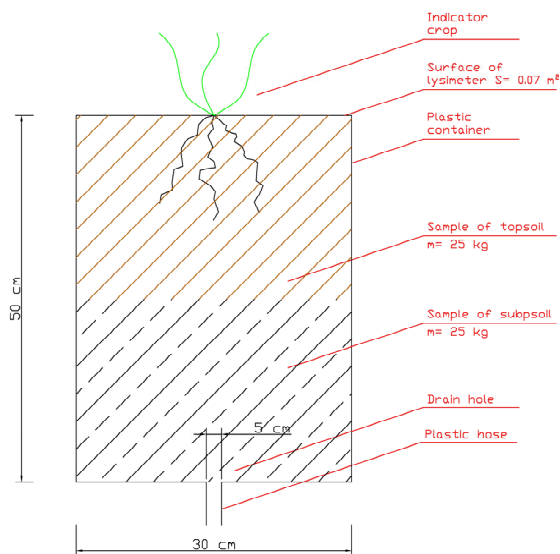


Fig. 2 Lysimeter – experimental container

Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10mm) and homogenized. Topsoil and subsoil were prepared separately. Each Lysimeter had one drain hole and PVC hose for collecting soil solution. Hose leads into the plastic bottle. All lysimeters were buried into the ground (Fig. 3). Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft (Fig. 4). Lysimeters were completed

and filled in October 2012. *Deschampsia caespitosa* was used as a model plant (Fig. 3) to determine the effect of microbial activities, fertilizers and weather on plant production. The model crop was planted into each lysimeter on the 9th of November 2012.



Fig. 3 Lysimeters



Fig. 4 Control shaft

Five variants (V1 – V5) of the experiment were prepared; each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variants: V1 (control – without fertilizers) and V2 (60% of recommended doses of N). Variants V1 and V2 were irrigated by water in May, June and July. Regular irrigation was necessary to maintain optimum of soil moisture. Second group consists of three variants, V3 (control – without fertilizers), V4 (60% of recommended doses of N) and V5 (60% of recommended doses of N and 100% of recommended doses of C_{org}), were not irrigated. But all variants were exposed to precipitation. For this reason, these variants were exposed to periods of drought and intensive rainfall that occurred in the area of our interest in June and July 2013.

Information on the applied fertilizers: Nitrogen was applied as a liquid fertilizer DAM 390. DAM 390 is a solution of ammonium nitrate and urea with an average content of 30% nitrogen (1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and 1/2 is in the form of urea). One hundred liters of DAM 390 contain 39kg of nitrogen. Organic carbon

(C_{org}) was applied as organic fertilizer Lignohumate B (LG B). Lignohumate is defined by [4] as a product of chemical transformation of liginosulfonate. This material is completely transformed into the final product: solution containing 90% of humic salts (1:1 ratio of humic and fulvic acids).

B. Determination of Mineral Nitrogen and Phosphate

Soil solution was collected into plastic bottles, which were placed in the control shaft (Figs. 3 and 4). The amount of the solution was monitored three times per week. If a solution was found in a bottle, it was taken for the determination. Samples were stored at 4°C before the determination.

Concentration of mineral nitrogen (N_{min}) was measured using distillation-titration method by [13]. Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of MgO. Nitrate nitrogen was determined in the same manner using Devard's alloy. Concentration of NH_4^+ -N and NO_3^- -N was calculated:

$$\text{mg } NH_4^+ \text{ or } NO_3^- - N = \left(\frac{\text{normality of standart HCl}}{0.03571} \right) \times 0.5 \times \text{titration} \quad (1)$$

The value of N_{min} was calculated as the sum of the detected ammonium and nitrate forms.

Concentration of phosphates (PO_4^{3-}) was measured by spectrophotometric method Hach Lange No. 8048 (in accordance with USEPA). Ten milliliters of soil solution was inserted into two cells. First cell was used as blank sample and second cell was used for determination. Therefore, ascorbic acid was added into the second cell. Subsequently, the second cell was shaken for 180s. After shaking, the measurement was carried out. A blank sample was measured at first. After that, sample with acid was determined. This method was realized on the device "Hach Lange DR 2800".

Determination of N_{min} and PO_4^{3-} was performed after each sampling of the soil solution and in each sample. The results obtained from the analyses of soil solution were expressed in mg of N_{min} or PO_4^{3-} per m^2 (mg/m^2).

C. Determination of Total Nitrogen and Carbon in Microbial Biomass

Microbial biomass has been defined as the part of the organic matter in soil that constitutes living microorganisms smaller than $5-10\mu m^3$. Soil microbial biomass is considered to be a pool for subsequent delivery of nutrients – nitrogen, phosphorus etc. [8]. Therefore, we consider the content of nitrogen and carbon in microbial biomass (N_{mic} and C_{mic}) to be an important indicator of microbial activity in the soil.

Content of C_{mic} and N_{mic} was measured using fumigation-extraction method [5]. N_{mic} was measured by [18] and C_{mic} was measured by [1]. The samples of rhizosphere soil were taken from each lysimeters in April (before application of fertilizers) and in June 2013 (after application of fertilizers, irrigation and long period of drought). After soil sampling, samples were stored at 4°C. Before determination of C_{mic} and N_{mic} , soil

samples were sieved through a sieve (grid size 2mm). The results were expressed in μg of C or N per g of soil ($\mu g/g$).

D. Plant Biomass Production

After 180 day of growth, aboveground biomass of indicator plant was harvested. The obtained biomass was dried at 105°C to constant weight. During experiment, plant biomass will be harvested twice a year (May and September).

E. Statistical Analysis

Potential differences in values of plant biomass production, microbial activities, concentration of mineral nitrogen and phosphate in soil solution (difference before and after application of fertilizers) were identified by one-way analysis of variance (ANOVA) in a combination with the Tukey's test. Regression was used for testing relationship between the increasing amount of leached nutrients and the content of N in microbial biomass. All analyses were performed using Statistica 10 software. The results were processed graphically in the program Microsoft Excel 2010.

III. RESULTS AND DISCUSSION

A. Concentration of Mineral Nitrogen and Phosphates in Soil Solution

The compounds of phosphorus (P) and nitrogen (N) are necessary for plant growth and the presence of microbial activity in the soil. From January to July 2013, the concentration of mineral nitrogen (N_{min}) and phosphate (PO_4^{3-}) were measured in soil solution. The solution was captured from individual lysimeters.

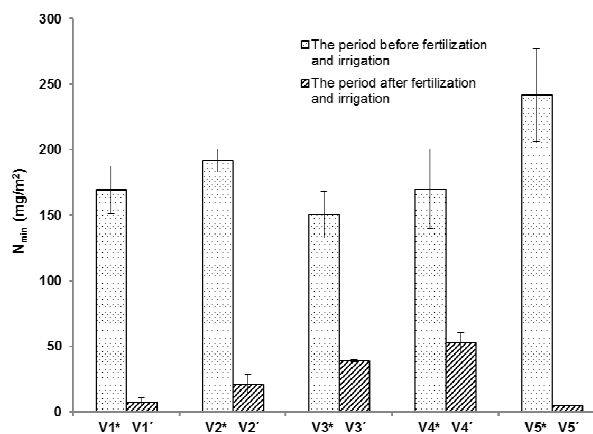


Fig. 5 Concentration of N_{min} in soil solution (mean \pm SD, $n = 3$)

Measured values were divided into two groups (V^* and V'). The concentration values of N_{min} and PO_4^{3-} before fertilization and irrigation (weighted average from January to April) are listed in the first group (V^*). Conversely, the second group (V') contains values that were measured after application of fertilizers and irrigation or long period of drought (weighted average from May to July). Individual variants are detailed in the methodology.

Fig. 5 shows concentration of N_{\min} in individual variants. This graph indicates a significant difference ($P < 0.05$) in content of N_{\min} before and after application of fertilizers in soil solution. The highest concentration of N_{\min} was measured in variant $V5^*$ (241 mg N/m^2) and the lowest in the same variant after applied fertilizers ($V5' = 4.57 \text{ mg N/m}^2$). Organic Carbon (C_{org}) was applied in this variant. Conversely, concentration of N_{\min} was about 200% higher in variant without addition of C_{org} ($V2'$, $V3'$ and $V4'$). Various scientific studies [3]-[6] confirm that C_{org} is source of energy for soil microorganisms and its application has a positive effect on microbial activities in soil. The increase of microbial activity has a direct impact on retention of N_{\min} in soil because soil microorganisms may enable the N_{\min} to be available for plants or they immobilize and store it in their bodies. But this immobilized N can afterwards be released and be leached. So building up a higher soil microbial biomass is also a risk. Therefore, this fact will be necessary to examine in further experiments.

The highest concentrations of N_{\min} were measured at the end of the first period (from January to April). These values were influenced by the original content of nutrients and vitality of plants in the fall 2012. Conversely, the lowest concentrations of N_{\min} were measured at the end of the second period (from May to June). The measured values were compared with the experiment of Decauret et al. [2]. In this experiment, authors tested fate of urine nitrogen in three soils throughout a grazing season. *Lolium perenne* L. was used as an indicator plant. Fifty-two g of N/m^2 was applied into each variant (three types of soil) and concentration of total N was measured in soil solution. The highest concentration of total N was found in every variant in fall of first year of experiment. In spring and summer, the nitrogen was taken by plant from soil. But in fall, plants reduced their activity. Therefore, the nitrogen could be leached from the soil. This is also applied for the present experiment. *Deschamsia caespitosa* L. had the highest activity after planting in the fall. In winter and at the beginning of growing season, *Deschampsia caespitosa* showed minimal activity. In combination with increased precipitation, this fact allowed leaching of N_{\min} from soil.

The relative proportion of soil P in inorganic and organic forms can vary from 10% to 90%, however, their relative solubility in soil solution, subsurface flow, and overland flow can also be dramatically different depending largely upon soil chemical conditions. Clearly, the loss of P in inorganic and organic forms is a characteristic driven by the nature and properties of the soil [11].

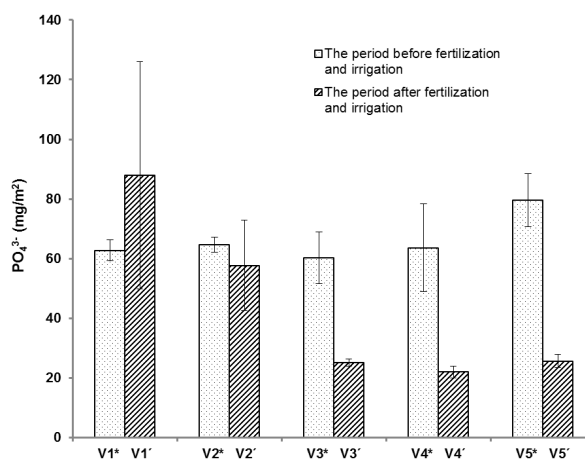


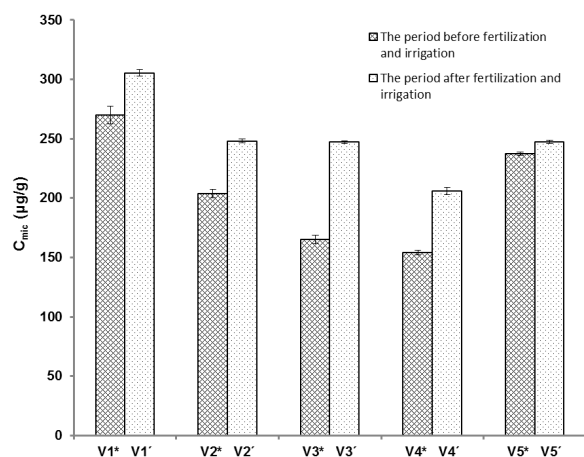
Fig. 6 Concentration of PO_4^{3-} in soil solution (mean \pm SD, $n = 3$)

Fig. 6 displays that concentration of PO_4^{3-} in the period before and after fertilization. The lowest concentration was always measured after fertilization. Besides variant $V1^*$, the highest concentration of PO_4^{3-} (87.98 mg/m^2) was measured here. In the period before fertilization (group V^*) significant differences ($P < 0.05$) were not detected between individual variants of the experiment. In the period after fertilization (group V'), significant differences were measured only between variants with irrigation ($V1$ and $V2$) and variants without irrigation ($V3$, $V4$ and $V5$). Values were significantly higher in irrigated variants than in non-irrigated variants. The differences between $V3$, $V4$ and $V5$ were not significant. The same applied to variants $V1$ and $V2$.

Data presented in Fig. 6 indicate that irrigation of $V1$ and $V2$ has a negative impact on leaching of PO_4^{3-} from soil. Conversely, the concentration of PO_4^{3-} was lowest in variants which were exposed to the weather ($V3$, $V4$ and $V5$). Likely reasons for this are: (a) Soil microorganisms did not have enough energy and nutrients so that they cannot store PO_4^{3-} in their bodies. (b) Topsoil had low water content ($V3$, $V4$ and $V5$) and hydrophobic film was created on the surface of these soils. Therefore, precipitations did not soak up and the soil solution could not be formed. The results and conclusions in [3], [7], [9], [14], [17] confirmed the possibility of *a* and *b*, which are listed above.

B. Content of Carbon and Nitrogen in Microbial Biomass

The chloroform fumigation-extraction method was used to estimate C_{mic} and N_{mic} in soil samples, which were removed from each lysimeters in April (before fertilization) and in June 2013 (after the period of intensive rainfall and drought). Measured values were divided into two groups (V^* and V' - see Materials and Methods).

Fig. 7 Content of C in microbial biomass (mean \pm SD, $n = 3$)

The above Fig. 7 shows a significant ($P < 0.05$) changes in content of C_{mic} in microbial biomass. The highest content of C_{mic} was measured in V1' (305.42 $\mu\text{g/g}$) after fertilization and the lowest content was measured in V4* (153.97 $\mu\text{g/g}$) before fertilization.

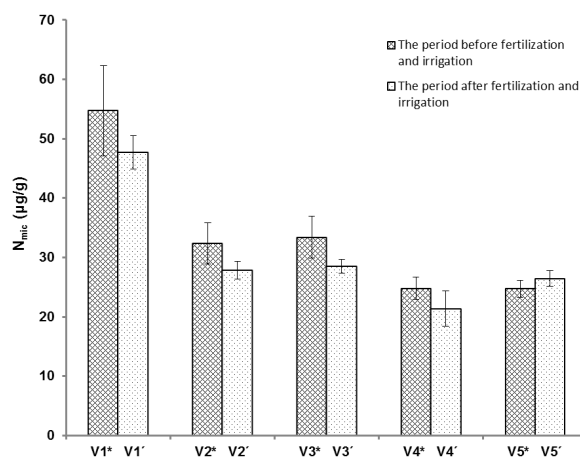
TABLE I
CONCENTRATION OF MINERAL NITROGEN IN SOIL SOLUTION AND CONTENT OF C AND N IN MICROBIAL BIOMASS

Variants	N_{min} (mg/m^2)	\pm SD	C_{mic} ($\mu\text{g/g}$)	\pm SD	N_{mic} ($\mu\text{g/g}$)	\pm SD	C:N
V1*	169.39	17.9	269.91	7.61	54.74	1.65	4.9
V2*	191.91	8.5	203.83	3.46	32.35	1.43	6.3
V3*	150.52	17.80	165.27	3.54	33.40	1.60	5.0
V4*	170.02	30.47	153.97	1.89	24.78	1.27	6.2
V5*	241.77	35.55	237.54	1.44	24.73	1.38	9.7
V1'	7.61	3.21	305.42	2.81	47.70	0.84	4.9
V2'	20.91	7.47	248.20	1.52	27.86	1.26	6.3
V3'	39.06	1.16	247.26	1.15	28.49	0.28	5.0
V4'	52.85	7.52	205.91	3.00	21.36	0.37	6.2
V5'	4.57	0.60	247.27	1.33	26.42	0.71	9.7

Consider data in Table I. These data indicated a relationship between the loss of N_{min} from the soil and the content of C in microbial biomass (C_{mic}). In accordance to our hypothesis, the highest content of C_{mic} was found in variant with sufficiency of soil water and with the lowest loss of N_{min} . This was confirmed by the regression analysis ($R = 0.4373$; $P < 0.01$; $F = 6.6203$).

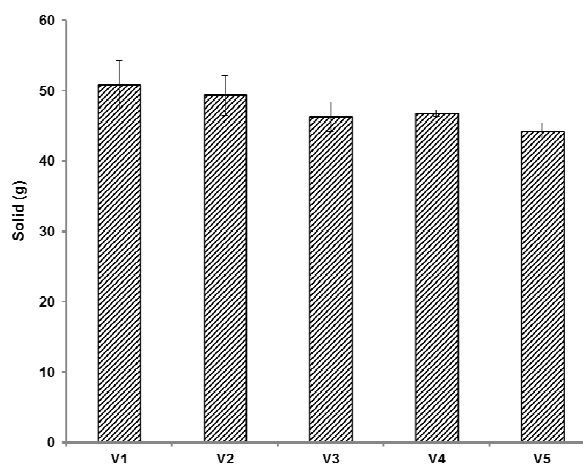
The following Fig. 8 displays significant ($P < 0.05$) changes of the content of N_{mic} in microbial biomass. The highest values were measured before fertilization. Conversely, in period from April to June 2013, the decrease in N_{mic} was found. Moreover, differences between variant with (V1 and V2) and without irrigation (V3, V4 and V5) were detected in both periods. There is the assumption that the changes in content of C_{mic} and N_{mic} have impact on the loss of N_{min} from rhizosphere soil, because quantities C_{mic} and N_{mic} reflect microbial substrate availability. So these changes indicate an increase in microbial C and a decrease in microbial N availability from the first to the second period. This is in accordance with plant

development, N uptake and production of C-rich root exudates of *Deschampsia caespitosa* during the second period. This assumption must be verified in the following periods (July – October 2013; November 2013 – January 2014; February – April 2014). For example, some authors [8], [10], [15], [14], [18] confirm, that the content and loss of nutrients in soil have a direct impact on soil microbial activities, SOM and soil fertility.

Fig. 8 Content of N in microbial biomass (mean \pm SD, $n = 3$)

C. Production of Plant Biomass

After 180 days, on the 18th of June, *Deschampsia caespitosa* (indicator plant) was harvested and its production is the main influence indicator of intensive rainfall and drought on plant production for each variant of the experiment. Fig. 9 shows the complete production of plant biomass for the first period of the experiment (November 2012 – June 2013).

Fig. 9 Production of plant biomass (mean \pm SD, $n = 3$)

Measured values of plant biomass production do not show significant differences between the individual variants. The highest values of plant biomass production were detected in variant V1 (50.8g) with the lowest losses of N_{min} for the whole

period ($V1 = 177.01 \text{ mg N/m}^2$). Based on these results, we can state that the production of *Deschampsia caespitosa* was a poor indicator of the impact of rainfall and drought on plant production.

IV. CONCLUSION

This contribution presents the first results of a long-term pot experiment. Therefore, these results must be interpreted with caution. The measured values indicate the influence of fertilization and climatic conditions on microbial biomass in soil. Based on these results, we can conclude, that there is an association between content of C (N) in microbial biomass and leaching of N_{\min} from the soil. We assume that the decrease in microbial activity causes a loss of mineral nitrogen from rhizosphere soil. The authors stress that the experiment was conducted in specific conditions and it should be repeated as a field and laboratory experiment.

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Jakub Elbl was born in Brno, Czech Republic on August 1986. He received the Ing. (Engineer) degree in Agricultural sciences (Agroecology) from Mendel University in Brno, Czech Republic in 2012. He is a PhD student and Junior researcher at Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition (Faculty of Agronomy, Mendel University in Brno). His doctoral research is focused on microbial activities in soil and their influence on leaching of nutrients from soil into underground water. Further, his research interests include influence of weather conditions on microbial activities in soil. He has been involved in national and international research projects (SONDAR - Soil Strategy Network in the Danube Region; NAZV – National Agency for Agricultural Research; Project "The possibilities for retention of reactive nitrogen from agriculture in the most vulnerable infiltration area of water resources"; IGA – Internal Grant Agency Faculty of Agronomy MENDELU; RIS - Innovation vouchers: Project "The necessary conditions for the use of pre-treated waste water for irrigation of the soil, while preventing groundwater pollution").