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Effects of Drought on Microbial Activity in Rhizosphere, Soil Hydrophobicity and Leaching of Mineral Nitrogen from Arable Soil Depending on Method of Fertilization

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Abstract**—**This work presents the first results from the long-term laboratory experiment dealing with impact of drought on soil properties. Three groups of the treatment (A, B and C) with different regime of irrigation were prepared. The soil water content was maintained at 70 % of soil water holding capacity in group A, at 40 % in group B. In group C, soil water regime was maintained in the range of wilting point. Each group of the experiment was divided into three variants (A1 = B1, C1; A2 = B2, C2 etc.) with three repetitions: Variants A1 (B1, C1) were a controls without addition of another fertilizer. Variants A2 (B2, C2) were fertilized with mineral nitrogen fertilizer DAM 390 (0.140 Mg of N per ha) and variants A3 (B3, C3) contained 45 g of C_p per a pot.

The significant differences (ANOVA, $P<0.05$) in the leaching of mineral nitrogen and values of saturated hydraulic conductivity (K_{sat}) were found. The highest values of K_{sat} were found in variants (within each group) with addition of compost (A3, B3, C3). Conversely, the lowest values of K_{sat} were found in variants with addition of mineral nitrogen. Low values of K_{sat} indicate an increased level of hydrophobicity in individual groups of the experiment. Moreover, all variants with compost addition showed lower amount of mineral nitrogen leaching and high level of microbial activity than variants without. This decrease of mineral nitrogen leaching was about 200 % in comparison with the control variant and about 300 % with variant, where mineral nitrogen was added. Based on these results, we can conclude that changes of soil water content directly have impact on microbial activity, soil hydrophobicity and loss of mineral nitrogen from soil.

Keywords**—**Drought, Microbial activity, Mineral nitrogen, Soil hydrophobicity.

I. INTRODUCTION

QUALITY and healthy soil is an essential prerequisite for ensuring production and non-production functions of agriculture. The primary consequence of quality and soil health is soil fertility. In recent years, Czech farmers have faced to decline of soil fertility and degradation of land resources. The direct causes of soil fertility depletion include: climate changes (long period of drought – precipitation totals are the same, but their layout has been changed), cultivation of fragile and marginal lands, soil erosion and decrease in the organic matter application (decreasing content of organic matter in soil – soil organic matter; SOM).

Soil organic matter (SOM) consists of a wide variety of plant and animal tissues in various stages of decomposition and it is necessary for soil fertility. The importance of organic matter (OM) is immense in supporting a biological population in the soil. OM has a profound effect on the number and kinds of organisms that are present in soil [39]. Soil microorganisms decompose SOM into simple nutrients, which are immediately used by plants or stored in their bodies. Therefore, they are an important part of the land that is indispensable to achieve quality and healthy soil – sustainable soil [33], [34].

Drought threat has significant consequences for belowground carbon and nutrient cycling [28] and thus significantly affects microbial activity in soil. The soil microbial community is involved in numerous ecosystem functions, such as nutrient cycling and organic matter decomposition. Its potential for rapid growth and turnover means that the microbial community is a more-reactive component of a terrestrial ecosystem to external stress compared to plants and animals [22]. Soil microorganisms are responsible for depolymerization of soil organic matter, which is necessary for nitrogen cycle in soil [33]. There is a direct correlation between microbial activity in the soil and the ability of soil organic-mineral complex retain of reactive nitrogen, which enters into soil from external flows, such as fertilization or atmospheric deposition [19], [34].

Moreover, the long period of drought has negative impact on fluctuations of soil moisture. The issue of extreme fluctuations of soil moisture affects activity of soil microorganisms and their influence on soil hydrophobicity [7], [11], [13]. It was clarified that soil hydrophobicity is caused by organic compounds, which remain on the surface of soil particles after the death of microorganisms. Soil water repellency is a widespread phenomenon, which affects infiltration as well as soil water retention and plant growth. It can be responsible for enhanced surface runoff, erosion and preferential flow [32]. The above information and other authors [7], [9], [11], [13] confirm that there is a complex of interactions between: (a) the formation of hydrophobic films on soil particles, (b) reduction of microbial activity, (c) leaching of nutrients from the soil sorption complex, (d) stability of soil aggregate and (e) content of water in soil.

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Therefore, the main reason for examining the impact of drought on soil hydrophobicity is its effect on the erodibility of the soil, the soil fertility and availability of nutrients.

Study of the effect of drought on soil quality is very important for agriculture in the Czech Republic, because the decline of soil fertility has become a major problem in recent years [15]. The area of our interest is the protection zone of underground drinking water source Březová nad Svitavou, which is responsible to protect this source against contamination, however, measures are not effective there. Evidence can be seen from increasing mineral nitrogen concentrations in the drinking water from this area. Soil sampling was performed only there, because we expect changes of weather conditions in future there. Currently, these changes were observed: precipitations total are the same, but their layout has been changed, there are long periods of drought and short periods of intensive rainfall. Therefore, significant changes in fluctuations of soil moisture are expected [16].

The aim of our research was to investigate the effects of drought on important parameters of soil quality: microbial activity and ability of soil to retain nutrient (nitrogen). There is hypothesis; the drought causes a decrease in microbial activity, which is subsequently reflected by increased soil hydrophobicity and leakage of nitrogen from soil. And this effect of drought can be significantly influenced by the method of fertilization. The present hypothesis was tested by pot experiment carried out under controlled conditions.

II. MATERIALS AND METHODS

A. *Experimental Design*

Effects of drought on microbial activities in rhizosphere soil, soil hydrophobicity and leaching of mineral nitrogen (N_{min}) depending on method of fertilization were tested by the pot experiment. Twenty-seven PVC tubes (see Fig. 1) were used as experimental containers and located in the growth box (phytotron; see Fig. 2). During the whole experiment, all containers with indicator plant *Deschampsia caespitosa* (one plants per experimental containers) were kept in a growth box at 24°C (day temperature), 20°C (night temperature) and 65% humidity (for all 24h) with a day length of 12 h (light intensity 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$).

Each experimental container had the same proportions (height was 55 cm and diameter was 15 cm). These containers were filled with 3 kg of topsoil and 7.5 kg of subsoil into layers. Soil sampling was done on the 25th of May 2013 in accordance with ČSN ISO 10 381-6 (ČSN - Czech Technical Standard) in the area of our interest – protection zone of underground source of drinking water Březová nad Svitavou. Local annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9°C mean of annual air temperature. Topsoil and subsoil were prepared (homogenized) separately; moreover soil samples were sieved through a sieve (grid size of 10 mm).

Samples of compost (C_p) were obtained from the company "CKB" a. s. C_p sampling was performed on the 15th of March 2013. Experimental containers were located in the growth box from the 1st of July 2013 to the 31th of January 2014. Before being stored in containers, prepared samples of compost were stored in a thermostat at a temperature of 3°C and soil samples were preincubated at 18.5°C in laboratory for 30 days.

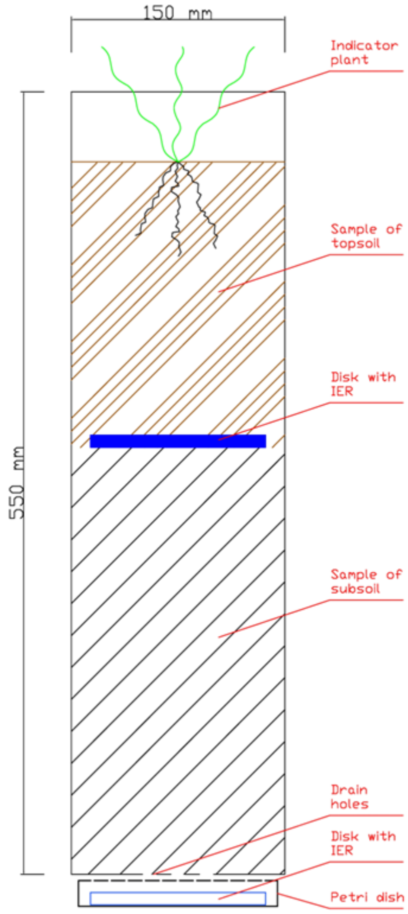

Fig. 1 Experimental container

To demonstrate effect of drought on microbial activity in rhizosphere soil, soil hydrophobicity and leaching of mineral nitrogen from arable soil, three groups of experiment A, B and C with different regime of irrigation were prepared. The complete overview is shown in the Table I.

The water content in soil was maintained at 70% of soil Water Holding Capacity (WHC) in group A, at 40% in group B. WHC was determined for top soil and subsoil according [14] as ability of soil samples (100 g) to soak up a certain amount of water for 2 h and then draining for 2 h. After that time, the soil sample was weighted and this value represents weight of soil sample achieving "100%" of WHC. The value of WHC was recalculated to the amount of topsoil and subsoil, which were used for filling of experimental containers (the same amount of subsoil 7.5 kg and topsoil 3 kg to all containers). Experimental containers were first filled with subsoil (7.5 kg), and then demineralized water was added in different quantities for the groups A and B to achieve a desired value of WHC (70% WHC and 40% WHC). The same procedure was used after filling the container with topsoil (3 kg). After filling and achieving the required values of WHC, experimental containers were weighted. Subsequently measured weight was maintained throughout the experiment by irrigation.

TABLE I
DISTRIBUTION OF THE LABORATORY EXPERIMENT

Group	Characteristic	Variants	Characteristic
		A1	Control
A	70 % WHC	A2	0.140 Mg N/ha
		A3	50 Mg C_p/ha
		B1	Control
B	40 % WHC	B2	0.140 Mg N/ha
		B3	50 Mg C_p/ha
		C1	Control
C	Wilting point	C2	0.140 Mg N/ha
		C3	50 Mg C_p/ha

Soil water regime was maintained in the range of wilting point in group C. Indicator plant was supplemented by salad (*Lactuca sativa* L.): one indicator plant and salad per one experimental container. The soil water content (in this group) was maintained at 70 % of WHC (containers have the same weight as in group A) at the beginning of the experiment. Subsequently, these containers were not irrigated until plants (salad) began to wilt. After reaching the point of wilting, plants were irrigated by one-off dose of demineralized water at the same weight as in the group A and again, these containers were not irrigated before reaching the wilting point.

Fig. 2 Location of experimental containers in a growth chamber

Each group of experiment was divided into three variants (A1, B1, C1; A2, B2, C2 etc.) with three repetitions: Variants A1 (B1, C1) were controls without addition of another fertilizer. Variants A2 (B2, C2) were fertilized with mineral nitrogen fertilizer DAM 390 (one hundred liters of DAM 390 contain 39 kg of nitrogen - 1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and ½ is in the form of urea). Recommended dose of N_{min} for extensive grass ecosystem was applied there (0.140 Mg N/ha). Variants A3 (B3, C3) contained 45 g of C_p per pot, this dose of C_p is in accordance with ČSN EN 46 5735 representing 50 Mg/ha. C_p was applied into topsoil. Used mineral fertilizer DAM 390 and C_p are registered for agriculture use in the Czech Republic.

B. Determination of Basal Respiration

Basal respiration (BAS) was determined by measuring the CO_2 production from soils incubated in serum bottles for 24 h. Field moist soil (15 g) was weighed into each of three 120-ml serum bottles. Bottles were sealed with butyl rubber stoppers and incubated at 25°C. After 3 and 24 h, a 0.5 ml sample of the internal atmosphere in each bottle was analyzed by gas chromatography (Agilent Technologies 7890A GC System equipped with a thermal conductivity detector). Respiration was calculated from the increase in CO_2 during the 21 h incubation period (24 – 3 h). At the end of measurements, the total headspace volume of each replicate bottle was determined by the volume of water required to fill the bottle. The measured amounts of CO_2 were corrected for the gas solved in the liquid phase. The results are expressed per gram of dry soil and hour [36].

C. Determination of Saturated Hydraulic Conductivity

Soil water repellency is a widespread phenomenon, affecting infiltration as well as soil water retention [32]. Therefore, saturated hydraulic conductivity (K_{sat}) was calculated based on the measured volume of water that infiltrated into the soil - cumulative infiltration, which was measured using Mini-Disk Infiltrometer (MDI) according [29].

The value of K_{sat} was determined by (1) modified according [37] originally [40].

$$K_{sat} = C_2 / A_2 \tag{1}$$

where: C_2 [$m \cdot s^{-1}$] is the function of the soil water content θ and suction (h_0) [cm]. A_2 is dimensionless coefficient. This parameter was determined by Van Genuchten equations, which were described by [40]. K_{sat} is very important parameter for the determination of soil hydrophobicity degree, because high soil hydrophobicity slows water infiltration – the value of K_{sat} is lower and conversely. K_{sat} was measured at an interval of thirty days during the experiment.

D. Determination of Mineral Nitrogen Leaching

Leaching of N_{min} (consisting of NH_4^+-N and NO_3^--N) was measured using Ion Exchange Resins (IER), which were placed into plastic PVC discs and located into and under each experimental container (see the Fig. 2).

The discs were made from plastic (PVC) tubes. Each disc was 75 mm in diameter and 5 mm thick. From both sides of each disc, nylon mesh was glued (grid size of 0.1 mm). Mixed IER (CER – Cation Exchange Resin and AER – Anion Exchange Resin in ratio 1:1) were then placed into the inner space of annular flat cove [25].

After exposition in and under the experimental containers, discs were dried at laboratory temperature 18.5°C for seven days. N_{min} was extracted from IER (individual discs) using 100 ml of 1.7 M NaCl. Distillation-titration method [27] was used for the determination of released N_{min}. The results were expressed in mg of N_{min} per m^2 (surface of experimental containers).

E. Statistical Analysis

Potential differences of values of CO_2 production, saturated hydraulic conductivity and leaching of mineral nitrogen were identified by one-way analysis of variance (ANOVA) in a combination with the Tukey´s test. All analyses were performed using Statistica 10 software. The results were processed graphically in the program Microsoft Excel 2010.

III. RESULTS AND DISCUSSION

A. Microbial Activity in Rhizosphere Soil

Respiration is probably process the most closely associated with life. It is aerobic or anaerobic energy-yielding process. In the cell, reduced organic or inorganic compounds serve as primary electron donors and imported oxidized compounds serve as terminal electron acceptors [4]. Therefore, soil respiration represents one of the most important indicators of microbial activity in soil.

The flux of carbon from soils to the atmosphere occurs primarily in the form of CO_2, and is the result of ‘soil respiration’. Soil respiration represents the combined respiration of roots and soil micro and macro-organisms [30]. Soil respiration was measured as Basal Respiration (BAS) in soil samples collected from rhizosphere.

BAS is the steady rate of respiration in soil, which originates from the turnover of organic matter (predominantly native carbon). The rate of BAS reflects both the amount and the quality of the carbon source. Moreover, the soil water content, oxygen concentration and the bioavailability of carbon are the main factors that regulate soil respiration [4].

Fig. 3 shows differences within each group of experiment between individual variants and between all variants of the experiment. Production of CO_2 reached a peak at the group A, variants A3 (0.62 µg/g·h) in comparison to other variants, this difference is significant. Conversely, the lowest value was found in group B, variants B1 (0.09 µg/g·h), but this difference is significant only if compared to variants of groups A and C. The highest production of CO_2 was caused by sufficient water content in the soil and organic carbon (C_{org}) representing a source of energy for soil microorganisms. The importance of C_{org} content and water content in soil for soil respiration was confirmed by [4], [8], [15], [31].

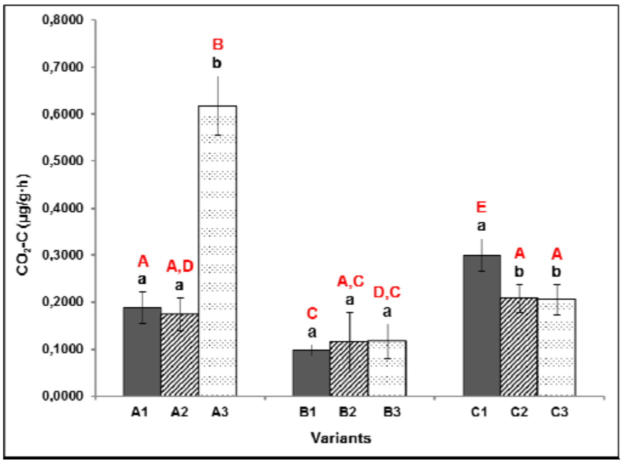

Fig. 3 Basal respiration (mean ±SD, *n* = 3)
Different small letters indicate a significant differences (P<0.05) between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants (regardless groups)

The measured values indicate important influence of water content in soil on soil respiration. Consider the Fig. 3; values of BAS were higher in group A and C compared to group B. The water content in soil was maintained at 70% of WHC in group A and at 40 % in group B. Soil water regime was maintained in the range of wilting point in group C. The above information indicates that the variant B was exposed to permanent deficits in content of soil water in comparison to other groups. The content of soil water of the group A was maintained at recommended level and the content of soil water of the group C was replenished by a single dose of water to achieve wilting point. On the one hand, system plants-soil were exposed to stress by drought, on the other hand, this system was always dosed with irrigation water, which modified the value of WHC from wilting point to optimal water content (70% of WHC).

The optimal water content in soil for respiration is thought to be 50–70% of the soil water-holding capacity [8]. This fact, together with the content of C_{org} is the main reason for the differences in BAS between individual groups. The results indicate that the drought in combination with precipitations can affect microbial activity. However, distribution of precipitation is important as demonstrated by the results obtained in group C. Correlation of soil respiration rates with mean annual air temperatures, mean annual precipitation and with interaction of these two variables were confirmed by [28].

B. Effect of Drought on Soil Water Repellency

Many regions of the world are predicted to experience water scarcity due to more frequent and more severe droughts and increased water demands compared to recent decades. Water use efficiency by plants can be negatively affected by SWR. SWR is not a static soil property, because the soil water content can alter the wetting properties. SWR evolves during dry periods and that it can disappear during wet periods [24].

TABLE II
IMPACT OF DROUGHT AND METHOD OF FERTILIZATION ON SOIL HYDROPHOBICITY

Group	Variants	K_{sat} (cm/s)	±SD	Differences within same group	Differences between all variants
A	A1	0.00071	0.00004	a	A
	A2	0.00076	0.00004	a	A
	A3	0.00111	0.00003	b	B
B	B1	0.00049	0.00015	a	C
	B2	0.00043	0.00012	a	C
	B3	0.00106	0.00028	b	A,B,D
C	C1	0.00080	0.00008	a	A,D
	C2	0.00073	0.00005	a	A,D
	C3	0.00092	0.00021	a	D

Different small letters indicate a significant differences ($P<0.05$) between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants (regardless groups).

Hydrophobicity (water repellency) reduces the affinity of soils for water [35]. Therefore, the infiltration method was used to estimate degree of SWR (or soil hydrophobicity), which were expressed based on value of K_{sat}. The values of K_{sat} are summarized in the Table II. These data indicate influence of water regime in soil and method of fertilization on SWR. The significant highest values were found in variants with addition of C_p (A3, B3, C3).

Many authors [13], [29], [32], [35] point the fact that content of organic matter (OM) in the soil have directly affected the formation of soil hydrophobicity. C_p contains a lot of OM, but in different forms, which are useful as a source of nutrients for soil microorganisms and subsequently also for plants [12], [39]. Therefore, the application of compost contributes to the development of microbial activity and thus to the development of soil organic - mineral complex, which allows better uptake and utilization of soil water. The positive effect of C_p application on microbial activity in soil, content of OM, biochemical and on its biophysical properties were confirmed by [12], [21], [23], [26].

Consider values of K_{sat} in variants with addition of mineral nitrogen (A2, B2, C2), which are lower than values in variant, where C_p was applied. These differences are significant ($P<0.05$) in group A and B. Again, data from the Table II show the influence of soil water content on soil properties, in this case, on SWR. Identical situation as in the case of soil respiration was found. The lowest degree of SWR was found in group A with sufficient content of soil water and in group C, where periodic changes in WHC were maintained. The relationship between hydraulic conductivity and soil hydrophobicity was confirmed by [2], [38], but accurate quantification of this relationship has not been described yet. Thus, accurate determination of degree of hydrophobicity is not possible yet.

C. *Leaching of Mineral Nitrogen from Arable Soil*

The chemical elements nitrogen (N), carbon, phosphorus, oxygen and sulfur are all necessary for life. N has the greatest total abundance in Earth's atmosphere, hydrosphere, and biosphere from all of these elements. In nature, N is divided into two groups: nonreactive and reactive. Nonreactive N is N_2; reactive N (N_r) includes all biologically, photochemically, and radiatively active N compounds in Earth's atmosphere and biosphere. Thus, N_r includes inorganic reduced forms of N – for example ammonium nitrogen, nitrate nitrogen etc. [20]. The most important kind of N_r in the soil is the N_{min}, which is formed by nitrate and ammonium nitrogen [34].

The most dangerous are nitrates, because they are very mobile in the soil. They have a negative charge and soil sorption complex has minimal affinity for negatively charged substances. Therefore, mineral nitrogen from arable land can quickly contaminate for example underground sources of drinking water or surface water [34], [41]. The above information confirms the importance of mineral nitrogen in arable soil. Monitoring the movement of N_{min} is thus necessary to identify the potential risk of climate change in relation to the method of fertilization on potential decrease in soil fertility and environmental contamination.

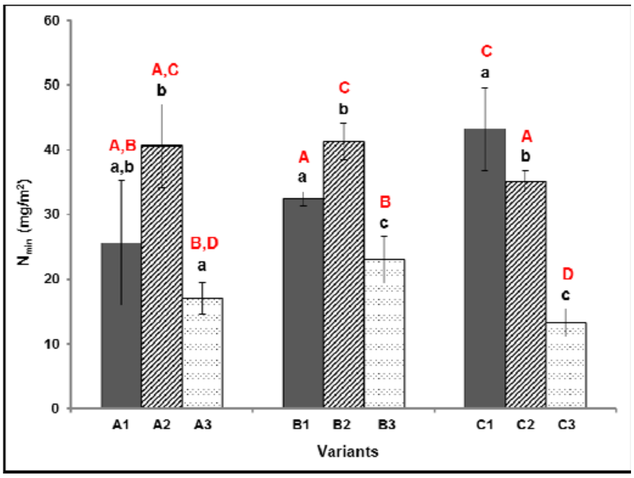

Fig. 4 Leaching of mineral nitrogen (mean ±SD, *n* = 3)
Different small letters indicate a significant differences ($P<0.05$) between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants (regardless groups).

N_{min} leaching from individual experimental containers was determined by using IER; measured values are presented in Fig. 4. Leaching of N_{min} was significantly decreased by application of C_p within the same group. The Fig. 4 shows how values of leaching of N_{min} increase in variants with mineral fertilization addition (group A and B) and in variant without fertilization (group C). The highest values of N_{min} detection in individual group were practically the same in comparison with other groups. In group A, the highest value was found in variant A2 (40.60 mg/m^2); in group B, the highest value was found again in variant B (41.20 mg/m^2) and in group C, the highest value was detected in variant C1

(43.20 mg/m^2). This data indicated that drought did not have directly affected amount of leaching of N_{min}. The effect of drought was secondary, because the primary influence was the method of fertilization. Consider the Fig. 4 – leaching of N_{min} in group A and B: the highest loss of N_{min} was found in variant with mineral fertilization addition. This is an evidence of influence of fertilization method on leaching of N_{min} during the standard variation of soil moisture. The loss of N_{min} was caused only by processes in rhizosphere. On the contrary, values of N_{min} leaching in group C confirm the influence of extreme variation and method of fertilization on loss of N_{min} from arable soil. The highest amount of N_{min} was found in control variant which were not fertilized, because N_{min} was leached by high single doses of water. These doses of water were used to simulate long periods of drought which is suddenly interrupted by intensive rainfall. Moreover, difference between variant C1 and C2 was low. Conversely, the difference between variant C2 and C3 (addition of C_p) was greater than 240 %.

The role of water balance (content of water in soil) in system plant-soil and its influence on movement of N_{min} in rhizosphere is very important for understanding the values that were measured in group C. Some authors [3], [17], [18] state that the extreme fluctuations in soil moisture have negative impact on loss of N_{min} from rhizosphere. Soils with a lack of OM are then the most vulnerable. Effect of fertilization method – nitrogen fertilization on increase in leaching of N_{min} from arable soil was confirmed by [5], [10], [19], [20], [34]. Positive effect of OM application on microbial activity and microbial immobilization of ammonium and nitrate nitrogen were confirmed by [1], [6]. Moreover positive effect of C_p as a source of C_{org} and OM on decrease in leaching of N_{min} was published in [12], [39]. The Above manuscripts explain results listed in the Fig. 3. In particular, these manuscripts and monographs confirm the influence of fertilization method and climate conditions (period of drought and intensive rainfall) on nitrogen losses from arable soil.

IV. CONCLUSION

The changes of weather conditions and climate represent a potential threat for agriculture in the Czech Republic in future. Therefore, studying the effect of different aspects of these changes is necessary to create adaptations that may help to minimize negative effects of these changes.

This study presents the part of results of a long-term laboratory experiment and therefore, these results must be interpreted with caution. From this contribution, it can be concluded that period of drought has a negative effect on loss of mineral nitrogen from soil, microbial activity and soil hydrophobicity. Based on these results, we can conclude, that there is an association between changes of WHC (caused by drought) and formation of soil hydrophobicity.

Moreover, the results of this study confirm that the negative effect of drought period can be affected by method of fertilization. These findings give support to new methods of fertilization, such as an application of organic waste compost. The authors stress that the experiment was conducted in specific conditions and it should be repeated as a field experiment.

ABBREVIATIONS

AER	– Anion exchange resin
BAS	– Basal respiration
CER	– Cation exchange resin
C_{org}	– Organic carbon
C_p	– Compost
IER	– Ion exchange resin
NaCl	– Sodium chloride
N	–Nitrogen
N_2	– Nonreactive nitrogen
N_{min}	– Mineral nitrogen
NH_4^+-N	– Ammonium nitrogen
NO_3^--N	– Nitrate nitrogen
N_r	– Reactive nitrogen
OM	– Organic matter
SOM	– Soil organic matter
SWR	– Soil water repellency
WHC	– Water holding capacity

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