The comparison of microbial activity in rhizosphere and non-rhizosphere soil stressed by drought

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Abstract: This work presents the analysis of the influence of drought on microbial activity in rhizosphere and non-rhizosphere soil. Microbial activity was expressed as basal respiration (BR). 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 maximum capillary capacity (MCC) in group A and 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. Significant differences in BR between individual variants were found in both rhizosphere and non-rhizosphere soil. The highest BR was always measured in variants A3 where soil water regime was maintained at 70% of MCC and C_p was applied. Differences in values of BR were detected between rhizosphere and non-rhizosphere soil. Unfortunately these differences were not significant. Based on these results, we can conclude that the drought in combination of method of fertilization has direct effect on microbial activities in soil, but this effect does not cause significant changes in microbial activities of rhizosphere and non-rhizosphere soil.

Key-Words: Drought, Microbial Activity, Rhizosphere Soil, Mineral Nitrogen, Arable Soil,

Introduction

While the effects of drought are well documented, a proper working definition of drought is less clear. Drought is a complex phenomenon that is difficult to accurately describe because its definition is both spatially variable and context dependent. Drought can be classified into three categories: meteorological, agricultural, and hydrological drought [1]. The wide variety of sectors affected by drought, its diverse geographical and temporal distribution, and the demand placed on water supply by human-use systems make it difficult to develop a single definition of drought and its influence [2]. Drought should be perceived as a natural aspect of climate under all climatic regimes as it occurs in both humid and arid areas (clearly with different impacts unique to the existing ecosystems). Central Europe is not frequently thought of as being a particularly drought-prone region in the European context with the exception being the Panonian Basin that, in part, includes eastern Austria and a large part of Hungary [3]. Therefore, there are not enough scientific papers dealing with the effects of drought on soil properties in the condition of central Europe.

Drought threat has significant consequences for belowground carbon (C) and nutrient cycling (N etc.). It may affect soil processes through changes in C allocation to roots and foliage as well as C turnover in the rhizosphere [4] and thus significantly affect microbial activity in rhizosphere and non-rhizosphere soil. The rhizosphere is an ill-defined a few millimeters thick that surrounds plant roots. Healthy soil is teeming with microscopic and larger organisms that perform many vital functions including converting dead and decaying matter together with minerals as plant nutrients. Therefore microbial activity represents important indicator of healthy soil [5]. Only healthy soil is resistant to negative phenomena: depletion of soil fertility, soil degradation and soil erosion.

The rhizosphere is a living space for soil microorganisms, which are very important for soil health and fertility. Plants produce root exudates which stimulate heterotrophic growth and lead to local competition for inorganic nutrients between

roots and microorganisms [6], [7]. Conversely, non-rhizosphere soil is not affected by plants roots and root exudations. Therefore there is lower level of microbial activity and soil fertility but this part of soil is necessary for the stability of the soil aggregates and resistance of soil to negative phenomena's (soil erosion, leaching of nutrients etc.). In the present work, we focused on the comparison of effect of drought on microbial activity in rhizosphere and non-rhizosphere soil. Moreover we looked for a link between the decrease in microbial activity and an increase in loss of mineral nitrogen from soil.

These hypotheses were tested: (1) the recurring period of drought has negative impact on microbial activity in both rhizosphere and non-rhizosphere soil; (2) drought – decrease in soil water content has a greater negative impact on microbial activity in non-rhizosphere zone than in rhizosphere zone; (3) microbial activity affects the soil´s ability to retain mineral nitrogen – decrease in microbial activity results in increase in leaching of mineral nitrogen.

Material and Methods

Experimental design

The above hypotheses were tested by pot experiment, which was carried out according Elbl et al. [8]: Twenty-seven PVC tubes (see Figure 1) were used as experimental containers and located in the growth box (phytotron; see Figure. 1). During the whole experiment, all containers with indicator plant *Deschampsia caespitosa* (one plant 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 $\mu mol \cdot m^{-1} \cdot s^{-1}$). Three groups of experiment A, B and C with different regime of irrigation were prepared. The complete overview is shown in the Table 1.

Table 1 Distribution of the laboratory experiment

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

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 Nmin for extensive grass ecosystem was applied there (0.140 Mg N/ha). Variants A3 (B3, C3) contained 45 g of Cp per pot. This dose of Cp is in accordance with ČSN EN 46 5735 representing 50 Mg/ha. C_p was applied into topsoil. Used mineral fertilizer DAM 390 and Cp are registered for agriculture use in the Czech Republic. 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 Dykyjova [9]. After filling and achieving the required values of WHC, experimental containers were weighted. Subsequently measured weight was maintained throughout the experiment by irrigation. 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. 1 Experimental containers in a growth box

Determination of CO_2 production

Basal respiration (BR) was determined by measuring the CO_2 production from soils incubated in serum bottles for 24 hours. Field moist soil (15 g) was weighed into each of three 120 cm^3 serum bottles. Bottles were sealed with butyl rubber stoppers and incubated at 25°C. After 3 and 24 hours 0.5 cm^3 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. At the end of the measurements, the total headspace volume for each replicate bottle was determined by measuring the volume of water required to fill the bottle. The results are expressed per gram of dry soil and hour [10]. BR was measured in soil sample which were collected from rhizosphere and non rhizosphere zone.

Determination of mineral nitrogen leaching

The loss of mineral nitrogen (N_{min}) was measured according Elbl et al. [8] and published in Elbl et al [8]. These results were used for reference purposes only: examination of a link between the decrease in microbial activity and formation of N_{min}.

Statistical analysis

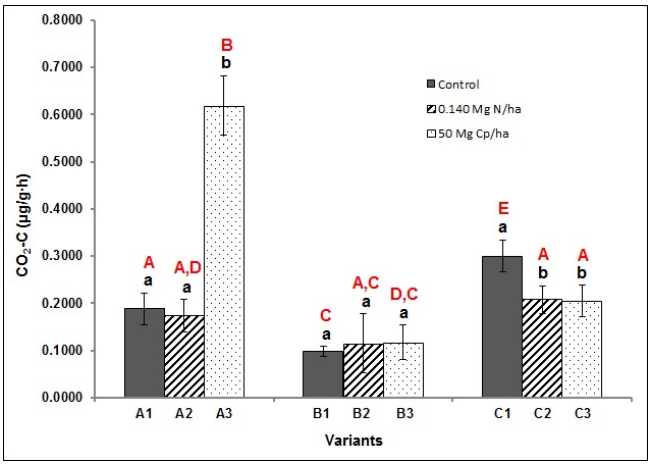
The potential differences in microbial activity between individual variants and groups of experiment were analysed by one-way analysis of variance (ANOVA; $P<0.05$) in combination with post-hoc Tukey`s HSD test ($P<0.05$). The potential differences in microbial activity between rhizosphere and non-rhizosphere soil were tested by t-test ($P<0.05$). The relationship between level of microbial activity and loss of Nmin was tested by regression analysis. All data were analyzed in the Statistica CZ 10 software. Graphic processing of measured data was performed in Microsoft Excel 2010.

Results and Discussion

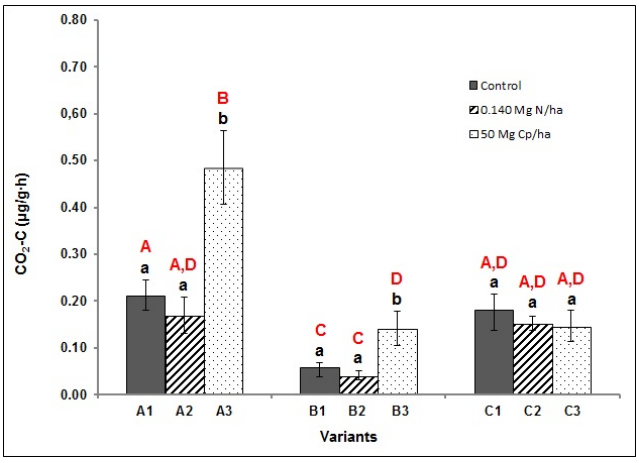
In soil microbial ecology, the actual (basal respiration, BR) and the potential microbial respirometric activity (substrate-induced respiration, SIR) are well-established and widely used method for measurement of microbial activity [12]. Soil respiration is one of the most important indicators of microbial activity in the soil. During the experiment, two types of respiration were measured: basal and substrate indicated respiration. The only values of BR are presented in this work.

Fig. 2 Basal respiration (mean ± SD, n = 3)

A) Rhizosphere soil



B) Non-rhizosphere soil



Comment for the Fig. 2: different small letters indicate significant differences ($P<0.05$) between individual variants within the same group and different uppercase letters indicate significant differences between all individual variants (regardless groups).

Microbial activity in rhizosphere and non-rhizosphere soil

Soil microbial communities play a critical role in ecosystem processes, such as carbon cycling, nutrient turnover, or the production of trace gases [12]. The BR was chosen as microbial indicator because it represents metabolic activity of soil microbes and thus their activity. The influence

of plants on the soil microbial community can be especially important in agricultural systems in which cash and cover crop selection can vary from short rotations (2-3 years) that might include one or two crop species to longer rotations (6-10 years) which might include six or eight species including both annual and perennial crops [13]. Therefore, we focused on microbial activity in zone with or without influence of plant roots.

Figure 2 presents significant differences in microbial activity in rhizosphere and non-rhizosphere soil between individual variants and all variants of experiment. The significant ($P<0.05$) highest microbial activity was found in variant A3 for both soil types. Conversely, the significant lowest microbial activity was observed in variants B for rhizosphere soil and in variant B2 at no-rhizosphere soil. The optimal water content in soil at respiration is from 50 to 70% of the soil WHC. This fact, together with the content of C_{org} in variants A3 is the main reason for the differences in BR between individual groups. Positive effect of C_p addition on microbial activity in soil was confirmed by [14][15].

The results indicate that the drought in combination with precipitations and type of fertilization (mineral/organic type) can affect microbial activity. Influence of method fertilization and soil properties (pH, soil water content, soil type, soil organic matter content, etc.) on soil microbial activity in rhizosphere and non rhizosphere soil was confirmed by [16], [17], [18].

Comparison of microbial activity in rhizosphere and non-rhizosphere soil

The rhizosphere is the plant root–microorganism interactional site. The rhizosphere is technically difficult to be defined for studying the root-induced physical–chemical, microbial properties in this zone. Generally, the rhizosphere has a thickness of 1–2 mm, but functionally, the rhizosphere can be defined as the soil physically and chemically influenced by growth and activity of the root [19].

Table 2 Comparison of microbial activity in rhizosphere (RH) and non-rhizosphere soil (NH)

Group	Variants	BR	Mean differences	t	p
A	A1	RH NR	-0.0235	-1.9742	0.1870
	A2	RH NR	0.0041	0.1215	0.9143
	A3	RH NR	**0.1333**	6.0836	0.0251
B	B1	RH NR	0.0408	1.6155	0.2476
	B2	RH NR	0.0734	1.3811	0.3013
	B3	RH NR	-0.0245	-0.7684	0.5225
C	C1	RH NR	**0.1204**	11.4530	0.0075
	C2	RH NR	0.0548	1.3754	0.3028
	C3	RH NR	0.0591	3.5260	0.0718

Comment for the Tab. 2: The means of differences are significant at the level 0.05. These differences are shown in bold. The microbial activity in RH and NH was always compared for one experimental site.

The Fig. 2 and Table 2 show complete overview of basal respiration in rhizosphere and non-rhizosphere soil. Significant differences in microbial activity were found between individual variants of experiment either in the rhizosphere or in the non-rhizosphere soil. Significant differences between rhizosphere and non-rhizosphere soil were detected only in variants A3 and C1. The microbial activity was higher in rhizosphere zone except variants A1 and B3 but these values were not significant. These situation was caused by root exudes which have direct impact on soil microbial communities.

Positive effect of root exudes on microbial activity in rhizosphere soil was confirmed by Maul et al. [13] Bloem & Hopkins [17] and Wenhao et al. [19].

Relationship between level of microbial activity and leaching of mineral nitrogen

Microbial communities in soil consist of a great diversity of species exploring their habitats by adjusting population abundance and activity rates to environmental factors. Soil microbial activities lead to the release of nutrients available for plants, and are of crucial importance in biogeochemical cycling [17].

Geisseler [20] state, that nitrogen is essential for the growth and activity of plants and soil microorganisms. Soil microorganisms can use a wide range of N compounds. These include inorganic compounds such as ammonium-N and nitrate-N, as well as organic molecules such as amino acids and small peptides.

Consider data in the Table 3 and 4 and data which are presented in the Figure 3. These data indicate the significant influence of microbial activity on leaching of N_{min}. In accordance to our hypothesis, the increase in microbial activity causes a reduction in nitrogen leaching and conversely. Relationship between microbial activity and utilization/leaching of N_{min} from soil was studied and confirmed by Geisseler [20] and Turner [21]. The significant differences in influence of microbial activity were not found between rhizosphere and non-rhizosphere soil.

Table 3 Regression analysis of the relationship between the level of microbial activity in rhizosphere soil and leaching of mineral nitrogen

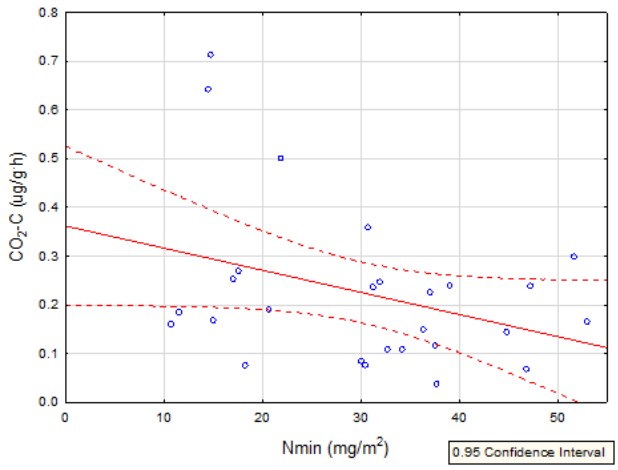
Parameter	Value
Multiple regression	0.34828
R-squared value	0.12130
Adjusted R-squared	0.08615
Statistical power	3.45119
Probability	0.07502
Standard error	12.06465

Table 4 Regression analysis of the relationship between the level of microbial activity in non-rhizosphere soil and leaching of mineral nitrogen

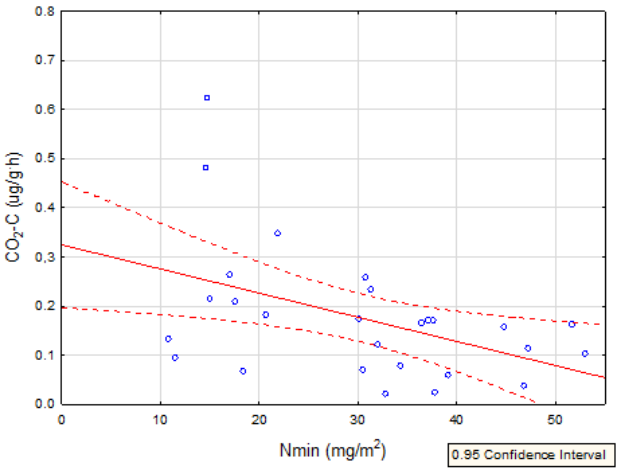
Parameter	Value
Multiple regression	0.45873
R-squared value	0.21043
Adjusted R-squared	0.17885
Statistical power	6.66296
Probability	0.01610
Standard error	11.43640

Fig. 3 Graphic processing of obtained data from regression analysis

A) Rhizosphere soil



B) Non-rhizosphere soil



Conclusion

In conclusion, our results suggest the influence of drought on microbial activity in soil and relationship between microbial activity and leaching of N_{min}. We assume that the decrease in microbial activity causes a loss of mineral nitrogen from soil. The authors stress that the experiment was conducted in specific laboratory conditions and it should be repeated as a field experiment.

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