RESPIRATION ACTIVITY AND DEVELOPMENT OF ARBUSCULAR MYCORHIZA IN LYSIMETRIC EXPERIMENT WITH DIFFERENT TYPE OF FERTILIZATION

[a]LUKÁŠ PLOŠEK, [b]MICHAELA STROBLOVÁ, [c]JAROSLAV ZÁHORA, [d]JAKUB ELBL, [e]ANTONÍN KINTL, [f]JAROSLAV HYNŠT

Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic
email: [a]lukas.plosek@mendelu.cz,
[b]michaela.stroblova@mendelu.cz, [c]jaroslav.zahora@mendelu.cz,
[d]jakub.elbl@mendelu.cz, [e]antonin.kintl@mendelu.cz,
[f]jaroslav.hynst@mendelu.cz

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Abstract: This paper deals with the effect of organic and mineral (nitrogen) fertilizers on the respiration activity and roots colonization by arbuscular mycorrhizal fungi. Soil respiration is an important component of terrestrial carbon cycling and one part of the biological properties of soil. Another biological property is arbuscular mycorrhiza (AM) which is one of mycorrhizal symbiosis types which are formed between plant roots and fungi. It was prepared seven lysimetric variants (n = 3) with different type of fertilization (compost and mineral nitrogen). We detected high differences between basal respiration (BR) and substrate induced respiration (SIR) after first year of the experiment. BR significantly decrease and SIR increase in most of variants. Influence of compost addition to roots colonization of winter wheat by AM fungi has not been confirmed. The highest roots colonization was detected in variant without the addition of fertilizers.

Keywords: basal respiration, substrate induced respiration, arbuscular mycorhiza

1 Introduction

Composting is one of the oldest methods for processing of organic waste. Also in modern times this process is more and more often taken into consideration in waste management strategies (Kopéc *et al*., 2013). The advantages of the use of organic wastes such as compost as fertilizers are evident. Their use would reduce the consumption of commercial fertilizers which need in their production high cost and energy (Romero *et al*., 2013). Compost amendment improves physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (Diaz *et al*., 2007). The present work´s goal was to evaluate effects of addition of compost and N on respiration activity (CO_2 production) and mycorrhizal roots colonization. Respiration activity of composts is one of many indicators of their maturity and usefulness for application. The degree of compost maturity also affects its fertilizer value. Arbuscular mycorrhiza (AM) is one of mycorrhizal symbiosis types which are formed between plant roots and fungi (Stroblová *et al*., 2005). The soil represents a natural source of arbuscular-mycorrhizal fungi propagules (spores, soil mycelium and infected root parts) which are of importance for the origin of mycorrhizal infection (Vázquez *et al*., 2002).

2 Material and methods

2.1 Experimental design

Effect of addition of different types of fertilizers was tested by pot experiment, previously detailed by Elbl *et al*. (2013).

Twenty one 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 588.47 mm 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 25 kg of subsoil, 25 kg of topsoil (arable soil) and with compost in selected variants. See Figure 1.

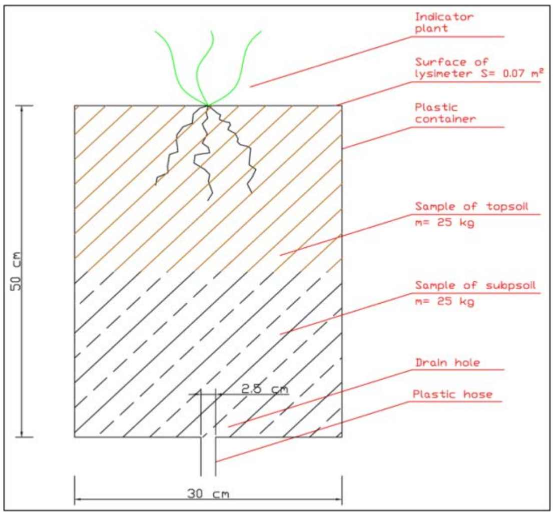

Fig. 1: Detail of experimental container – lysimeter according to Elbl *et al.* (2013)

Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) 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. Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft. Lysimeters were completed and filled in October 2012. Winter wheat was used as a nodal plant to determine the effect of addition of different types of fertilizers, microbial activities and weather on plant production. Winter wheat (22 grains into each of lysimeters) was planted in the end of October.

Seven variants of the experiment were prepared, each one in three repetitions:

- C1 – arable soil with the addition of 100 % of recommended dose of N,
- C2 – arable soil without the addition of fertilizers,
- K1 – arable soil with the addition of 100 % of recommended dose of compost,
- K2 – arable soil with the addition of 100 % of recommended dose of compost and 25 % of recommended dose of N,
- K3 – arable soil with the addition of 100 % of recommended dose of compost and 50 % of recommended dose of N,
- K4 – arable soil with the addition of 100 % of recommended dose of compost and 100 % of recommended dose of N,
- K5 – arable soil with the addition of 200 % of recommended dose of compost.

Information on the applied fertilizers: Compost (Černy drak) samples were taken from the Central Composting Plant in Brno and it is registered (under the Fertilizers Law) for agriculture use in the Czech Republic. 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 ½ is in the form of urea). One hundred liters of DAM 390 contain 39 kg of nitrogen. Recommended dose in Czech Republic of compost is 5 kg m^{-2} per 5 years and of nitrogen is 140 g m^{-2} per year for winter wheat.

2.2 Basal and substrate induced respiration

Basal respiration (BR) was determined by measuring the CO_2 productions from soil samples incubated in serum bottles for 24

h. Soil samples (15 g, humidity 20 %) was weighed into each of five 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, equipped with a thermal conductivity detector). Respiration was calculated from the increase in CO_2 during 21-h incubation period (24-3h). At the end of measurements, the total headspace volume for each replicate bottle was determined by measuring the volume of water required to fill the bottle. The measured amounts of CO_2 were corrected for the gas dissolved in the liquid phase. The results are expressed per gram of dry matter and hour (Simek *et al*., 2011). Substrate induced respiration (SIR) was determined by measuring the CO_2 production from the soil samples incubated in serum bottles for 3 h after addition of glucose. Field-moist soil sample (5 g, humidity 20 %) was added to 3 replicate serum bottles as described for the determination of BR in the previous paragraph, and 2 ml of a glucose solution was added to each bottle (4mg C g^{-1}). Bottles were sealed with butyl rubber stoppers, and incubated at 25 °C. After 2 and 4 h, a 0,5-ml sample of the internal atmosphere was analysed by gas chromatography (see previous paragraph). SIR was calculated from the CO_2 increase during the 2-h incubation period (4-2 h). The bottles were further processed as described for BR measurement (Simek *et al*., 2011).

2.2 Mycorrhizal root colonization

Mycorrhizal root colonization was evaluated in root samples taken from root systems of experimental plants. Plant roots were stored before processing proper in the fixative solution FAA (ethanol 50 %, acetic acid, formaldehyde). Staining of roots by 0.05% trypan blue in lactoglycerol (Koske & Gemma, 1989) was used for the evaluation of mycorrhizal infection, and the percentage of root colonization by AM fungi was estimated microscopically by means of a modified slide method (Giovanneti & Mosse, 1980). Mycorrhizal colonization of roots was evaluated in all cases in five replicas from each repetition of soil sample.

2.3 Statistical analysis

Potential differences in the values of roots colonization and respiration activity were analyzed by one-way analysis of variance (ANOVA) in combination with the Tukey´s test. All analyses were performed using Statistica 10 software. The results were processed graphically in the program Microsoft Excel 2007.

3 Results and Discussion

3.1 Basal and Substrate induced respiration

Soil respiration is an important component of terrestrial carbon cycling and can be influenced by many factors that vary spatially (Martin & Bolstad, 2009). Basal respiration is defined as a production of released CO_2 from the soil for a time without the addition any substrate.

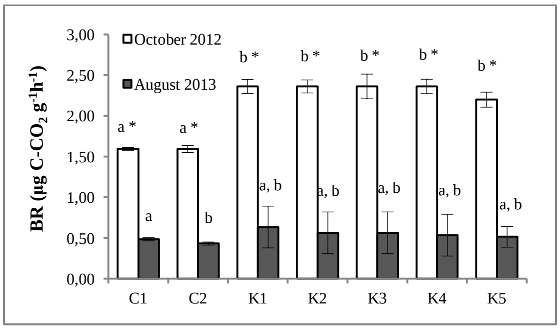

Fig. 2: Basal respiration (BR). Values are means and standard errors of three replications. Different letter indicate significant difference in one period and * indicate significant differences in one variant (ANOVA, P < 0.05).

Figure 2 presents results of basal respiration. We can see that CO_2 production is significantly higher at the beginning of the experiment. After one year of the experiment BR decreased three to four times. Addition of compost at the beginning of the experiment significantly increase BR. On the other hand increased dose of compost did not increase CO_2 production.

Substrate induced respiration method uses the soil organism´s physiological respirations reactions to substrate addition, as a means of quantifying microbial biomass in soils. Microorganisms need mainly organic carbon in the form of soil organic matter for their development (Elbl *et al.*, 2013). The organic carbon (C_{org}) is a source of energy. Martin & Bolstad (2009) confirm influence of C_{org} content on soil respiration.

Beesley (2014) confirmed that addition of compost increased respiration activity. But this activity decrease during the time (it is connected with the dose of compost and with the temperature of soil)

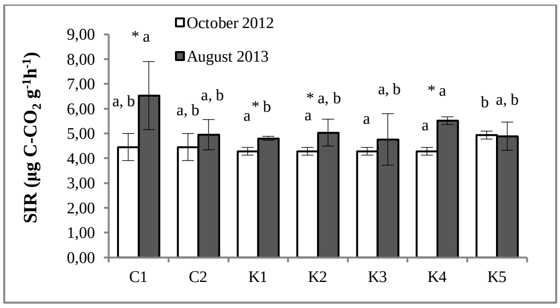

Fig. 3: Substrate induced respiration (SIR). Values are means and standard errors of three replications. Different letter indicate significant difference in one period and * indicate significant differences in one variant (ANOVA, P < 0.05).

The above figure 3 shows results of substrate induced respiration. After one year SIR increase (significantly in variants C1, K1, K2 and K4). But there were no major differences between variants in the same period.

3.1 Mycorrhizal root colonization

Importance of AM is evident - this symbiosis improves water and nutrient intake, reduces infestation of the host plant by root pathogens etc.

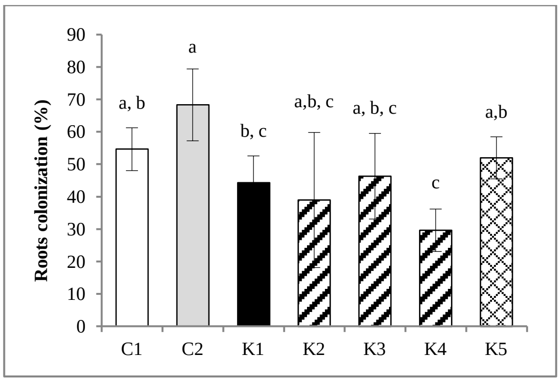

Fig. 4: Roots colonization of winter wheat by AM fungi. Values are means and standard errors of three replications. Different letter indicate significant difference in one period and * indicate significant differences in one variant (ANOVA, P < 0.05).

The above figure 4 shows differences between variants and roots colonization. You can see that combination of addition of compost and escalating dose of decrease roots colonization by

AM fungi. Azcón *et al.* (2003) and Stroblová *et al.* (2005) present confirm that high addition of nitrogen and phosphorus can have a negative effect on AM roots colonization. Stroblová *et al.* (2005) highlight that important factor for colonization of AM fungi is a type of cultivated crops.

The colonization of winter wheat roots by AM fungi varied of 30 - 68 %. The highest percentage of colonization was found in variant without the addition of fertilizers (C2). The lowest percentage of root colonization was found in variant with 100 % recommended dose of compost and nitrogen (K4).

4 Conclusions

Our lysimetric experiment showed the effect of different types of fertilization on respiration activity and roots colonization by AM fungi. Increased dose of compost did not demonstrate positive effect on roots colonization of winter wheat by AM fungi. The highest roots colonization by AM fungi was detected in variant without fertilization. Addition of compost significantly increase basal respiration, on the other hand nitrogen addition increase substrate induced respiration. Based on the results of selected biological properties is optimal to combine fertilization with compost and reduced doses of mineral fertilizers (especially nitrogen fertilizer). The reason is that compost is source of C_{org} and support development of microbial community and nitrogen (in mineral fertilizers) is a limiting factor for this development.

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