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BIOFERTILIZERS

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Comparison of effects exerted by bio-fertilizers, NPK fertilizers, and cultivation methods on soil respiration in Chernozem soil

Comparación de los efectos ejercidos por los biofertilizantes, fertilizantes NPK y los métodos de cultivo sobre la respiración del suelo en el suelo de Chernozem

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Resumen

La respiración del suelo es un indicador importante de la actividad microbiana; los procesos de respiración y descomposición del suelo a nivel mundial liberan anualmente a la atmósfera un total de 220 mil millones de toneladas de dióxido de carbono. Por lo tanto, los estudios sobre los aspectos del ciclo del carbono del suelo para optimizar las emisiones de dióxido de carbono agrícola o mejorar el secuestro de carbono contribuyen una práctica agrícola sostenible. En este artículo se presentan los efectos de la aplicación de biofertilizantes (*Bacillus megaterium, Bacillus circulans,* y *Pseudomonas putida*) en la respiración del suelo, en el suelo chernozem. Los experimentos se realizaron en la Estación Experimental de Látókép, perteneciente a la Universidad de Debrecen, Hungría. Además, estos resultados se compararon con los hallazgos de estudios anteriores relacionados con aplicaciones comerciales de fertilizantes NPK (en cuatro dosis: $N_{60}P_{45}K_{45}$;$N_{120}P_{90}K_{90}$;$N_{180}P_{135}K_{135}$; y $N_{240}P_{180}K_{180}$), y dos métodos de cultivo (arado, aflojado, RTK en filas y RTK entre filas); estas investigaciones se llevaron a cabo en la misma estación experimental. Los resultados indican una menor tendencia a la respiración del suelo cuando se aplican biofertilizantes en comparación con los fertilizantes NPK comerciales, lo que permite disminuir la emisión de CO_2 en el medio ambiente. También se discutió un cambio unitario en los diferentes métodos basados en la absorción de álcalis (Oxitop y Witkamp) para facilitar la comparación de los datos adquiridos recientemente con los resultados anteriores de experimentos de fertilización a largo plazo.

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Palabras clave: respiración del suelo, CO_2, biofertlizantes, fertilizantes, suelo chernozem, respiración del suelo, Hungría, Ecuador.

Abstract

Soil respiration is a significant indicator of soil microbial activity; global soil respiration and decomposition processes release yearly to the atmosphere a total of 220 billion tons of carbon dioxide. Therefore, studies on the whole- or one particular aspect of soil carbon cycle aiming at optimizing agricultural carbon dioxide emissions or improving carbon sequestration contribute to a sustainable agriculture practice. In this paper we present the effects of biofertilizer application (*Bacillus megaterium, Bacillus circulans*, and *Pseudomonas putida*) on soil respiration in chernozem soil. Experiments were performed at Látókép Experimental Station, belonging to the University of Debrecen, Hungary. Additionally, we compare our results with findings of prior studies related to commercial NPK fertilizer applications (in four doses: $N_{60}P_{45}K_{45}$; $N_{120}P_{90}K_{90}$; $N_{180}P_{135}K_{135}$; and $N_{240}P_{180}K_{180}$), and two different cultivation methods (ploughed, loosened, RTK in rows, and RTK between rows); these investigations were conducted at the same experimental station. Our results indicate lower tendency for soil respiration, when biofertilizers are applied as compared to commercial NPK fertilizers, which enables to decrease CO_2 emission in the environment. We also discuss a unit change in different alkali absorption-based methods (Oxitop and Witkamp) to facilitate comparability of recently acquired data with results of previous long-term fertilization experiments.

Keywords: Soil respiration, CO_2, biofertilizer, fertilizer, chernozem soil, soil respiration, Hungary, Ecuador.

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1 Introduction

Increase in CO_2 emissions is a contributor to global climate change (Gratani et al., 2016; Ashok et al., 2019). Unfortunately, carbon dioxide levels exceed the Earth's response rate to assimilate and process the emission within the carbon cycle (Lajtha, 2017). Metropolitan century has arrived in which a broad suite of sustainability challenges has to be addressed in both urban (Elmqvist et al., 2019) and rural areas (Lowy and Mátyás, 2020). If considering that over 50% of the world's population is concentrated in urban areas, and it is expected that by year 2050 over two thirds will live in cities, the problem of mitigating the concentration of atmospheric CO_2 is considerable (Gratani et al., 2016). Furthermore, to satisfy increasing food needs of people living in urban areas, agriculture must become increasingly productive. Evaluated carbon emissions in all stages of the world's food system was firmly carried out by Vermeulen et al. (2012). Authors reported that food system (from fertilizer application and cultivation to food storage and packaging) is responsible for up to one-third of anthropogenic greenhouse gas emission (Thornton, 2012). Therefore, it is a proposed viable alternative and strategy based on absorbent materials from biomass (Moral et al., 2018) and sustainable agricultural solutions.

The global atmospheric concentration of carbon dioxide (CO_2), ranging from 278 to 391 ppm, nitrous oxide (N_2O), from 2.5 to 1803 ppb, methane (CH_4), from 270 to 342 ppb (Team et al., 2014) and fluorinated gases have shown continuous increase since pre-industrial times and have contributed significantly to global warming, as stated in 2007 by the Intergovernmental Panel on Climate Change (IPCC). Unfortunately, atmospheric greenhouse gas (GHG) concentrations continue to rise, and out of GHGs, CO_2 concentration is increasing at fast pace. For maintaining global warming below $2^{\circ}C$ relative to pre-industrial levels, by 2050 the global anthropogenic GHG emissions should reduce by 40%, relative to 2010. Also, it is envisaged the increase of existing biological carbon pools for carbon sequestration (Team et al., 2014).

Global trends allow the implementation of alternatives to mitigate CO_2 production and enable the conversion of algae biomass into biofuels and the use of biofuels for replacing fossil fuels (Eloka-Eboka and Inambao, 2017). Sequestration of CO_2 was evaluated in the context of fertilizers, the loading of $NaCl$ and $NaOH$, the intensity of light and its effects on algae-biomass growth, lipid productivity and CO_2 sequestration by Kumar et al. (2018). Agriculture contributes to three primary GHG emissions: CO_2, CH_4, and N_2O. Relevant to this topic is that soil can be regarded as a sink for CO_2 via carbon sequestration and its conversion into biomass products and soil organic matter (Johnson et al., 2007; Fekete et al., 2014). For example, a change from the use of conventional tillage practices to less intensive ones (e.g., no-tillage) has been proposed to mitigate CO_2 emission from agricultural soils. In Mediterranean areas, it is observed an increased carbon pool in the soil, when reducing or eliminating tillage (Álvaro Fuentes and Cantero-Martínez, 2010). Xiao et al. (2020) reported results on addition of N-increased soil respiration (RS) by 7,1% ($P < 0{,}05$) in all biomes. Positive SR response in farmland (27,0%, $P < 0{,}05$) was significantly greater than in grassland and forest biomes, indicating that SR in anthropogenic ecosystems could be more sensitive to nitrogen enrichment. Nevertheless, it is still unclear, whether there is a similar pattern in SR response and of its components to the deposition of N in grasslands with a state of variable degradation (Zeng et al., 2018).

Recent experiments conducted with carbonate compounds in synthetic calcareous soil treated with biomass ashes, originating from a gasification power plant, showed that 16.5 g of CO_2 per kg of biomass ash were fixed. Without plant cultivation 19.7 g of CO_2 per kg of bottom biomass were fixed (López et al., 2018). This organic carbon fixation shows significant promise for carbon sequestration. Another important issue in soil carbon cycle is the extent of soil respiration (Kotroczó et al., 2018; Fekete et al., 2011), which is a reliable indicator of microbial activity proceeding in soil. Soil, plant, and animal respiration and decomposition with 28,56% of CO_2 natural emissions are the second source of CO_2 after ocean atmospheric exchange. Both processes (respiration and decomposition) release carbon dioxide as a byproduct, equal to 220 billion tons of CO_2 freed by soil organisms over one year (Denman et al., 2007). Soil respiration processes typically take place in plant roots, bacteria, fungi, and soil animals to produce the energy needed for their survival. Also considered as soil respiration is the one going on

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below-ground, decomposing buried organic matter (like roots, leaves, and animals). Carbon dioxide is released in both processes (Denman et al., 2007).

The increase in atmospheric nitrogen deposition (N) should also be considered, which impacts carbon (C) and the nutrient cycle in forest ecosystems. Findings by Peng et al. (2020) indicate that high N input increased the C content in the soil surface by reducing soil respiration. This occurred mainly by an improved stabilizing of soil organic matter, rather than reducing the microbial biomass of the soil.

Research conducted by Chen et al. (2020) established that extracellular enzymes involved in the C, N, and P cycle did not respond to the addition of N. Concentrations of extractable Ca^{2+} from the soil were reduced by adding N, while other extractable cations (Fe^{3+}, Al^{3+}, Mg^{2+}, K^{+}, and Na^{+}) were unaffected. Carbon contained by microbial biomass and the total abundance of microbes, bacteria, and fungi (phospholipid fatty acid and PLFA) was reduced by nitrogen addition, but the extracellular enzymes involved in the C, N, and P cycle did not respond to nitrogen addition. In forests, the buildup of microbial waste and its relationship with the accumulation of organic carbon in the soil has been affected by the addition of N and P, over a monitoring time frame of seven years. It produced changes in both the structure of the microbial community and the enzymatic activity induced by the deposition of N and P. All these can alter the accumulation and composition of microbial residues in tropical forests rich in nitrogen. The fungal ratio also underwent changes: bacteria population increased by the addition of P, or N and P, while the proportion of fungal residues, including bacterial residues, decreased upon adding of phosphorus. The latter may be related to an imbalance in the decomposition process of microbial waste.

Li et al. (2020) found that the stocks of C and N were related to the depth of the soil, duration of conversion and the precipitation, while the response of P was insensitive to these factors. Evidence was also provided that C, N, and P losses were correlated with physicochemical properties of the soil (pH, sand, silt, and clay). Changes in response ratios of C/N, C/P, and N/P indicated that soil C and N were more sensitive to grassland conversion, than phosphorus was.

Vast scientific research focuses on GHG emissions, based on evaluating different factors, such as soil types, cropping, irrigation, and fertilizer management (Glatzel et al., 2004; Kong et al., 2013; Singla et al., 2014; Singla and Inubushi, 2014). Different land uses impact significantly the production and consumption of GHGs (Baldock et al., 2012), including soil temperature (Rustad et al., 2001), soil moisture (Inubushi et al., 2005), aboveground plant biomass, and soil microbial biomass, SMB (Inubushi et al., 2005) and soil total carbon/nitrogen (C/N) ratio (Xu et al., 2008). Temperature changes may increase the decomposition rate of soil organic carbon, SOC (Powlson, 2005; Iqbal et al., 2010). Soil organic carbon returns to the atmosphere as CO2 via respiration, while soil organisms use organic materials as a source of energy and nutrients (Baldock et al., 2012). SMB encompassing microbial biomass carbon (MBC) and nitrogen (MBN) can serve as an early indicator of changes in soil properties (Glatzel et al., 2004). Given that soil respiration is important parameter of soil microbial activity, it can serve as an essential component of sustainable agriculture.

Aims of sustainable agriculture are to secure humankind's present food and textile needs, without compromising the resources of future generations. Involved in sustainable agriculture are: farmers, food processors, distributors, retailers, consumers, and waste managers. In addition, research scientists working in sustainable agriculture are guided by interdisciplinary approach. They combine a blend of disciplines, encompassing biology, chemistry, engineering, economics, and community development. They rely on project management and corporate social responsibility (Melendez et al., 2018a,b; Melendez and Gracia, 2019). All these groups seek to integrate three main objectives into their work: (i) a healthy environment, (ii) economic profitability, as well as (iii) social justice and fair trade (Francis and Porter, 2011).

Currently, many different microbial biofertilizers are available for agricultural use since they enhance plant growth and productivity. They improve soil fertility directly, by adding to the soil beneficial microbial inoculants, and indirectly, via stimulating soil microorganisms (El-Yazeid et al., 2007). Phylazonit is a bio-fertilizer brand released by Phylazonit Ltd., which contains an optimized

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ratio of bacterial strains for soil injection (*Bacillus megaterium*, *Bacillus circulans*, and *Pseudomonas putida*). *Bacillus megaterium* is a cosmopolitan bacterium, which lives in an extended habitat, from soil to seawater, sediment, rice paddies, honey, fish, and dried food. It can easily grow in both simple and complex media, being mainly aerobic gram-positive, spore forming bacteria. *B. megaterium* is amongst the largest size known bacteria, with a cell length of up to 4 μm and a diameter of 1.5 μm. *B. megaterium* has at least up to 100-fold greater volume than *Escherichia coli* (De Vos et al., 2009; Vary et al., 2007; Bunk et al., 2010). Recently, it has become increasingly popular in the field of biotechnology for its recombinant protein production capacity. For the purpose of intra- and extracellular protein synthesis, several vectors were constructed and commercialized (MoBiTec GmbH, Germany). *B. megaterium* is also used in industry, where it produces biotechnologically relevant substances that grow on cheap substrates, and are non-pathogenic (they do not produce endotoxins associated with an outer membrane), unlike *E. coli*. Therefore, *B. megaterium* opens an avenue toward challenging biotechnological approaches (Vary et al., 2007; Bunk et al., 2010).

Bacillus circulans is a gram-positive rod that is motile by flagella. Cell size is in the range of 2.0-4.2 μm x 0.5-0.8 μm. *B. circulans* is a facultative anaerobic organism, so that it can make ATP (adenosine triphosphate) by aerobic respiration, when oxygen is present, but it also can switch to anaerobic respiration, when oxygen is absent. It can grow in the range of pH 6-9, but pH 7 is optimal for its evolution in an optimum temperature range of 30-37°*C*. This bacterium produces endospores, which allow bacteria to lie dormant for extended periods of time in harsh living conditions, but under favorable conditions they can reactivate themselves into vegetative stage. *B. circulans* bacteria are reported as plant growth promoting rhizobacteria (Gordon et al., 1973). *Pseudomonas putida* is a rod-shaped, flagellated, gram-negative bacterium found in most soil and water habitats, where oxygen is present. It grows optimally at 25-30°*C*. Given that *Pseudomonas putida* promotes plant development, it is used in bioengineering research to develop biopesticides and to the enhance plant health. The root surface, rhizosphere allows bacteria to prosper from the root nutrients; *P. putida* induces plant growth and protects plants from pathogens (Espinosa-Urgel et al., 2000).

Recently, changes in several physical-chemical (Bautista et al., 2017; Jakab, 2020) and microbial soil properties (Mátyás et al., 2015; Sándor et al., 2020b) have been investigated in the region. Mátyás et al. (2016) examined the effect of different NPK doses on soil microbial activity and microbial biomass. Sándor et al. (2020b) explored how different cultivation methods affected soil respiration and enzymatic activity. In this study, the effects of a Phylazonit biofertilizer on soil respiration were addressed and compared to the extent of CO_2 emission caused by commercial chemical NPK fertilizers, biofertilizers, and different cultivation methods.

2 Materials and Methods

This investigation was conducted on a calcareous chernozem soil, in a multifactorial experiment at Látókép Experimental Station, the Center of Agricultural Sciences, the University of Debrecen. This station is in Eastern Hungary, 15 km away from the city of Debrecen. The area is known for the aeolain loess of Hajdúsag area, its geographical coordinates being $47°33'55{,}36''N; 21°28'12{,}27''E$. Annual yield fluctuations are primarily determined by the moisture content of soil in the month of July, and the water supply in May (Brebbia and Bjornlund, 2014). Experiments were performed from March to April 2016. The soil in the area can be classified as loamy and nearly neutral. Phosphorus supply of the soil is medium, while its potassium content is medium or good (Brebbia and Bjornlund, 2014).

Experimental plots were set up randomized in 4 replications per each measurement, in two sets. First set corresponds to measurements carried out on April 5, 2016, and the second on April 19, 2016. $15L/ha$ of Phylazonit were injected according to the manufacturer's specifications. Concentration of bacteria was $109/cm^3$. Phylazonit contains an optimized ratio of bacteria strains, *Bacillus megaterium*, *Bacillus circulans*, and *Pseudomonas putida*. Soil moisture content was measured gravimetrically by drying soil samples at 105°*C* for 24 h, according to the protocol described in A. (1970). pH was determined potentiometrically in distilled water, for soil/water ratio of 1:2.5 (w/w), according to Buzás (1988), by using a glass electrode attached to a Mo-

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del Seven2Go Advanced Single-Channel Portable pH Meter (Mettler, Toledo), suited for pH and conductivity measurements. Silt and clay fraction were determined according to Buzás (1988). Physical-chemical and microbial soil properties and enzymatic activities are reported in the supporting material.

The experimental design for soil respiration was completely randomized, treatments were setup in incubators at 25°*C* for 180 h, in dark. These were placed in laboratory bottles (250 mL) equipped with a tight screw cap, 0.1 M NaOH (10 mL), and then a sterile gauze pad was filled with soil sample (10 g), and placed inside the bottle. After 2, 3, and 10 days, respectively, the amount of CO_2 absorbed by the residual alkali solution was determined by potentiometric titration with aqueous 0.1 M HCl solution, using phenolphthalein as the indicator. CO_2 outputs were calculated by Equation 1, as described by Witkamp (1966).

$$mg(CO_2) = V * M * 22 \quad (1)$$

Where $mg(CO_2)$ is the mass of captured CO_2 (mg), V is the volume of HCl used in titration against saturated KOH solution (mL), M is the molarity of HCI ($mol\ L^{-1}$). Results are interpreted for respiration of 100 g soil samples over 10 day period, so the unit in Witkamp method is: $mg\ CO_2 \cdot (100g)^{-1} \cdot (10\ \text{day})^{-1}$.

Moisture multiplication factors of control samples are 1.40, and 1.26 for treated samples, respectively. Factor of KOH solution was 1.09,- while HCl solution had a factor of 0.93. An induced method was also used, in which 0.10 g glucose was added to the soil samples. Each treatment was replicated in quadruplicate. In addition, experimental results were compared to the findings of prior studies conducted at the same experimental station. In previous experiments, effects of commercial fertilizer doses of $N_{60}P_{45}K_{45}$; $N_{120}P_{90}K_{90}$; $N_{180}P_{135}K_{135}$; and $N_{240}P_{180}K_{180}$ (Mátyás et al., 2015) and of different cultivation methods (ploughed, loosened, RTK in rows, and RTK between rows) (Sándor et al., 2020a) on physical, chemical, and microbiological soil properties were assessed on the long term, in fertilization experiments that spanned over 30 years. Two methods for determining soil respiration were also compared, the Witkamp and Oxitop incubations (Bautista et al., 2017). Student's t-test was applied for statistical analysis, using SPSS (version 26) to reveal possible relevant differences in control and treated (biofertilizer) samples.

3 Results and discussion

Typically, induced methods are applied in studies related to microbial activity to reveal differences between various treatments. In this case, without induced method (added glucose) differences between control and treated samples are obvious. Differences can be observed between control and treated samples, starting the 2nd day of incubation (Table 1).

Table 1. Average results of soil respiration in incubation over 2, 3, and 10 days (mg $CO_2 \cdot 100\text{g}^{-1} \cdot 10\ \text{day}^{-1}$). Control = absolute control that does not stand for either biofertilizer treatment or induced method. Control + glucose = no biofertilizer was added, but induced method was applied. Treated = bio-fertilzer was applied. Treated + glucose= biofertilizer was added, and induced method was applied.

				Soil respiration (mg $CO_2 \cdot 100\text{g}^{-1} \cdot 10\ \text{day}^{-1}$)		
	Soil moisture content (%)	pH (H_2O)	Silt and clay fraction (%)	2nd day	3rd day	10th day
Control	20.11-21.02	6.9	37.5	85.2	77.4	143
Control + glucose				148.5	173.5	259.9
Treated				130.1	84.9	141.1
Treated+ glucose				137.3	194.8	265.7

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Raw data (results in repetitions and factors) are provided in Supporting Material. Nevertheless, there is a decrease in soil CO_2 production in treated samples, starting the 2nd and 3rd days of incubation. This depletion in CO_2 production was assessed in all experiments, performed in quadruplicate. It is assumed that the phenomenon originates from the presence in soil of CO_2 consuming microbes or methanotrophs, which utilize the CO_2 produced periodically. This assumption is verified by a prior study (Bautista et al., 2017). However, this finding isunusual, because such bacteria are typically present in seawater and paddy soils, rather than in well ventilated chernozem soils with optimal moisture content (between 20.11 and 21.02 wt %) (Table 1). Values radically increased from the third to the seventh day of incubation. By the 10th day, there are no more notable differences between control and treated samples (Table 1); furthermore, no statistically significant differences were found between control and treated (Phylazonit) samples (at significance level 0.05).

In Figure 1, the results obtained on CO_2 emission dynamics were compared to findings reported previously in the scientific literature. In prior studies (Bautista et al., 2017; Sándor et al., 2020b), samples were collected from both irrigated and non-irrigated plots. Considering that soil moisture content strongly affects CO_2 production, it is assumed that results coming from prior studies had similar soil moisture content to the ones of the current research. Accepted range was: 19-21 wt% (within the optimal range on this soil type). In a prior study the effect of the same biofertilizer on soil respiration was examined at the same experimental station (Bautista et al., 2017). However, authors applied a different alkaline absorption method; they used Oxitop bottles, and their results were expressed in another unit, namely: CO_2 mL/L. To compare study findings obtained with different methodologies, the followings were considered:

(i) the amount of CO_2 in Oxitop bottles is calculated from the oxygen consumed by decomposition process that involves oxygen, carbon, and carbon dioxide participating in the respiration process (Oxitop manual).

(ii) formation of a CO_2 molecule requires one C atom and one O_2 molecule.

To compare prior study findings with these, all results are expressed in $CO_2 \cdot (100\ \mathrm{g})^{-1} \cdot (10\ \mathrm{day})^{-1}$ according to Equation 2.

$$\mathrm{ResultsOxitop}(ml/L) = 11{,}136363 * \mathrm{WitkampCO_2}(mgCO_2) \quad (2)$$

In studies conducted by Mátyás et al. (2015) and Sándor et al. (2020b) CO_2 values are expressed in $CO2 \cdot (100\ \mathrm{g})^{-1} \cdot (10\ \mathrm{day})^{-1}$, so there was no need for unit change. Generally, titration is carried out only after 10th day of incubation (comparable to the before mentioned prior studies). Additionally, in the present study the soil CO_2 production was measured along the entire incubation process; this is the reason why results are also shown for 2nd and 3rd days of incubation.

It can be stated that the highest NPK dose ($N_{240}P_{180}K_{180}$) causes the most intensive soil respiration as compared to bio-fertilizer application or different cultivation methods. Differences are observed between different cultivation methods; highest CO_2 values belong to samples from RTK in rows. Soil respiration values measured by two methods (Witkamp and Oxitop) are of the same order of magnitude, although Witkamp-based values are greater on each incubation day. Apart from the extremely high values of CO_2 measured by Witkamp's method on the second day of incubation, the increase in CO_2 from day 3 to day 7 is remarkable in the framework of both methods: Witkamp and Oxitop. During the incubation period, the increase of soil respiration was 60,17%, within Witkamp's method, and 54,87%, when Oxitop bottles were used. This pattern is notable if considering that experiments were performed quadruplicate, hence they are statistically relevant. These results validate a modern method (Oxitop) with a well-established, proven method (Witkamp).

4 Conclusions

This study indicates a lower tendency for soil respiration when bio-fertilizers are used, as compared to commercial NPK fertilizers. Hence, by means of bio-fertilizers CO_2 emissions can decrease to the environment. Nevertheless, long term experiments and field trials are needed for better understanding

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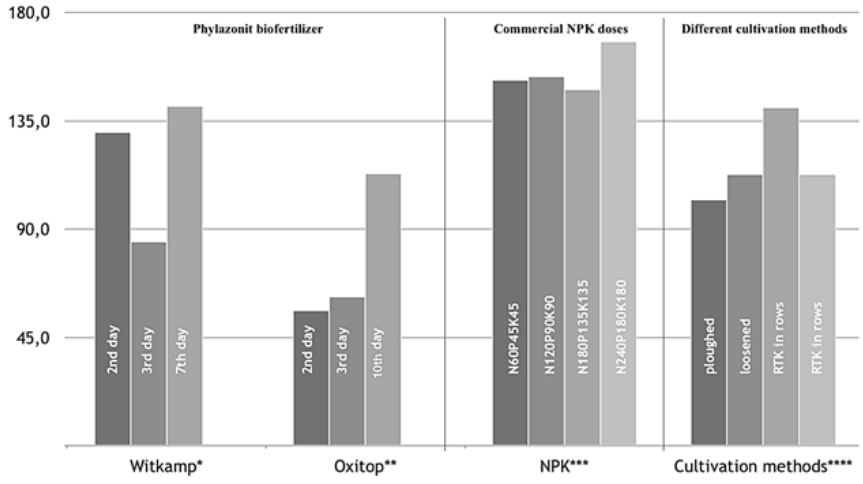

Figure 1. Soil respiration results after 2, 3, and 10 days of incubation ($mg\ CO_2 \cdot (100\ g)^{-1} \cdot (10\ day)^{-1}$)), collated from own measurements and prior study findings. *own results related to effect of Phylazonit on soil respiration by the Witkamp method; **effect of Phylazonit on soil respiration by Oxitop bottles (Bautista et al., 2017); ***commercial NPK fertilizers in doses of $N_{60}P_{45}K_{45}$; $N_{120}P_{90}K_{90}$; $N_{180}P_{135}K_{135}$; and $N_{240}P_{180}K_{180}$ (Mátyás et al., 2015); ****different cultivation methods: ploughed, loosened, RTK in rows, and RTK between rows (Sándor et al., 2020b,a) on soil respiration.

and possibly reveal statistical differences between bio-fertilizers and chemical fertilizers-treated soil.

With the unit change in CO_2 results obtained in this study by two different alkali absorption-based methods (Oxitop and Witkamp) the aim is to contribute to improving comparability of scholarly studies, which apply different methodologies to determine soil respiration. This is particularly useful for examining changes in microbiological soil properties over long-term fertilization experiments, where even 30 years old results are being compared with new findings. Unit change applied and discussed here allows to compare studies based on different methods, allowing the adoption of new methods offered by the evolving technology. Data acquired by new methods can be incorporated with studies performed by traditional methods over the past decades.

Competing interests

No competing interests were disclosed.

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