RESEARCH NOTE

Unexpected results in Chernozem soil respiration while measuring the effect of a bio-fertilizer on soil microbial activity [version 1; peer review: 1 approved, 1 approved with reservations]

Gabriela Bautista ^{1,2}, Bence Mátyás ^{2,3}

¹Department of Agricultural Chemistry and Soil Sciences, University of Debrecen, Debrecen, Hungary

²Grupo de Investigación Mentoría y Gestión del Cambio, Universidad Politécnica Salesiana, Cuenca, Ecuador

³Grupo de Investigación en Ciencias Ambientales, Universidad Politécnica Salesiana, Quito, Ecuador

V1 First published: 03 Nov 2017, 6:1950
<https://doi.org/10.12688/f1000research.12936.1>
 Latest published: 18 Dec 2017, 6:1950
<https://doi.org/10.12688/f1000research.12936.2>

Abstract

The number of studies investigating the effect of bio-fertilizers is increasing because of their importance in sustainable agriculture and environmental quality. In our experiments, we measured the effect of different fertilizers on soil respiration. In the present study, we were looking for the cause of unexpected changes in CO₂ values while examining Chernozem soil samples. We concluded that CO₂ oxidizing microbes or methanotrophs may be present in the soil that periodically consume CO₂. This is unusual for a sample taken from the upper layer of well-ventilated Chernozem soil with optimal moisture content.

Keywords

bio-fertilizer, soil respiration, Chernozem, OxiTop

Open Peer Review

Approval Status

	1	2
version 2		
(revision)		
18 Dec 2017		view
version 1		
03 Nov 2017	view	view

1. **Ankit Singla**, Regional Centre of Organic Farming, Bhubaneswar, India

2. **Muhammad Aslam Ali**, Bangladesh Agricultural University, Mymensingh, Bangladesh

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Gabriela Bautista (gebautista89@gmail.com)

Author roles: **Bautista G:** Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Mátyás B:** Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2017 Bautista G and Mátyás B. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Bautista G and Mátyás B. **Unexpected results in Chernozem soil respiration while measuring the effect of a bio-fertilizer on soil microbial activity [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2017, 6:1950 <https://doi.org/10.12688/f1000research.12936.1>

First published: 03 Nov 2017, 6:1950 <https://doi.org/10.12688/f1000research.12936.1>

Introduction

Research related to the benefits of microbes as biofertilizer has become increasingly important in the agricultural sector. This is due to their potential role in achieving higher crop yields while minimizing negative impact on the environment. It is well known that bio-fertilizers increase plant yield and improve soil fertility¹⁻³. Soil respiration is an important indicator of soil microbial activity⁴⁻⁶. In our experiments, we measured the effect of different chemicals⁷⁻⁹ and a bio fertilizer on soil microbial activity, using both well-established and novel methods under laboratory conditions. We present some unexpected results from a setup in which Chernozem soil samples were examined.

Methods

The phylazonit bio-fertilizer (produced by Phylazonit.Ltd, Hungary) was used for testing (15 l/ha). It has the following composition: *Bacillus megaterium*, *Bacillus circulans*, *Pseudomonas putida*, in an optimized ratio for soil injection. Number of bacteria: 10^9 piece/cm³.

A total of 24 soil samples were collected near Debrecen, Hungary on the 19th April 2016, from an upper layer (0–20 cm) of Chernozem soil (47° 33' 55.36" N; 21° 28' 12.27" E). All samples had optimum moisture content with 19–21 percent. Soil moisture content was determined gravimetrically by drying the soil at 105 degrees C for 24 hours according to Klimes-Szmik, 1970¹⁰. The experimental design was completely randomized, treatments were applied at 25 °C. An OxiTop OC110 respirometer was used to quantify the release and capture of CO₂, automatically determined by the device after the biological oxygen demand (BOD) required for the degradation of organic matter has been measured. We used a 500 ml glass bottle system, following the protocol in the manual (<https://www.wtw.com/en/service/downloads/operating-manuals.html>).

10g of soil sample were placed into OxiTop flasks, capped with the sensor heads according to Barrales-Brito *et al.*, 2014¹¹. 2.5 g of CO₂ absorber (sodalime) were then added to a tank, to absorb the generated CO₂ according to Barrales-Brito *et al.*, 2014¹¹. Induced samples were given 0.1g of glucose. Each treatment was replicated four times. As Figure 1 shows, four samples were always measured in parallel: Absolute control (does not contain fertilizer, nor added glucose), Induced control (contains added glucose), Treated (contains bio-fertilizer) and Induced treated (contains bio-fertilizer and glucose). The Oxitop automatically provides the values related to CO₂ production.

Results

The treated samples produced more CO₂ than the controls, as expected. Each repeat with the exception of one showed growing CO₂ values (Figure 1), as the pressure continuously decreased in the bottle due to gas (oxygen) consumption. One sample produced unexpected results (Figure 2). In the first 12 hours, the treated samples produced more CO₂ than the controls in each measurement. Following this, a fluctuation in the values was observed.

Dataset 1. Average values for a number of different soil properties

<http://dx.doi.org/10.5256/f1000research.12936.d182655>

Dataset 2. Average values of produced CO₂ (ml/l) with different treatments. 'Control' does not contain fertilizer, nor added glucose. 'Control+Glucose' contains 0,1 g of added glucose. 'Biofertilizer' contains Phylazonit biofertilizer. 'Biofertilizer+Glucose' contains Phylazonit biofertilizer and 0,1 g of added glucose

<http://dx.doi.org/10.5256/f1000research.12936.d182663>

Figure 1. Differences in CO₂ production of treated and control soil samples. The induced method was also applied in order to differentiate between the control and the treated soil samples to become detectable sooner. Glucose was applied as inducer. As expected the CO₂ values increase or stagnate.

Figure 2. This sample shows CO_2 values periodically decreasing in all conditions. After examining the Oxitop device's operation, this pattern became more interesting for us, as the device quantifies CO_2 production by measuring BOD required for the degradation of organic matter. From the decreasing CO_2 values, we conclude that there was oxygen production and/or CO_2 consumption in the Oxitop bottles.

Dataset 3. Comparison of produced CO_2 (ml/l) in the sample in which unexpected (periodically decreasing CO_2) values can be observed. 'Control' does not contain fertilizer, nor added glucose. 'Control+Glucose' contains 0,1 g of added glucose. 'Biofertilizer' contains Phylazonit bio-fertilizer. 'Biofertilizer+Glucose' contains Phylazonit bio-fertilizer and 0,1 g of added glucose

<http://dx.doi.org/10.5256/f1000research.12936.d182664>

Discussion

In a closed system where the pressure decreases due to oxygen consumption, the values must increase or stagnate with the passage of time, but this was not the case with one of the samples (Figure 2). Here, a decrease in CO_2 occurred. The following possible reasons were excluded:

- Presence of algae: there was no light in the incubator, so there was no photosynthesis.
- Changing pressure caused by changing temperature: the temperature was constant in the setup.
- Absorption by the water in the sample: all other samples that produced increasing amount of CO_2 had the same or comparable moisture content.

One reason that seemed more likely was that CO_2 oxidizing microbes or methanotrophs may have been present in the soil, periodically using the produced CO_2 . This is unusual since most of studies report the presence of these bacteria in seawater¹², paddy fields¹³ or industrial processes¹⁴ and not in well-ventilated Chernozem soil. Further genomics research could detect the bacterial strains that consumed the CO_2 in this soil.

Data availability

Dataset 1: Average values for a number of different soil properties. DOI, [10.5256/f1000research.12936.d182655](https://doi.org/10.5256/f1000research.12936.d182655)¹⁵.

Dataset 2: Average values of produced CO_2 (ml/l) with different treatments. 'Control' does not contain fertilizer, nor added glucose. 'Control+Glucose' contains 0,1 g of added glucose. 'Biofertilizer' contains Phylazonit bio-fertilizer. 'Biofertilizer+Glucose' contains Phylazonit bio-fertilizer and 0,1 g of added glucose. DOI, [10.5256/f1000research.12936.d182663](https://doi.org/10.5256/f1000research.12936.d182663)¹⁶.

Dataset 3: Comparison of produced CO_2 (ml/l) in the sample in which unexpected (periodically decreasing CO_2) values can be observed. 'Control' does not contain fertilizer, nor added glucose. 'Control+Glucose' contains 0,1 g of added glucose. 'Biofertilizer' contains Phylazonit bio-fertilizer. 'Biofertilizer+Glucose' contains Phylazonit bio-fertilizer and 0,1 g of added glucose. DOI, [10.5256/f1000research.12936.d182664](https://doi.org/10.5256/f1000research.12936.d182664)¹⁷.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

We are grateful to the Department of Agricultural Chemistry and Soil Sciences at University of Debrecen for providing the experimental setups.

References

1. El-Yazeid A, Abou-Aly H, Mady M, *et al.*: **Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray.** *Res J Agr Biol Sci.* 2007; **3**(4): 274–286.
[Reference Source](#)
2. Garg V, Kukreja K, Singla A: **Efficacy of native rhizobial strains for nodulation and plant biomass production in berseem (*Trifolium alexandrinum* L.).** *AgricInternational.* 2016; **3**(1–2): 89–95.
[Reference Source](#)
3. Garg V, Kukreja K, Gera R, *et al.*: **Production of indole-3-acetic acid by berseem (*Trifolium alexandrinum* L.) rhizobia isolated from Haryana, India.** *Agr Sci Digest.* 2015; **35**(3): 229–232.
[Publisher Full Text](#)
4. Anderson TH, Domsch KH: **The metabolic quotient for CO₂ (qCO₂) as a specific activity parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass of forest soils.** *Soil Biol Biochem.* 1993; **25**(3): 393–395.
[Publisher Full Text](#)
5. Allison SD, Czimczik CI, Treseder KK: **Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest.** *Glob Chang Biol.* 2008; **14**(5): 1156–1168.
[Publisher Full Text](#)
6. Cachipundo-Ulcuango CJ, Requelme N, Gualavisi Cachiguango OM, *et al.*: **Community use of water and land for sustainable production of pasture.** *La Granja: Journal of Life Sciences.* 2017; **26**(2): 142–154.
[Publisher Full Text](#)
7. Mátyás B, Kátai J, Horváth J: **Comparative analysis of certain soil microbiological characteristics of carbon cycle.** *Acta Agraria.* 2016; (69): 137–142.
[Reference Source](#)
8. Horváth J, Mátyás B, Kátai J: **Impact of agronomic factors on urease enzyme activity in a long term fertilizer experiment.** *Acta Agraria.* 2016; (68): 43–48.
[Reference Source](#)
9. Mátyás B, Tállai M, Kátai J, *et al.*: **The impact of fertilisation on a few microbiological parameters of the carbon cycle.** *Acta Agraria.* 2015; (64): 45–50.
[Reference Source](#)
10. Klimes-Szmik A: **A talajok fizikai tulajdonságainak vizsgálata. Talaj és trágyvizsgálati módszerek.** 1970; (48): 83–161.
11. Barreles-Brito E, Etchevers-Barra J, Hidalgo-Moreno C, *et al.*: **Determinación in vitro de la emisión de CO₂ en muestras de mantillo in vitro determination of CO₂ emission in forest litter.** *Agrociencia.* 2014; **48**(7): 679–690.
[Reference Source](#)
12. Bae SL, Kwak K, Kim S, *et al.*: **Isolation and characterization of CO₂-fixing hydrogen-oxidizing marine bacteria.** *J Biosci Bioeng.* 2001; **91**(5): 442–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Singla A, Inubushi K: **CO₂, CH₄ and N₂O production potential of paddy soil after biogas byproducts application under waterlogged condition.** *Int J Agric Environ Biotech.* 2013; **6**(2): 233–239.
[Reference Source](#)
14. Kurek I, Reed JS, Dyson L, *et al.*: **Engineered CO₂-Fixing Chemotrophic Microorganisms Producing Carbon-Based Products and Methods of Using the Same.** Patent, US20170152533 A1. 2017.
[Reference Source](#)
15. Bautista G, Mátyás B: **Dataset 1 in: Unexpected results in Chernozem soil respiration while measuring the effect of a bio-fertilizer on soil microbial activity.** *F1000Research.* 2017.
[Data Source](#)
16. Bautista G, Mátyás B: **Dataset 2 in: Unexpected results in Chernozem soil respiration while measuring the effect of a bio-fertilizer on soil microbial activity.** *F1000Research.* 2017.
[Data Source](#)
17. Bautista G, Mátyás B: **Dataset 3 in: Unexpected results in Chernozem soil respiration while measuring the effect of a bio-fertilizer on soil microbial activity.** *F1000Research.* 2017.
[Data Source](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 29 November 2017

<https://doi.org/10.5256/f1000research.14027.r27583>

© 2017 Ali M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Muhammad Aslam Ali

Department of Environmental Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

1. Why did the authors select Phylazonit biofertilizer? Does it contain any methanotrophs bacterial spp. or any electron acceptors? Didn't find the composition.
2. Why not investigate the CO₂ production rate with varying levels such as 0.5%, 1% and 5% substrates application in soils?
2. What were the initial content of organic carbon, total nitrogen, soil pH, redox status (Soil Eh) and microbial composition of the collected 24 soil samples?
3. How did the researchers control the pressure within the glass bottles during the experimental period?
4. How did the authors maintain moisture levels or water filled pore space uniformity in the 24 soil samples containing glass bottles?
5. Why didn't you collect the gas samples evolved from the soils in glass bottles at varying time hours?
6. Why didn't you follow the light/dark conditions in the Incubator where the glass bottles were kept?
7. All the Figures are not clear, no contrasting colors or bullets with lines used to differentiate the treatments.
8. How were soil microbial activities assessed? Methanogens and methanotroph's relative intensity were not found in this script, which are the major focus related to the current research topic.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Soil GHGs flux measurement, soil microbes & environment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2017

Gabriela Bautista, University of Debrecen, Debrecen, Hungary

Dear Prof. Muhammad Aslam Ali

We are trying to answer your questions, and submit a second version of the manuscript in order to clarify the following points.

1. Why did the authors select Phylazonit biofertilizer? Does it contain any methanotrophs bacterial spp. or any electron acceptors? Didn't find the composition.

The Phylazonit Ltd. provided the biofertilizer for test.

The Methods chapter begins with the information related to the composition: "*Bacillus megaterium*, *Bacillus circulans*, *Pseudomonas putida*, in an optimized ratio for soil injection". We did not say that the fertilizer contains methanotrophs bacterial spp. or any electron acceptors. That's why the results presented in the paper are unexpected.

2. Why not investigate the CO₂ production rate with varying levels such as 0.5%, 1% and 5% substrates application in soils?

This Research note discusses only unexpected results come from an experiment that was carried out using Oxitop devices. This is part of a project in which more methods are applied. In an other method (using liquid-alkaline absorption) is possible to setup the different levels, but that is not part of the discussion of the present paper. Using Oxitop bottles only one level is possible for the setup.

2. What were the initial content of organic carbon, total nitrogen, soil pH, redox status

(Soil Eh) and microbial composition of the collected 24 soil samples?

In the Dataset 1: Average values for a number of different soil properties you can find the main physical, chemical and microbial soil properties such as pH (H₂O), pH (KCl), Organic carbon. We will extend the dataset with the Total Nitrogen in the second version of the paper.

3. How did the researchers control the pressure within the glass bottles during the experimental period?

The Oxitop automatically measures the changes in the bottles due to gas consumption by its sensor, there is no needed to apply external measurement.

4. How did the authors maintain moisture levels or water filled pore space uniformity in the 24 soil samples containing glass bottles?

The measurement was carried out in closed system (bootles), it is not possible to open the bottles during the measurement.

5. Why didn't you collect the gas samples evolved from the soils in glass bottles at varying time hours?

The Oxitop continously measures the changes. As Fig.1 and Fig.2 show during 168 hours the gas oxygen consumption/ CO₂ production were measured.

6. Why didn't you follow the light/dark conditions in the Incubator where the glass bottles were kept?

In order to avoid the effect of the photosynthesis by algaeas. We were interested in soil bacteria activities only.

7. All the Figures are not clear, no contrasting colors or bullets with lines used to differentiate the treatments.

We do not understand this question. In Both figures we used different colors and bullets.

- Control (absolute): Blue
- Control + glucose: Red
- Treated: Green
- Treated + glucose: Purple

8. How were soil microbial activities assessed? Methanogens and methanotroph's relative intensity were not found in this script, which are the major focus related to the current research topic.

This paper was submitted as a Research note. Research notes are often *preliminary studies*.

descriptions of unexpected and perhaps unexplained observations or lab protocols. We concluded that "Further genomics research could detect the bacterial strains that consumed the CO2 in this soil."

Gabriela Bautista, Bence Mátyás

Competing Interests: No competing interests were disclosed.

Reviewer Report 20 November 2017

<https://doi.org/10.5256/f1000research.14027.r27580>

© 2017 Singla A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ankit Singla

Regional Centre of Organic Farming, Bhubaneswar, Odisha, India

Bautista and Matyas observed unexpected results in Chernozem soil respiration following the different fertilizer treatments. I think, the values of Dataset 2 could be directly included in the main content of the paper, if possible. The title of Dataset 1 should be "Average values for various properties of Chernozem soil".

I have answered 'partly' to the question '*Are all the source data underlying the results available to ensure full reproducibility?*' as soil ecosystems are very diverse and results could vary under different environmental conditions.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research