BRIEF REPORT

Effect of herbicides on soil respiration: a case study conducted at Debrecen-Látókép Plant Cultivation Experimental Station [version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract
Measuring the effect of herbicides on the natural environment is essential to secure sustainable agriculture practices. Amount of carbon dioxide released by soil microorganisms (soil respiration) is one of the most important soil health indicators, known so far. In this paper we present a comprehensive quantifying study, in which we measured the effect of 14 herbicides on soil respiration over 16 years, from 1991 to 2017, at Debrecen-Látókép Plant Cultivation Experimental Station. Investigated herbicides contained different active ingredients and were applied in various doses. It was found that 11 out of the examined 14 herbicides had a detrimental effect on soil respiration.

Keywords
CO2 emission, Chernozem, Herbicides, Látókép, Debrecen, soil respiration

Open Peer Review

Reviewer Status ✓ ? ✓

	Invited Reviewers		
	1	**2**	**3**
version 1 19 Nov 2020	✓ report	? report	✓ report

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2. **Fa-Jun Chen**, Nanjing Agricultural University, Nanjing, China
3. **Istvan Fekete**, University of Nyíregyháza, Nyíregyháza, Hungary

Any reports and responses or comments on the

article can be found at the end of the article.

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Author roles: Sándor Z: Conceptualization, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation; **Kincses I**: Formal Analysis, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Tállai M**: Conceptualization, Investigation, Methodology, Project Administration, Software, Writing – Original Draft Preparation; **Lowy DA**: Formal Analysis, Methodology, Project Administration, Validation, Writing – Review & Editing; **Melendez JR**: Investigation, Methodology, Project Administration, Validation, Writing – Original Draft Preparation; **Guananga Diaz NI**: Investigation, Methodology, Project Administration, Writing – Original Draft Preparation; **Guevara Iñiguez LE**: Investigation, Methodology, Writing – Original Draft Preparation; **Cuenca Nevarez G**: Data Curation, Investigation, Methodology, Writing – Original Draft Preparation; **Talledo Solórzano V**: Investigation, Methodology, Resources, Supervision, Writing – Original Draft Preparation; **Kátai J**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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Introduction

Carbon dioxide (CO_2) is an important greenhouse gas, which affects significantly global warming and climate change (Rastogi *et al.*, 2002). Approximately 30% of the total CO_2 emissions are released by agricultural activities. It is notable that agricultural CO_2 emissions increased by 27% over two decades, from 1970 to 1990 (Lal, 2004).

Primary sources of soil CO_2 emissions are root respiration and degrading of organics by soil microorganisms. Soil microbial activity mainly depends on soil properties, including soil temperature, organic matter and soil moisture content (Smith *et al.*, 2003). Increasing scientific attention is focused on understanding the role of the soil microbial community (Bautista *et al.*, 2017; Cho-Tiedje, 2000; Mátyás *et al.*, 2018; Mátyás *et al.*, 2020) and nutrient cycles (Jakab, 2020; Sándor *et al.*, 2020). It has been documented that different cultivation technologies significantly impact soil microbiological activity (Sándor *et al.*, 2020).

Different chemicals (such as fertilizers and/or herbicides) are utilized in agricultural technologies. Use of herbicides constitutes an integral part of crop production, and one should be aware that they cause a "secondary effect" on both soil life and so called "non-target" organisms (Kecskés, 1976). Sensitive organisms are killed after using herbicides, and their remains are easily decomposed by the surviving microorganisms (Cervelli *et al.*, 1978). At present, the selection criteria for allowed chemicals is more rigorous and stricter than over past decades, and they are restricted to smaller concentrations (Inui *et al.*, 2001). Soil microbes play a major role in maintaining soil quality (Mendes *et al.*, 2018; Wang *et al.*, 2008).

In this paper, we discuss carbon dioxide emission levels of chernozem soil at the Debrecen-Látókép Plant Cultivation Experimental Station, where herbicides were applied to control the weeds. We compare results of carbon dioxide production in treated plots to untreated control parcels.

Methods

First, we conducted a literature review on types and doses (L-ha^{-1} or kg-ha^{-1}) of herbicides (Molnár & Ocskó, 2000; Ocskó, 1991; Ocskó *et al.*, 2017) that had been applied from 1991 to 2017 at Debrecen-Látókép Plant Cultivation Experimental Station (47°33' 55.36" N; 21°28' 12.27" E). The type of soil is calcareous chernozem; according to the International Classification (WRB) it is designated as Calcic Endofluvic Chernozen (Endosceletic). Prior absolute control soil was measured; control soil did not receive any treatment or fertilizers.

Soil CO_2 was measured in triplicate by NaOH absorption. Experiments were performed between 1991 and 2017. In 1991, 2000, 2008 and 2017 soil samples were obtained two weeks after the herbicide(s) was applied. For incubation, 10 g of soil was weighed and placed in a polyester bag (0.1 mm ⌀ holes), from where CO_2 could escape. One took 500 mL laboratory glassware in which 10 cm^3 of 0.1 M NaOH solution (Sigma-Aldrich, USA) was introduced to absorb the released carbon dioxide. Soil samples were hung above the NaOH solution, and the glass containers were sealed tightly. Since CO_2 has a greater density than air, it sunk in the container, and was absorbed by the alkaline solution. After an incubation period of 7 days, the remaining alkali solution was back titrated with 0.1 M HCl (Sigma-Aldrich, USA), in the presence of phenolphthalein, and then with methyl orange indicator. From the volume of equivalence one can calculate the amount of CO_2 formed during soil respiration, according to Equation 1.

$$\text{mg}(CO_2)\cdot 10\ \text{g}^{-1}\cdot 7\ \text{day}^{-1} = (\text{C-S})\cdot f(\text{NaOH})\cdot f(\text{HCl})\cdot 2.2 * \text{dm} \quad (1)$$

where, C: 0.1 M/ dm^3 HCl loss for methyl orange indicator (Sigma-Aldrich, USA); S: 0.1 M dm^3 HCl loss for phenolphthalein indicator (Sigma-Aldrich, USA); f: 0.1 mol dm^{-3} HCl and a 0.10 mol dm^{-3} NaOH factor; 2.2: titer (1 mL 0,1 mol dm^{-3} HCl equivalent 2.2 mg CO_2); dm: multiplication factor for dry soil.

Data analysis was performed using Microsoft Excel 2003 (mean values and standard deviation). Two-factor variance analysis was performed to obtain the significant effect on measured parameters. Significant differences were accepted at the level 1%, but the evaluation was calculated by LSD 5% values, as widely accepted in agricultural research.

Results and discussion

In 1991, three herbicides were applied, and even the basic doses were high. Results were compared to the control; CO_2 production was significantly reduced at single doses and a further decrease was experienced at 2–3 times greater doses. Consequently, CO_2 production declined gradually with increasing doses of herbicides. The smallest production was obtained at 3 times the dose of Anelda Plus 80 EC, its value being only 59% of the control (Table 1).

In 2000, six different active ingredients were used, and their effect examined. Much lower doses were applied, half and one third of the ones used in 1991. As compared to the control, soil respiration decreased significantly in all treated plots, after laboratory incubation. The lowest results were obtained with Acenit 880 EC; when 3x dose was used, only the 64% of the control being achieved.

In 2008, a significant decrease was found for the treated soil relative to the control. In the treatment with triple dose, only 74% of the control was measured. The herbicides used in 2008 are no longer authorized, as they were withdrawn from the market.

In 2017, three herbicides were examined. Out of them, Figaro TF, which contained glyphosate agent, was no longer authorized. When this herbicide was applied, CO_2 production decreased significantly. Carbon dioxide production did not change considerably in Andengo and Capreno treatments; there was a slight increase in treatments with Andengo and decrease in treatments with Capreno.

Table 1. Herbicides' doses and soil respiration measured.

Year	Herbicide dose / Herbicide	Initial herbicide dose ($L\ ha^{-1}$)	1x	2x	3x
			Soil respiration (mg CO_2 · 10 g^{-1} · (7 day)$^{-1}$)		
1991	Control	None	23.5		
	Alirox 80 Ec	5-8	22.81	21.75	18.37
	Anelda Plus 80 EC	5-9	20.15	16.25	13.91
	Vernolate 80 EC	6-8	18.34	17.55	15.91
2000	Control	None	14.25		
	Dual 720 EC	2.5 -3.5	11.34	12.67	11.56
	Frontier 900 EC	1.5-2.0	10.4	10.21	10.14
	Hungazin PK	1.4-2.8*	12.43	10.11	9.36
	Dual Gold 960 EC	1.4-1.6	10.52	10.37	10.21
	Proponit 8720 EC	1.5-2.5	12.4	11.71	10.54
	Acenit 880 EC	2.0-2.6	9.22	9.57	9.17
2008	Control	None	15.47		
	Merlin SC	0.16-0.20	15.41	15.38	15.49
	Wig EC	3.5-4.5	12.86	13.27	11.46
2017	Control	None	19.18		
	Adengo	0.40-0.44	19.42	19.47	19.44
	Capreno	0.25-0.30	18.87	19.16	18.96
	Figaro TF	2.0-5.0	17.94	17.72	16.3

* $kg\ ha^{-1}$ (quantity given in different units)

Conclusions

We can conclude that CO_2 production decreased significantly in the soil for 11 out of the 14 herbicides. With two herbicides, Merlin SC (izoxaflutol) and Capreno (Isoxadifen-ethyl, tembotrione), there was no significant change of treated soils relative to the untreated soil, and there was only one herbicide Adengo (Bayer, Germany), which increased soil respiration slightly, but not significantly. The main sources of CO_2-emissions from soil is the respiration of plant roots and of the microbial community. Therefore, a significant decrease of CO_2 emission indicates a change in these parameters. One can recommend for use those chemicals, which do not cause major changes in the microbial community and do not affect life conditions of other live organisms.

Data availability

Underlying data

Figshare: Supporting data CO2 soil respiration, https://doi.org/10.6084/m9.figshare.13125290.v1 (Dama Research Center Limited, 2020).

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Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 1

Reviewer Report 26 February 2021

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✔ **Istvan Fekete**
Institute of Environmental Sciences, University of Nyíregyháza, Nyíregyháza, Hungary

"Primary sources of soil CO_2 emissions are root respiration and degrading of organics by soil microorganisms."

- the above sentence needs some citatioms: e.g Béni et al. 2017
  Beni Áron; Kate, Lajtha ; János, Kozma ; István, Fekete, (2017), Application of a Stir Bar Sorptive Extraction sample preparation method with HPLC for soil fungal biomass determination in soils from a detrital manipulation study, JOURNAL OF MICROBIOLOGICAL METHODS 136 pp. 1-5.[1]
  PMID: **28238755, DOI: 10.1016/j.mimet.2017.02.009**

"Soil microbial activity mainly depends on soil properties, including soil temperature, organic matter and soil moisture content Smith *et al*., 2003"

- A more recent citation would also be helpful.
  e. g. Fekete et al. 2021
  (Fekete, I ; Berki, I ; Lajtha, K ; Trumbore, S ; Francioso, O ; Gioacchini, P ; Montecchio, D ; Várbíró, G ; Béni, Á ; Makádi, M et al. (2021) How will a drier climate change carbon sequestration in soils of the deciduous forests of Central Europe?, BIOGEOCHEMISTRY 152 pp. 13-32. , 20 p.), https://doi.org/10.1007/s10533-020-00740-0[2]

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How many repetitions did the measurements take, when the soil respiration values in Table 1 were determined? If average values were used then standard error or standard deviation values should also be included.

It would also be useful to indicate in the table which values differed significantly from the control.

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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?
No

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

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?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Soil ecology

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.

Reviewer Report 07 January 2021

https://doi.org/10.5256/f1000research.29886.r75106

?

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In this manuscript, the authors carried out experiments to study the effect of herbicides on soil

respiration. In this study, they measured the effect of 14 herbicides on soil respiration over 16 years, from 1991 to 2017, at Debrecen-Látókép Plant Cultivation Experimental Station.This is an interesting and novel topics for the readers.
There were some errors and shortcomings, which were following as:

- Q1: Title: In this title, it is no necessary to give the testing site. So it should be changed as “Effect of herbicides on soil respiration: a case study”.

- Q2: Abstract: Give more results of the measured indexes, not just the description of detrimental effects!

- Q3: M&M: Soil CO2 was measured in triplicate by NaOH absorption. Experiments were performed between 1991 and 2017. Why was just soil samples were obtained two weeks after the herbicide(s) in 1991, 2000, 2008 and 2017? What about the results of the other measuring years? And what were the two factors in the two-favtor ANOVAs? Sampling years and herbicide treatment? This should be given in the data analysis.

- Q4: Results: Why were just the results in 1991, 2000, 2008 and 2017 given in this study? What about the other years' results from 1991 to 2017?

- Q5: Table 1: In this stable, it shows confused and not clear to say "Herbicide dose", "Initial herbicide dose" (L ha-1), and 1x, 2x and3x!

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?
Partly

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.

Reviewer Expertise: Plant Protection; Insect Ecology; Soil Ecology

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.

Reviewer Report 01 December 2020

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The authors provided greater clarity on the controls for their study, which measured the effect of 14 herbicides on soil respiration over 16 years from 1991 to 2017, and this study is highly interesting and readers will be intrigued by these results.

I have some minor comment that are:

- Creating a map with the sites for sampling to be studied with a mention of the crops grown during the study period;
- The number of doses should be indicated in the methods used;
- What about the standard deviation and LSD in Table 1.

In the future, I would like to use a phospholipid fatty acids (PLFAs) technique to study the biomass of the microbial community such as bacteria, fungi....etc.

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?
Partly

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

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Soil microbiology

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