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RESPONSES OF SOIL CO_2 EFFLUX TO BIOTIC AND ABIOTIC DRIVERS IN AGRICULTURAL SOILS

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Title: Responses of soil CO_2 efflux to biotic and abiotic drivers in agricultural soils

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1. BACKGROUND AND OBJECTIVES

Global warming has become a severe problem that cannot be overlooked (Pesce *et al.*, 2018). Rising temperature has significant effects on ecosystem's functioning, especially on carbon cycling. In fact, soil respiration (Rs) is a dominant process of the global carbon cycle and it has a significant influence on global radiative forcing (Qin *et al.*, 2014). Soil respiration and its components are under the control of a complex set of biotic and abiotic driving forces, and as croplands are one of the main sources of greenhouse gases into the atmosphere (Reichstein *et al.*, 2003) a study of the temporal dynamics of soil respiration has great significance. However, a wide range of studies proved that several factors, like vegetation (Balogh *et al.*, 2019), soil temperature, soil moisture, nutrient availability and N treatments, and agriculture management practices can affect soil respiration rates (Wang *et al.*, 2016).

The large uncertainty in Rs estimations could be caused by the fact that Rs is regulated by these multiple biotic and environmental factors (Hanson *et al.*, 2000) and because of the error of measurements (Nagy *et al.*, 2011). Soil respiration is the second-largest flux in the global C budget and returns as much as 50-90% of annual gross primary production (GPP) back into the atmosphere (Bahn *et al.*, 2008) depending on the cited drivers (Carbone *et al.*, 2011). Combined experiments (field and lab studies) could provide new insights into these effects. Among these factors, soil temperature and moisture are generally acknowledged as the dominant drivers of Rs but soil temperature is generally considered the most dynamic on both diurnal and longer time scales, therefore it is used in the majority of Rs models (Daly *et al.*, 2008) being a good predictor of the dynamics of the soil CO_2 flux rate.

Soil moisture influences the production of CO_2 both by directly affecting the activity of microorganism and plant roots and the diffusion of gases through the soil pores (Li, Ou and Chen, 2014), and indirectly affecting the change of the substrate supply and plant growth (Davidson, Janssens and Lou, 2006). The changes in soil water content can strongly modify the total soil respiration.

Rs is a highly complex process consisting of two main components including autotrophic (R_a) and heterotrophic respiration (R_h) (Balogh *et al.*, 2016). Plants are the most important autotrophs contributing to CO_2 efflux from soil by root

respiration, while heterotrophic respiration mainly comes from free-living soil microorganisms that subsisted by decomposition of soil organic matter (SOM) and organic matter in litter layer (Moyano *et al.*, 2009), and is primarily regulated by the root activity and plant photosynthate supply (Vargas *et al.*, 2011). Although the direct contribution of nematodes and soil macro-fauna (macroscopic invertebrates and small mammals) to R_h is small, they can greatly increase microbial respiration not only by fragmentation and comminution of plant residues but also by predation of some groups of microorganisms.

1.1. Objectives

The main goals of current study were:

1. To investigate the temporal dynamics of CO_2 efflux from the soil surface in a temperate cropland site during two years long study period.
2. To analyze the response of the soil respiration components to the main environmental factors of cropland systems such as soil temperature (Ts), soil water content (SWC), N fertilization and biotic drivers as plant growth. Both field and laboratory measurements were conducted within the framework of the study.

2. MATERIAL AND METHODS

2.1. Field measurements

2.1.1 Site description

The study was performed from November 2017 to November 2019 in cropland near Kartal (47.658°N, 19.532°E, 153 m a.s.l.) which is located in the middle part of Hungary. The site has a running eddy-covariance (EC) station since 2017 for CO_2/H_2O gas exchange and meteorological measurements. Gödöllő Experimental Farm Ltd. has the land management rights of the site and provided management data. The soil is chernozem type brown forest soil (WRB, 2015: chernozem) with 54.9% sand, 28.05% clay and 17.05% loam,

2.1.2 Field design and soil CO_2 exchange measurements

Ten PVC rings were installed one month before the flux measurement, the living weeds and the litter in the PVC ring were removed from the soil surface to avoid soil disturbance and ground vegetation respiration (Han *et al.*, 2014). PVC rings were inserted approx. 2.5 cm into the ground, leaving 2.5 cm above the ground to measure CO_2 efflux at each point. Fluxes of CO_2 were measured about bi-weekly/monthly between 10:00 and 12:00 h as the most suitable time of the day

for measurements during a two-year-long study period by closed chamber systems: Licor 6400 (LiCor, Inc. Lincoln, NE, USA) in 2017 and EGM-4 (PPSystems, Amesbury, USA) in 2018 and in 2019.

Net ecosystem exchange of CO_2 (NEE) was measured by eddy-covariance (EC) technique. The EC system at the Kartal site has been measuring the CO_2 and sensible and latent heat fluxes continuously since October 2017.

Table 1. Agricultural management practices during two years-long study period in Kartal site.

Study years	Crops type	Seedbed preparation date	Sowing Date	Fertilization date	Nitrogen application rate (kg N/ha)	Harvesting amount and date	Tillage date
2017	Winter wheat	02.10.2017	03.10.2017	01.10.2017	100 Kg/ha CAN 27%	- 14.07.2018	-
2018	rapeseed	31.08.2018	10.09.2018	15.03.2018 29.08.2018	140 Kg/ha Nikrol 30% 200 kg/ha NPK 15-15-15	7.04 t/ha 02.04.2019	01.08.2018
2019	Sorghum	26.04.2019	03.05.2019	03.05.2019 04.10.2019	200 kg/ha MAS 27% 100 kg/ha MAS 27%	9.38 t/ha 30.09.2019	-

2.1.3 Additional measurements

Soil temperature (Ts), Soil moisture (SWC), leaf area index (LAI), VIgreen and bulk density were measured in the same time with the CO_2 efflux measurement during the study period.

2.1.4 Microbial diversity

To investigate the microbial diversity of our five soil samples which was collected during the study period, microbiological counts (enumeration of a bacteria population, actinomycetes, ammonificans, fungi and denitrifying bacteria) and BIOLOG EcoPlate was used.

2.2. Lab measurements

2.2.1 Sampling of soil

Soil from the top 15 cm layer was collected from the same field and transported to the lab. Before establishing the laboratory experiments, the soil was air-dried, visible roots, large stones and organic residues were removed and the soil was passed through a 2-mm mesh size and then mixed thoroughly; PVC tubes (10.2 cm in diameter and 20 cm height) were used as pots filled up to 15 cm with about 1.6 kg of soil to achieve a bulk density of 1.30 g cm^{-3}. The top 5 cm of the tube was used as a soil respiration chamber during the measurements.

2.2.2 Lab experiment design and soil CO_2 exchange measurements

Three successive laboratory experiments using different treatments (SWC and N fertilization) in the presence and absence of plant were carried out in following order; the same protocol was used for the three experiments. For the 1st one we used bare and planted soils with wheat plants, Two levels of SWC was applied (20% and 25%) and different levels of N treatments (N0, N50, N75, N100, and N150) was used. For the 2nd experiment we increased the frequency of the measurements and SWC values were binned into two different categories during the data analysis: below 30% (15%, 20% and 25%) and above 30% (35% and 40%). Different levels of ammonium nitrate (N0, N75 and N150) were applied on the surface of the soil. And for the last experiment; beside the effect of these factors, we aimed to study the effect of glucose addition on soil CO_2 efflux. D (+) glucose monohydrate ($C_6H_{12}O_6$ .H_2O), was added to our bare and planted soil with the application of two levels of SWC (20 and 40%). These measurements were conducted in a controlled environment under 12/12 h day/night periods, 20 °C of air temperature and the soil water content of each pot was controlled and was adjusted one day before gas efflux measurement.

Closed chamber technique was used for measuring the emission of carbon dioxide and its isotopic composition by a Picarro G1101-i gas analyser. Each sample was measured for 20 minutes, CO_2 efflux was calculated using the slope of the concentration change during this period.

CO_2 efflux was calculated by the following equation:

$$F = \frac{n*\Delta C}{A}$$ Eq. 1

where *n* represents the number of mols (µmol) in the volume of the closed system, ΔC is the concentration change of the carbon dioxide (µmol mol^{-1} s^{-1}) and *A* is the area of soil in the PVC tube used in the lab experiment (m^2).

Keeling plots were constructed by plotting the $\Delta^{13}C$ of CO_2 in any given gas sample obtained at each collar with the inverse of the CO_2 concentration of each gas sample. To ensure linearity, only those y-intercepts from linear regressions with $r^2 > 0.20$ were used to calculate isotopic composition of the emitted CO_2

2.2.3 Cumulative gas effluxes

The cumulative emissions were calculated based on the measurements of lab experiment 2 using the following formula:

$$T = \sum_{i=1}^{n} \left[\frac{(X_i + X_{i+1})}{2} \times (t_{i+1} - t_i) \times 24 \times \frac{3600}{1000000} \times 44 \right]$$ Eq. 2

Where, *T* (g CO_2 m^{-2}) is the cumulative CO_2 flux, *X* (µmol CO_2 m^{-2} s^{-1}) is the average daily CO_2 flux rate, *i* is the i^{th} measurement, and $(t_{i+1} - t_i)$ is the number of days between two adjacent measurements.

2.3. Data Processing and Modeling

Data processing and statistical analysis were done in R (R Core team, 2018). Gaussian error propagation was used to calculate propagated uncertainties of the cumulative sums and for the averages and model parameters.

Three different soil respiration models were used during the data processing to describe the response of the different CO_2 fluxes to the main biotic and abiotic drivers.

In the Lloyd and Taylor model (model 1) soil temperature is the only driving variable:

$$F = a \times e^{\left(b \times \left(\frac{1}{56.02} - \frac{1}{(Ts - 227.13)}\right)\right)}$$ Eq.3

Where, *F* is the soil CO_2 efflux (µmol CO_2 m^{-2} s^{-1}), T_s is the soil temperature at 5 cm in Kelvin, *a* and *b* are the model parameters.

Model 2 additionally includes SWC (Balogh *et al.*, 2011):

$$F = a \times e^{\left(b \times \left(\frac{1}{56.02} - \frac{1}{(Ts-227.13)}\right)\right) + \left(-0.5 \times \left[\log\left(\frac{SWC}{c}\right)\right]^2\right)} \quad \text{Eq.4}$$

where, *SWC* is the volumetric soil water content (%) and *c* is a model parameter.

Model 3 is extended model 2 by adding VIgreen as a driving variable:

$$F = a \times e^{\left((d \times VIGreen) + b \times \left(\frac{1}{56.02} - \frac{1}{(Ts-227.13)}\right)\right) + \left(-0.5 \times \left[\log\left(\frac{SWC}{c}\right)\right]^2\right)} \quad \text{Eq.5}$$

where, *VIgreen* is the vegetation index and *d* is a model parameter.

3. RESULTS AND DISCUSSION

3.1. Field experiment

3.1.1 Seasonal variation of soil respiration

Figure 1 shows that the highest emissions of 7.04±0.44 µmol CO_2 m^{-2} s^{-1} were detected immediately after soil loosening in the fallow period in 27th of August 2018 at an intermediate soil water content of 26% and soil temperature of 23 °C. Soil respiration is typically related to air or soil temperature, soil water content and in more recent cases to substrate supply. During the tillering stage the air and soil temperature gradually increases, plants grow quickly, soil microbial activities are enhanced and root exudate production increases, providing suitable conditions for soil respiration (Tang *et al.*, 2018).

The second-highest emission of 5.72±3.72 µmol CO_2 m^{-2} s^{-1} was observed in 26th of June 2019 a few weeks after sorghum sowing and N fertilizer application, accompanied by higher soil water contents (42%) due to a heavy rainfall before the day of the measurement. The ample water availability in the soil, plant activity (VIgreen, 0.3) and high soil temperature (29 °C) all resulted in a peak in soil CO_2 emission rate (Figure 1).

According to previous studies the impacts of N addition on CO_2 efflux varied widely with the level of N addition resulting in contradictory viewpoints concerning whether N applied to soils (regardless of its forms) increases soil CO_2 production or not (Ramirez, Craine and Fierer, 2010). In addition, a previous study suggested that increased N supply significantly stimulated CO_2 emission and these conditions generally promoted autotrophic plant respiration of above- and belowground parts.

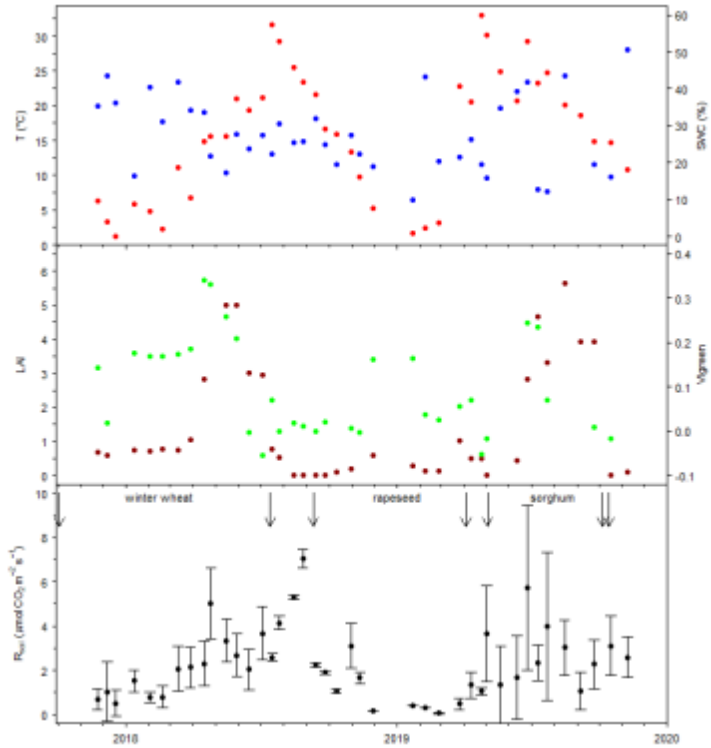

Figure 1. Top panel: Seasonal variations of soil moisture (SWC, %, blue dots) in the 0–7.5 cm soil layer, 5 cm depth soil temperature (T_s, °C, red dots). Middle panel: seasonal variations of leaf area index (LAI, $m^2\ m^{-2}$, brown dots) and VIgreen index (VIgreen, green dots). Lower panel: crop rotation of the measured field (Winter wheat, Rapeseed, Sorghum), and soil respiration (Rs, whiskers showing standard deviation) during the two-year-long study period. Downward-facing arrows indicate the timing of sowing and harvesting in the site.

Soil respiration decreased to 0.17±0.006 µmol CO_2 m^{-2} µmol CO_2 m^{-2} s^{-1} s^{-1} on 30th of November 2018. Accompanied by 19% of SWC and 5 °C of soil temperature, this lower efflux was due to the lack of vegetation in the field because the sowing of rapeseed at the beginning of autumn in 2018 wasn't successful. Kuzyakov (2006) mentioned that the vegetation may contribute strongly to the total CO_2 efflux by root and rhizo-microbial respiration. Another possible reason was the low temperature (Smith *et al.*, 2018), low temperatures slow down soil respiration by lowering rates of C cycling via autotrophic and heterotrophic respiration (Melillo *et al.*, 2011).

While it decreased substantially in the winter to 0.06±0.007 µmol CO_2 m^{-2} s^{-1} in 26th of February 2019 with 20% of SWC and at a temperature of 3 °C (Figure

1). This lower efflux was due to the low temperature and to the fact that the autotrophic respiration was generally very low or zero because there was no vegetation growing in the study site. However, the heterotrophic could maintain both catabolic (CO_2 production) and anabolic processes (biomass synthesis) under frozen conditions (Drotz *et al.*, 2010).

Soil respiration showed a positive correlation with soil temperature R=0.57, but no other investigated variable showed a significant correlation with soil respiration (Figure 2). Soil temperature was found to be the principal factor influencing soil respiration on both diurnal and longer time scales (Balogh *et al.*, 2019), it is used in the majority of Rs models (Lloyd and Taylor, 1994; Daly *et al.*, 2008; Zhang, Lei and Yang, 2013) due to its general effect on soil microclimate conditions and the biological activity of below-ground organisms (Yuste *et al.*, 2003; Dhital *et al.*, 2019).

The eventual influence on soil respiration by the variation of soil temperature as observed in the present study was similar to previous studies (Shen, Li and Fu, 2015; Bao *et al.*, 2016; Feng *et al.*, 2017).

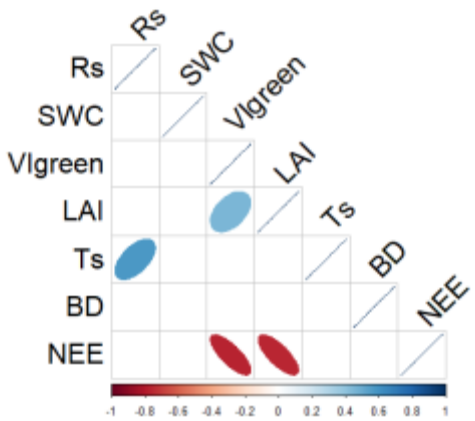

Figure 2. Correlation plot between soil respiration and SWC (soil water content), VIgreen (VIgreen index), LAI (leaf area index), Ts (soil temperature), BD (bulk density of the soil) and NEE (net ecosystem exchange of CO_2). Only statistically significant ($p < 0.05$) correlations are presented.

Using an exponential model (Model 1, Lloyd and Taylor (1994), Eq. 3) between CO_2 efflux and soil temperature, the goodness-of-fit was r^2=0.4 (Table 2). Using Lloyd and Taylor soil respiration model extended by a log-normal function of soil water content (Eq. 4) including SWC in the modelling, the goodness-of-fit

value had slightly improved (r^2=0.45). Furthermore, using soil respiration model extended by a log-normal function of soil water content and by an exponential function of VIgreen (Eq.5) was apt to represent better the response of soil respiration to these factors at our site with r^2= 0.54 (Table 2).

Table 2. r^2 values for soil respiration and the three drivers (Ts, SWC and VIgreen), and model coefficients for model 1, 2 and 3. Statistical significance levels of the coefficients and model fitting were p-value <0.0001 in all cases.

	r^2	a	b	C	d
Model 1	0.40	0.85	237.33	—	—
Model 2	0.45	1.54	242.31	71.66	—
Model 3	0.54	1.27	247.45	66.07	0.11

Fitted parameters of the three soil respiration models (model 1, 2 and 3, Table 2) show that Model (3) was the best fit because the r squared value improved with the increasing number of variables. The log-normal shape of soil moisture-respiration response was proposed before (Balogh *et al.*, 2011). The reflected green and red lights of the surface obtained by commercial digital camera (Canin Eos 350D) were used to calculate VIgreen, which changed with the different phenological stages of the vegetation during the seasons (Nagai *et al.*, 2014).

Relationship between soil respiration and ecosystem respiration

We also aimed to quantify the share of soil respiration in total ecosystem respiration (R_{eco}). Beside soil respiration R_{eco} has another major part, which is the respiration of the above-ground autotrophic (plant shoots) and heterotrophic (animals) components. While soil respiration is the largest component within R_{eco} (Claire L. Phillips *et al.*, 2017), the respiration of the above-ground parts of the plants can be significant as well. During the field work we measured soil respiration in different phenological stages, even when no plants were present (fallow periods). Therefore, we had a wide range of plant activity and aboveground respiration component. R_{eco} was calculated by partitioning NEE (Nagy *et al.*, 2011) , therefore it was measured continuously during the study. For the analysis, we selected the R_{eco} values measured in the same time (half-hour frequency) when manual soil respiration measurements were conducted.

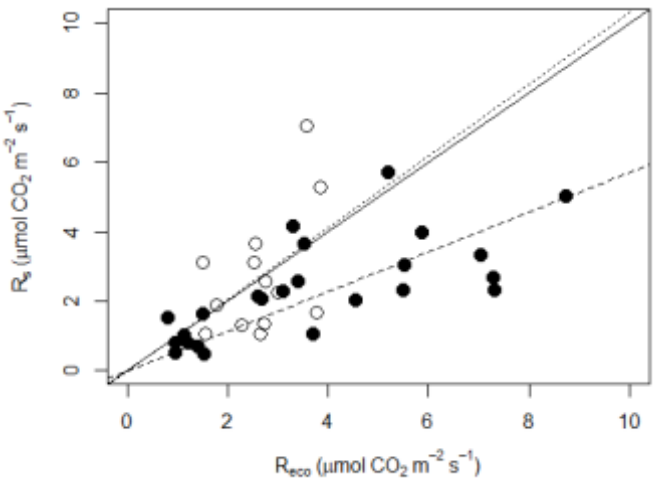

Figure 3. Average soil respiration as a function of ecosystem respiration during the study period, November 2017-November 2019, Kartal. Full circles represent measurements when leaf area index was greater, than 0.5 (LAI>0.5), while open circles represent measuring occasions when no, or small amount of plant biomass were present in the field (LAI<0.5). Solid line is 1:1 line, while dotted line is the linear regression between the variables when LAI<0.5, and dashed line represent the regression when LAI>0.5.

Figure 3 shows the regressions between Rs and R_{eco}. The slope of the linear regression for the whole dataset was 0.65 ($p<0.001$, not presented in Figure 3), but we split our dataset into two parts: when LAI was significant (LAI>0.5, full circles) and when there was no or very small plant biomass (LAI<0.5, open circles). Both regressions are significant ($p<0.001$), but the slope of the regressions is different: the slope was 0.57 in the first case and 1.03 in the latter. Therefore, the share of soil respiration in total ecosystem respiration was 57% on average when crops were present in the field, while ecosystem respiration originated from soil respiration, when crops were not present in the field. These results are similar to the findings of of Zhang *et al.* (2015), Myklebust, Hipps and Ryel (2008) and Claire L Phillips *et al.* (2017)

Microbial diversity

According to our microbiological results, the microbial counts showed that the highest total bacteria number, the highest number of fungi and the highest number of denitrifying bacteria in our soil was found during the summer of 2018.

For the average well color development measurement, the AWCDa of all soil samples displayed an apparent lag phase in the first 24 h. Then the average absorbance started to increase significantly, showing that the five soil microbial communities were able to metabolize organic substrates in BIOLOG EcoPlates, and concerning the six compound groups (carboxylic acids, carbohydrates, amino acids, polymers, miscellaneous, and amines/amides - plus a blank well as a control) exist in the BIOLOG EcoPlate, the current study revealed that carbohydrate was characterized by the highest metabolic activity, while the lowest activity was determined for amines/amides.

3.2. Laboratory experiments

3.2.1 First experiment

This experiment contained two different series with different levels of SWC and different amount of treatments, CO_2 efflux of the samples was measured weekly.

20% of soil moisture and three levels of NH_4NO_3 (N0, N50 and N100 – 0, 50 and 100 kg N ha^{-1}, respectively) was applied in the first series. Figure 4 shows that the CO_2 efflux in the first week was almost the same in all treatments in bare soil, while in planted soil the efflux in N100 was higher than N0 and N50. The second week of gas measurement , the CO_2 efflux in bare soil was around two times higher than planted soil, and there was no effect of N treatments. In the third week of gas measurement, the same phenomenon was observed as the first and the second week. In the fourth week of gas measurement, the efflux decreased at lower soil moisture in both bare and planted soils.

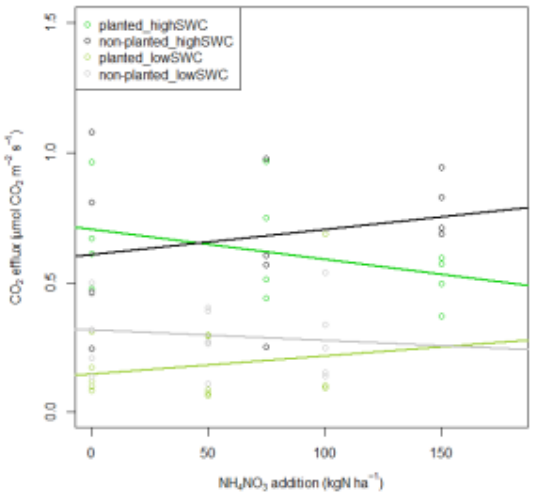

Figure 4. CO_2 efflux of different treatments (N0, N50, N75, N100 and N150) under two levels of soil moisture (20 and 25%) in planted and non-planted (bare soil) during four-five weeks long laboratory study period.

We repeated the experiement with slight changes in the treatments. 25% of soil moisture and three levels of NH_4NO_3 (N0, N75 and N150 – 0, 75 and 150 kg N ha^{-1}, respectively) was applied in the second series. In the first week and at higher soil moisture (25%) the CO_2 efflux was significantly higher, the efflux in bare soil was higher than planted soil in all treatments (N0, N75, N150), and there was no obvious effect of NH_4NO_3 in both planted and bare soil. In the second week of measurement the efflux was higher in planted soil than in bare soil in (N0 and N75). But it was lower in N100 in plant soil and high in bare soil. In the third week, the efflux increased significantly in bare and planted soil. In the fourth week the efflux increased in both soils (bare and planted). At the end of this experiment (fifth week) no significant changes were observed, the CO_2 efflux higher in bare soils than planted soils in both series and no effect of N treatments was found.

From the results, we can conclude that there was no effect of plant presence on the CO_2 efflux during four and five weeks of measurement. Although it was surprising, we must note that the plants were small during the first weeks having small root respiration. Also, soil CO_2 efflux was highest in the zero N application treatment so there was no effect of different treatments in this experiment which was the same with some of studies Liu *et al.* (2017) and He *et al.* (2018) who indicated that N addition alone exerts no obvious effect on Rs. Meanwhile, the soil CO_2 efflux was significantly higher at the higher soil moisture level (25%), moisture in soils is essential for both plant growth (Huxman *et al.*, 2004) and soil microbial activity, thus affecting carbon inputs as well as the decomposition of litter and soil organic matter, and hence heterotrophic respiration and carbon outputs (Moyano, Manzoni and Chenu, 2013).

3.2.2 Second experiment

3.2.2.1 Cumulative CO_2 efflux course with different levels of N treatment in the presence/absence of plants

Lab measurements were aimed at quantifying the effect of the presence/absence of plants, the effect of soil moisture and the effect of different N addition (0, 75, and 150 kg N ha^{-1}) on the cumulative CO_2 efflux. We found that more than three weeks after N fertilization (on day 21st after fertilization), the cumulative CO_2

efflux in bare soil at N75, N150 were 0.63±0.01 g CO_2 $m^{-2}$and 0.90±0.02 g CO_2 m^{-2}, respectively (Figure 5, left panel), which is higher than that in the N0 treatment (0.60±0.02 g CO_2 m^{-2}). However, much higher cumulative CO_2 effluxes were observed in planted soil samples in all treatments. These results suggest that N addition had a slight positive effect on soil respiration: cumulative efflux was 1.3 times higher in N75 and N150 than in N0 in planted samples, while CO_2 efflux of N150 treatment was 1.5 times higher than N0 in bare soil samples.

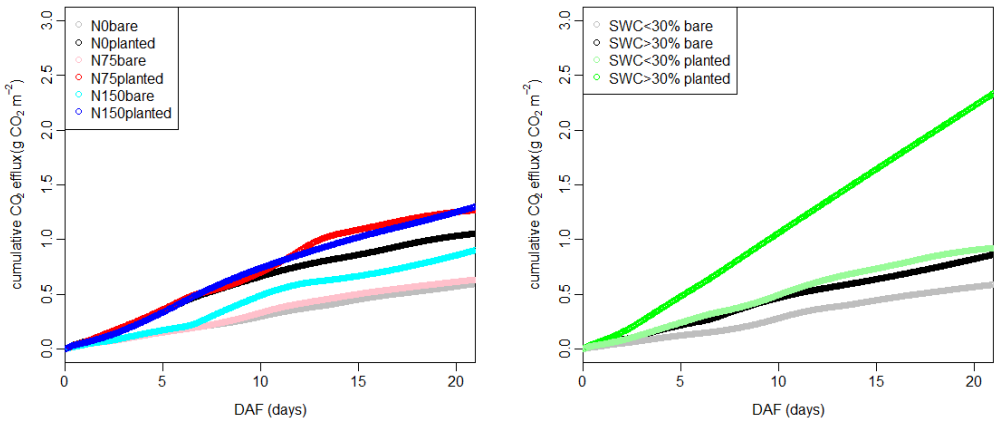

Figure 5. Cumulative CO_2 efflux (g CO_2 m^{-2}) courses across the 3 weeks long laboratory study period. CO_2 effluxes are separated by N treatments (left panel, 0, 75 and 150 kg N ha^{-1}) and by soil water content (right panel, >30% and <30%) in bare and planted soil.

The difference between planted and bare soil in our study was due to the activity of plants resulting in root respiration and the priming effects of root exudates on soil microbes (Kuzyakov and Larionova, 2005), which, in turn, improved soil nutrient content, (Savage, Davidson and Tang, 2013), and accelerated the decomposition of soil organic matter (Nakano, Nemoto and Shinoda, 2008). The CO_2 emission in our present study was found to have a positive correlation with the stand age of the plant and with N fertilizer rates: as the plants grew and more N was added more CO_2 was emitted. A previous study suggested that N addition stimulated CO_2 emission by promoting autotrophic plant respiration (above and below ground parts, Chen, Hooper and Lin, 2011) as well as heterotrophic respiration by microbes due to the accelerated decomposition of SOM which was discussed above.

3.2.2.2 Relationship between the cumulative CO_2 efflux and soil water content

Pearson's correlation of CO_2 efflux and soil moisture indicated that soil moisture was well correlated with CO_2 emission (R= 0.43). Figure 5, right panel shows that the cumulative CO_2 efflux increased with increasing SWC, the efflux was significantly (almost three times) higher in planted soils at higher soil moisture levels (>30% and after three weeks of N fertilization) than at the lower soil moisture levels and, similarly, three times higher than in bare soil at higher SWC. Therefore, the effects of plant presence and soil moisture on soil respiration had similar magnitude. In bare soil the cumulative CO_2 efflux was also significantly lower at the lower soil moisture level (<30%) than at higher soil moisture level (>30%).

Generally, soil CO_2 efflux increases as soil moisture increases but soil moisture content can significantly reduce soil CO_2 efflux at its highest (wet soil) by blocking CO_2 transport because of low soil effective porosity (Balogh *et al.*, 2019), and at its lowest (dry soil) (Wang *et al.*, 2016), by limiting respiration substrate availability and thereby it reduces soil respiration (Dhadli, Brar and Black, 2015). In our study the higher soil moisture levels (35% and 40%) could enhance respiration rates and no negative effect of high soil moisture was observed.

3.2.3 Third experiment

Carbon dioxide efflux dynamics with different levels of N fertilization and glucose addition in the presence/absence of plant.

This experiment was aimed to quantify the effect of glucose addition (carbon source) on the CO_2 efflux together with the effect of the biotic and abiotic drivers which were mentioned before. Figure 6, left panel shows that the CO_2 efflux before fertilization (–200, –100 and 0 h) was almost three times higher in planted soil with maize than in bare soil. After 12 hours of fertilization, the efflux increased, and it was higher in N75 in both bare and planted soil compared with N0 and N150. It was three times higher in planted soil in all treatment (N0, N75 and N150) than it was in bare soil.

The CO_2 efflux was almost stable after the period of 12 h from fertilization until 157 h. Then it started to increase again, it was six times higher in planted soil in all treatments (N0, N75 and N150) as compared to bare soil (Figure 6) but there were no significant differences between the fertilizer treatments.

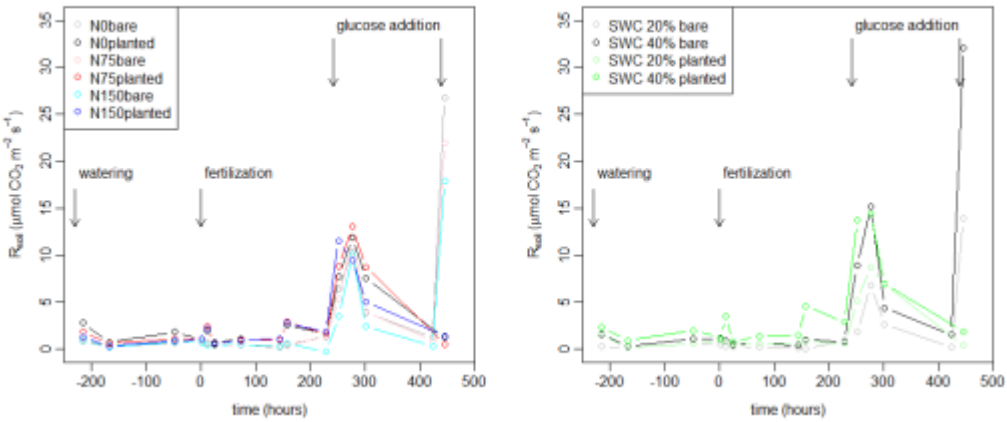

Figure 6. The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) dynamic during 4 weeks long laboratory study period. CO_2 effluxes are separated by N fertilization (left panel, 0, 75 and 150 kg N ha^{-1}) and by soil water content (right panel, 20% and 40%), addition of glucose in the25 the251 h in bare and planted soil and at 445 h in bare soil.

At 251 h the first portion of glucose was added for both bare and planted soil, the CO_2 efflux increased significantly and reached higher values, it was higher in planted soil than in bare soil in all treatments, there was a fertilizer effect in planted soil especially with N150 but there was no effect in bare soil. The effluxes continued to increase and reached higher values, than start to decrease after 276 h in both bare and planted soil. At 445 h the second portion of glucose was added just to bare soil samples. In the graph (Figure 6, left panel) we can see that the CO_2 efflux in bare soil increased significantly and reached higher values in the all three treatments (N0, N75 and N150).

We found that there was a positive effect of vegetation on CO_2 efflux with their activity resulting in root respiration and the priming effects of root exudates on soil microbes (Malek *et al.*, 2021), which is the same case in the previous experiment. According to our results, there was no clear effect of N applied to both bare and planted soil samples similarly to some studies indicating that N addition alone exerts no obvious effect on Rs (He *et al.*, 2018). However, in some of the samples we found an effect of N treatment which stimulated CO_2 efflux by promoting autotrophic plant respiration (Chen, Hooper and Lin, 2011) as well as heterotrophic respiration. But, glucose addition had a much larger positive effect.

Carbon dioxide efflux dynamics with two levels of SWC (20% and 40%) and glucose addition in the presence/absence of plant

Figure 6, right panel shows that the CO_2 efflux increased with increasing SWC and the CO_2 efflux before fertilization (–200, –100 and 0 h) was significantly higher in planted and bare soils at higher soil moisture levels (40%) than it was at lower soil moisture levels (20%). The CO_2 effluxes increased rapidly and reached a higher value when the first portion of glucose was added (251 h). Rs was higher in planted soil than in bare soil both at lower and higher SWC. After peaking at 276 h the CO_2 efflux started to decrease both in bare and planted soil at higher and lower soil moisture levels.

The CO_2 efflux in bare soil increased rapidly after the adding of the second portion of glucose (445 h) and reached even higher values than after the first addition. It was two and half times higher at higher SWC than at lower SWC with 32.11±6.56 and 13.89±6.43 μmol CO_2 m^{-2} s^{-1}Rs rates, respectively.

According to our results, we can conclude that there was a positive relationship between CO_2 efflux and soil moisture as it was discussed earlier. Therefore, the effects of plant presence and soil moisture on soil respiration had a similar magnitude. In this experiment, the higher soil moisture levels (40%) could enhance respiration rates and no negative effect of high soil moisture was observed. There was a positive effect of glucose addition in both bare and planted soils (Figure 6, right panel) at the two levels of SWC.

The glucose additions enhanced soil respiration rates at higher soil moisture in both bare and planted soils which is similar to the findings of Sánchez-Martín *et al.* (2008). Glucose often produces a rapid response in microbial activity (Bernal *et al.*, 2016) and leads to rapid metabolic changes in a wide variety of fast-growing bacteria that utilize it as a substrate (Hungate *et al.*, 2015). Studies showed that glucose was readily used by microbes as an energy source to produce enzymes that assist in the decomposition of organic molecules that are resistant to microbial degradation (Bernal *et al.*, 2016).

The CO_2 efflux (μmol CO_2 $m^{-2}s^{-1}$) and its isotopic signal difference between planted soil with maize and bare soils.

In Figure 7 we can see that before fertilization the soil CO_2 efflux was slightly higher in planted soil with maize than in bare soil. Also, before fertilization, the natural isotopic abundance of Rs ($\delta^{13}CO_2$) was higher in planted soil than in bare soil. 12 h after N fertilization the efflux increased in planted soil from

1.04±0.25 µmol CO_2 m^{-2} $s^{-1}$to 2.16±1.73 µmol CO_2 m^{-2} $s^{-1}$and there was no increment in bare soil, while the $\delta^{13}CO_2$‰ of planted and bare soil increased from –20.84±3.06‰ to –19.08±2.73‰ and –24.10±5.00‰ to –18.62±4.62‰.

CO_2 efflux was almost stable after the period of 12 h from fertilization in both soils until 157 h, then the efflux started to increase again, it was two times higher in planted soil compared with bare soil at 228 h (Figure 7). $\delta^{13}CO_2$ of planted soil and bare soil Rs increased and reached higher values: from 24 h to 228 h after fertilization it increased from –27.60±5.01‰ to –20.20±7.81‰ in bare soil and it was higher in Rs of planted soil increasing from –22.23±3.30‰ to –16.75±7.09‰.

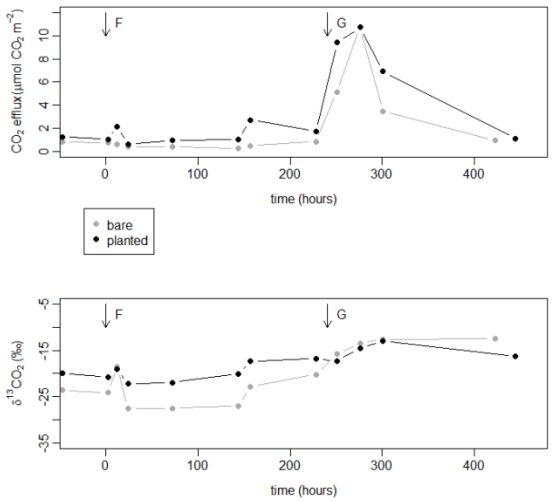

Figure 7. The CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) and its $\delta^{13}CO_2$ ‰ difference between planted soil with maize and bare soils in time with N fertilization (F) and glucose additionin the 251 h (G) during 4 weeks long laboratory study period.

251 h after fertilization the glucose was added for both bare and planted soil. Soil CO_2 effluxes increased significantly and reached higher values, it was higher two times in planted soil than in bare soil, than it decreased significantly to reach 1.11±0.91 µmol CO_2 m^{-2} $s^{-1}$for planted soil and 0.94±0.70 µmol CO_2 m^{-2} s^{-1} for planted soil. Meanwhile, the $\delta^{13}CO_2$ increased in bare soil when the glucose was added from –20.20±7.81‰ to –15.84±2.76‰, but it decreased in planted soil from –16.75±7.09‰ to –17.34±2.52‰. After that, $\delta^{13}CO_2$ increased

again in both bare and planted samples and reached similar values –2.44±1.00‰ and –13.00 ±1.16‰) in bare soil and in planted soil, respectively.

According to these results, we found a positive effect of plant presence, glucose addition and N fertilization on CO_2 efflux and on its stable isotope signal ($\delta^{13}CO_2$‰). The $\delta^{13}C$ of CO_2 respired by roots and other rhizosphere components may also be affected by utilization of fast or slow turnover carbon pools (Schnyder and Lattanzi, 2005) or allocation between growth vs. maintenance. The $\delta^{13}C$ of CO_2 respired by heterotrophic soil microorganisms depends on the substrates within soil organic matter utilized for decomposition. The artificial addition of glucose has changed the isotopic signal in our experiment; it increased in both planted and bare soils.

We separated the isotopic signals of Rs by the different treatments (Figures. 8-10). Isotopic signatures of soil respiration are a useful tool for estimating the contribution of its main components (Hopkins *et al.*, 2013) and for tracing the transfer of C in ecosystems (Högberg *et al.*, 2008) and thus have the potential to provide insights into the coupling of photosynthetic assimilation and soil respiratory fluxes. Plants contain less ^{13}C than the atmosphere due to processes discriminating against ^{13}C during CO_2 fixation. Maize is a C4 plant so it has a slightly higher delta value than soil and C3 plants (–18- –14‰). We used a C4 species planted in a soil with mixed origin (both C3 and C4 crops were grown in the last years). Figure 8 shows that the medians $\delta^{13}CO_2$ of planted soil with maize higher than in bare soil by –19.79 and –23.77, respectively and there was a significant difference between them. Maize is C4 plant and it has a slightly higher delta value than soil and C3 plants.

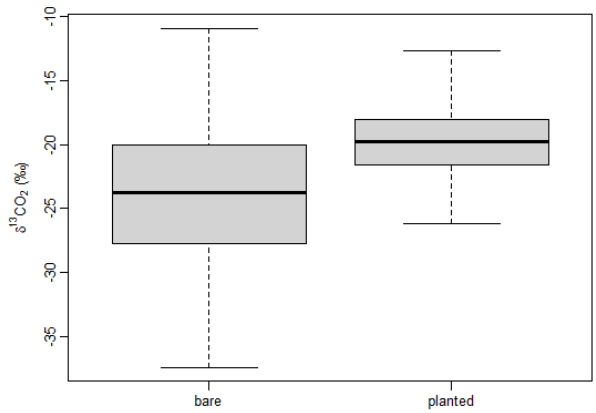

Figure 8. Isotopic signals of soil samples distinguished by the presence of maize plants (planted) and the absence of maize plants (bare).

Figure 9 shows that soil water content (two levels: 20% and 40%) also affected the isotopic signal of soil respiration, $\delta^{13}CO_2$ increased with increasing SWC, the $\delta^{13}CO_2$ was significantly higher at higher soil moisture levels (40%) with median of –20.2‰, than in lower soil moisture level (20%) with median of -22.65‰.

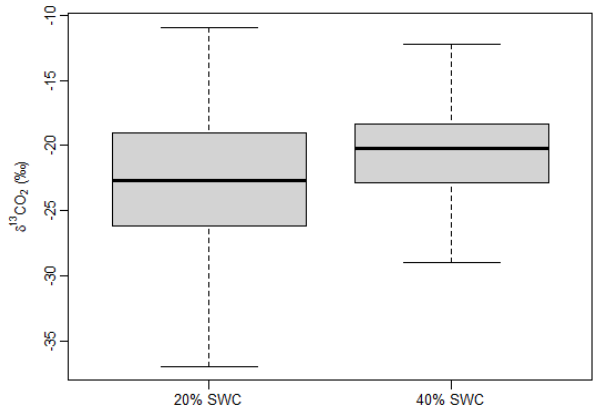

Figure 9. Isotopic signals of soil samples distinguished by SWC (20% and 40%)

According to the results, there was a significant difference in isotopic signals between the two levels of soil moisture. Water shortage can change the isotopic signal of soil respiration. In our study, the isotopic signal increased with increasing SWC suggesting that the C4 plant (maize) was more active at higher soil moisture level. Therefore, these results are similar to those measured in the grassland also supporting the hypothesis that heterotrophic respiration component is the less sensitive part of soil respiration during drought (Balogh *et al.*, 2016).

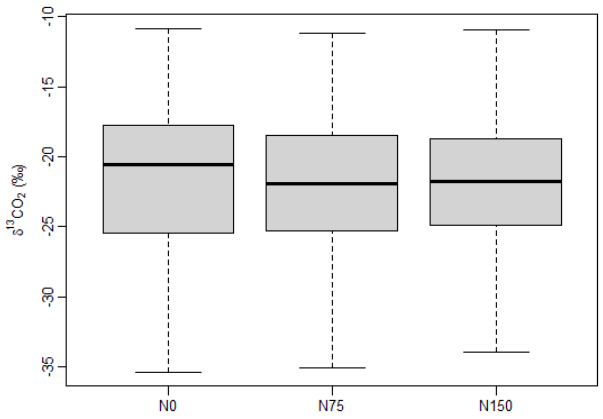

Figure **10**. Isotopic signals of soil samples of the three levels of NH_4NO_3 addition (N0, N75, and N150)

We found no effect of fertilization on $\delta^{13}CO_2$ (Figure 10) as there were no significant differences between the treatments, medians: –20.55‰ (N0), –21.89‰ (N75), –21.75 ‰ (N150).

4. CONCLUSION

Field and laboratory experiments were performed during a two-year-long study period (From November 2017 to November 2019) to quantify the different effects of principal biotic and abiotic drivers on soil CO_2 efflux and to investigate the temporal dynamics of CO_2 efflux from the soil surface. We found that the highest CO_2 emission rates occurred during summer and the lowest rates during the snow-covered winter period, and that soil temperature, soil water content, agricultural management practices and plant growth were the principal drivers playing a major role in the carbon cycle at this temperate cropland site.

We aimed to separate the effect of these drivers on CO_2 efflux in our laboratory study and we found that the CO_2 efflux in the N application was higher than that it was in zero-N treatment in both planted and bare soil in in most cases but sometimes there was no obvious effect of N treatment, therefore the presence of plants and their growth could explain the temporal variations in CO_2 efflux due to root biomass. On the other hand, significant positive correlations between CO_2 efflux and soil moisture were found, as soil moisture increases soil CO_2 efflux increases indicating that soil water content was the main factor limiting the rate of CO_2 emission from the soil. We found in one of our experiments that

the higher soil moisture levels (40 %) could enhance respiration rates and no negative effect of high soil moisture was observed. There was a positive effect of glucose addition also on CO_2 efflux in both bare and planted soils under different levels of SWC. At 40 % of SWC; glucose additions with low and high rates of N fertilization (N0, N75, and N150) significantly increased CO_2 emission, rather than reducing it. According to our microbiological results, the microbial counts showed that the highest bacteria population and the highest number of fungi were found during the summer of 2018.

For the average well color development measurement, the AWCDa of all soil samples displayed an apparent lag phase in the first 24 h. Then the average absorbance started to increase significantly, showing that the five soil microbial communities were able to metabolize organic substrates in BIOLOG EcoPlates, also the current study revealed that carbohydrate was characterized by the highest metabolic activity, while the lowest activity was determined for amines/amides.

We also studied the isotopic signal of the respired CO_2 and the effect of the major factors on that. We found that soil moisture and plant presence had a significant positive effect on $\delta^{13}C$, while N addition had no effect on that.

5. NOVEL SCIENTIFIC RESULTS

Field data of CO_2 emission from a temperate cropland soil located in the middle part of Hungary (Kartal) under conventional management (tillage, sowing, harvesting, loosening, fertilization) during different crops (winter wheat, sorghum, rapeseed, winter wheat) with parallel laboratory experiments on the same soil were performed during a two-years-long study period.

We found the following results:

1. We described the temporal variation of soil CO_2 efflux in a conventionally managed agricultural soil in Hungary. We found that soil respiration had 57% share on average in total ecosystem respiration during crop periods, while it had 100% share on average during fallow periods.
2. We described the response of soil respiration to temperature, soil moisture and plant activity by using three different soil respiration models. According to our results; Model (3) (with soil temperature, soil

moisture and VIgreen) was the best fit because the r squared value (from 0.40 to 0.54) improved with the increasing number of variables

3. We found that the impacts of N addition on CO_2 efflux varied with the level of N addition. Based on the field and lab data, we found a positive effect of fertilization on the CO_2 efflux of the soil, NH_4NO_3 stimulated CO_2 efflux by promoting autotrophic plant respiration as well as heterotrophic respiration.

4. We found that CO_2 efflux increased with increasing SWC. The efflux was significantly higher in both planted soils and in bare soils at higher soil moisture levels than at the lower soil moisture levels in all experiments and in the field as well. According to our results, higher soil moisture levels (35% and 40%) could enhance respiration rates and no negative effect of high soil moisture was observed in this soil

5. We found that the vegetation could contribute strongly to the total CO_2 efflux by root and rhizo-microbial respiration, therefore the presence of plants and their growth can explain the temporal variations in CO_2 efflux due to root biomass and its activity in croplands. According to our result a positive correlation with the stand age of the plant was found: as the plants grew more CO_2 was emitted.
6. Glucose additions, as easily decomposable carbon source enhanced soil respiration rates independently on soil moisture and plant presence. It was two times higher in planted soil comparing with bare soil. Glucose additions had stronger effect on soil CO_2 efflux than N fertilization by producing a rapid response in microbial activity and leads to rapid metabolic changes in a wide variety of fast-growing bacteria that utilize it as a substrate

7. Indices like AWCD, H´, E and D was very useful to describe the activity and diversity of microorganism population. The BIOLOG EcoPlate has been found to be a good indicator of reflecting changes of metabolic activity and/or potential functional versatility of microbial communities exposed to abiotic conditions. The AWCD reflects the oxidative ability of the microorganisms developed in Biolog, and it may be used as an indicator of microbial activity.

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LIST OF PUBLICATIONS

ACCEPTED PAPERS WITH IMPACT FACTOR:

1- Responses of soil respiration to different biotic and abiotic drivers in a temperate cropland.
Insaf Malek, Meryem Bouteldja, Katalin Posta, Szilvia Fóti, Krisztina Pintér, Zoltán Nagy, János Balogh. Eurasian Soil Science, accepted paper. Eurasian Soil Science, Eurasian soil science. https://doi.org/10.1134/S1064229321070097.

2- Temporal variability and drivers of nitrous oxide emissions from Central Hungarian croplands: field and pot experiments.
Meryem Bouteldja, **Insaf Malek**, Katalin Posta, Györgyi Kampfl, Szilvia Fóti, Krisztina Pintér, Zoltán Nagy, János Balogh Eurasian Soil Science, accepted paper.

PUBLISHED CONFERENCE PROCEEDINGS:

1- CO_2 efflux from agricultural soils in hungary.
Insaf MALEK, Meryem BOUTELDJA, János BALOGH, Katalin POSTA.
(18th Alps-Adria Scientific Workshop). Doi: 10.34116/NTI.2019.AA.43. p. 104-105

2- The effect of biotic and abiotic drivers on soil respiration in Kartal site.
Insaf MALEK, Meryem BOUTELDJA, Katalin POSTA, János BALOGH.
(ALPS Abstract Book – 19th Alps Adria Scientific Workshop Wisła, Poland, 29.04 – 05.01.2020). p 64. DOI: 10.34116/NTI.2020.AA

3- N_2O flux from planted and not planted cropland soils.
Meryem BOUTELDJA, **Insaf MALEK**, Katalin POSTA, Györgyi KAMPFL, János BALOGH.18th Alps-Adria Scientific Workshop. Doi: 10.34116/NTI.2019.AA.7. p. 30,31

4- Temporal variability of N_2O emission in agricultural field.
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5- Soil carbon balance in Hungarian crop rotation systems.
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