INVESTIGATION OF LABORATORY METHODS OF SOIL RESPIRATION MEASUREMENTS

Eszter Tóth[1], Györgyi Gelybó[1], Csilla Farkas[1]

[1]Institute for Soil Science and Agricultural Chemistry of CAR HAS, H-1022 Budapest, Herman O. Street 15, Hungary; e-mail: toth.eszter@agrar.mta.hu

Abstract
Soil carbon dioxide emission and soil carbon stocks in general have recently gained much scientific interest due to their special relevance to climate change. Agricultural soils provide the opportunity to decrease soil carbon loss or increase carbon sequestration also improving soil quality by the application of appropriate management techniques. To support development of such techniques it is essential to understand soil respiration and its responses to biotic and abiotic drivers using state-of-the-art methods with sound scientific background. We propose here a new method to study soil respiration on the laboratory scale.
In contrast with the widely accepted use of disturbed soil samples in laboratory analysis, we propose that undisturbed soil cores provide more reliable soil respiration data. We prove our concept investigating moisture effects on soil respiration, in order to ensure comparability of results from different soil textures, soil water potential should be used instead of volumetric soil water content.

KEY WORDS: soil CO2 emission, undisturbed soil columns, soil water potental, soil water content

Introduction

The increasing atmospheric concentration of most greenhouse gases, as a result of anthropogenic emissions is a well known fact. Most recently, new peak atmospheric CO_2 concentration of 400 ppm was recorded at Mauna Loa observatory, the first continuous monitoring station for atmospheric CO_2 levels. According to some scenarios, joint concentration of greenhouse gases will reach the 560 ppm (CO_2 equivalent) by 2030, which is two times higher than before the industrial revolution (Foody et al., 1996). Atmospheric CO_2 concentration (the 'airborn fraction', IPCC (2007)) however increases with half of the rate of anthropogenic emissions. The so-called residual sink is the increased carbon absorbing capacity of terrestrial vegetation and oceans as a response to several factors of the changing environment, increasing CO_2 concentration of the atmosphere, etc. Hence, biogeochemical cycles in general and the vegetation-atmosphere carbon exchange in particular are of current high priority research interest for the understanding of global climate change.
Terrestrial vegetation is currently a substantial sink for atmospheric carbon dioxide (CO_2) (Schimel, 1995) offsetting anthropogenic emissions. Although terrestrial ecosystems exchange 15 times more CO_2 with the atmosphere than anthropogenic emissions do, the sink capacity and sustainability of terrestrial ecosystems to store atmospheric C is not clear (Gonzalez-Meler et al., 2004; Friedlingstein et al., 2006). Majority of the carbon stored by terrestrial ecosystems - about 2000 Gt C - is located in the upper 1 m of the soils, which is more than the amount of carbon stored in the atmosphere (about 760 Gt C) (Fontain, 2007). It is clear that any changes in the capacity of the soil carbon pool can significantly increase the airborn fraction. As ecosystems respond to changes in climate and weather conditions (Ciais et al., 2005, Reichstein et al., 2008), the stability of the terrestrial C pool may be affected.

The net amount of C that can be retained by an ecosystem (NEP) is a function of GPP (Gross Primary Production) minus total ecosystem respiration (Re) that consists of plant (autotrophic) respiration (Rp) and respiration from heterotrophs (Rh). Current reliable measurements of GPP at different scales contrast with the poorly understood and measured Rp and Rh parameters.
Modeling these processes at different spatial and temporal scales is a challenge as it requires knowledge from many disciplines to understand key processes that govern NEP, GPP and Re. Currently, an incomplete understanding of carbon allocation within an ecosystem limits the capacity of models to predict ecosystem metabolism and the effects of global change on carbon cycling (Friedlingstein et al., 2006; Litton et al., 2007). The least understood parts of carbon balance are Rp and Rh. Extensive measurements and measuring protocol is required to assess the respiration part of carbon balance especially regarding the partitioning of Re between aboveground (plant) respiration and soil respiration (where both autotrophic and heterotrophic respiration contributes to CO_2 fluxes).

Overview of soil respiration measurement techniques

Although the first soil CO_2 emission measurements were carried out more than 100 years ago there are no standard methods for soil respiration measurements till now (Anderson, 1982, Nakayama, 1990). Soil respiration is extremely heterogeneous in time and space, both vertically and horizontally as influenced by soil properties and many other highly variable factors. The determinants of CO2 emission are diverse, since soil CO_2 emission includes the respiration of roots and heterotrophic microorganisms with different driving factors. Uncertainties of the existing measurement methods is basically associated with this huge variability (Smith et al., 2008).
Due to the high spatial and temporal variability of soil respiration, many measurement methods have been developed aiming at the investigation of SR on different scales dominated by different processes. In situ field measurements are usually based on the use of soil respiration chambers (open or closed, static or dynamic). These measurements investigate soil respiration and its relations with biotic and abiotic driving forces on the days to months scale (Reichstein and Beer, 2008). under natural circumstances, Since soil water content and soil temperature are the two main factors affecting soil CO_2 emission (Reich & Schlesinger, 1992,Lloyd & Taylor, 1994, Reichstein et al., 2002, Simek et al, 2004, Szili-Kovács, 2004,) these data must be also measured beside field measurements to evaluate the emission values.
On the laboratory scale, under controlled circumstances, the effect of individual factors can be investigated, while others kept constant. In laboratory experiments, root respiration is usually eliminated from measurement. At this scale, the governing processes are respiration of roots, bacteria, and fungi; population dynamics of microbes (with a characteristic time scale of seconds to days Reichstein and Beer, 2008). However, root respiration cannot be examined appropriately in laboratory since roots do not have any supply for respiration from photosynthesis after removing the plants. In the laboratory, dependency of soil organic matter decomposition on e.g. temperature and moisture conditions can be studied. During the interpretation of the result, this should be kept in mind.
The ideal methodology to be used should be determined by the scientific question addressed. Soil respiration on different time and spatial scales is driven by different processes that spans domains from microbiology to large scale weather and climate phenomena.
Soil CO_2 emission measurements in laboratory are usually carried out on disturbed soils without soil structure (Linn & Doran, 1984, Bowden et al., 1988, Cross & Grace, 2010, Bajgai et al., 2011, Serrano-Silva et al., 2011). However, parallel with destroying soil structure and breaking aggregates the effect of soil structure and pore size distribution on soil solid, fluid and aerial phases is eliminated so the effect of structure on soil biological properties and soil CO_2 emission cannot be prevailed. Not too many studies can be found in the scientific literature where soil CO_2 emission is measured from undisturbed soil columns (Priemé & Christensen, 2001, Obrist et al, 2010, Ruser et al, 2006, Szili-Kovács et al., 2009). However the importance of undisturbed soil structure was reported by many studies. Ruamps et al (2011) demonstrated with Phospholipid Fatty-Acid analyses that the structure of microbial community varies with pore size distribution and the microbial activity also significantly

changes simultaneously. Consequently with the use of soil samples with disturbed structure the information originated from the soil structural state got lost and the possibility of evaluation of measurement results in the function of soil hydrological properties is also decreased.
Our aim was to determine whether soil CO_2 emission measured from disturbed and undisturbed soil samples are significantly different. In order to prove that, two laboratory experiment was set up in the climatic room of Institute for Soil Science and Agricultural Chemistry.
Our other aim was to develop an experimental setup where soil CO_2 emission values can be evaluated in the function of soil water potential values. Soil CO_2 emission is usually studied as a function of soil temperature and soil water content. This way emission rate of different soil types cannot be compared because same water content values in soil with different texture and structure represent different soil water potential values. Nevertheless it also means that water is in different energetic state in the soil and circumstances for soil microbial community are also different.

Methods

Experimental site and treatments

Soil samples were collected from a peach orchard planted in 1992 on Raman brown forest soil formed on sandy loam (Mollic Cambisol, WRB 2006) near the city of Vác. Row spacing is 6 m, plant-to-plant distance is 4.5 m. There are two row types with different management in the orchard; every second row is disked (D, 12-15cm), the other rows are covered by grass (G). Disking and grass cutting are carried out in every third week. Annual total precipitation is 570 mm, 330 mm from that falls in the vegetation period the mean annul temperature is 10.8 ^{o}C. The ratio of the sand, loam and clay fraction in the upper 20 cm soil is 58 %, 23 % and 19 %, respectively. The main soil physical, chemical and biological properties are shown in Table 1.

Table 1. Main soil properties of the differently managed rows of the plantation

Soil properties	Disked row (D)		Grass-covered row (G)	
	0-5cm	5-10cm	0-5cm	5-10cm
pH (KCl)	7.3	7.3	7.8	8.0
pH (H_2O)	7.1	7.2	8.2	8.1
Total N [mg kg^{-1}]	1298		1805	
K_2O [mg kg^{-1}]	244		387	
P_2O_5 [mg kg^{-1}]	337		382	
WEOC [[µg C g^{-1} soil]	41.56	41.11	138.1	93.9
WEN[µg C g^{-1} soil]	1.48	3.16	10.58	7.01
Microbial biomass C[µg C g^{-1} soil]	52.0	32.9	234.5	87.0
Microbial biomass N[µg C g^{-1} soil]	9.1	8.8	50.0	17.0
Organic C [%]	0.98		1.32	
SIR [µg C g^{-1} soil h^{-1}]	4.64	3.76	21.78	6.38
Humus [%]	1.69		1.32	
Bulk density [g cm^{-3}]	1.35	1.47	1.18	1.43
Saturated soil water content [v%]	51.3	47.6	57.3	48.1
Field capacity [%]	31.2	32.5	38.1	33.2
Wilting point [%]	9.7	10.6	10.3	10.4

Soil sampling method and experimental set up

Since soil microbiological activity and soil respiration is the most intensive in the upper soil (Agbeko & Kita, 2007), for laboratory analyses soil samples from the upper 10 cm of soil with a volume of about 800cm^3 were used. PVC tubes (d = 10.5 cm, h = 20 cm) were carefully inserted into the soil into 10 cm depth then after digging around they were pulled out with the smallest possible disturbance of the soil inside. For the first experiment (*exp1*) soils from the grass-covered (G) rows of the experimental site were used. The reasoning was to exclude effects of disking (as a form of soil disturbance) that could potentially dampen the difference between disturbed and undisturbed columns. For *exp1* half of the columns were immediately closed from beneath than all the samples were transported into the lab. The soil was taken out from the other half of the tubes and from this soil disturbed soil samples with the same volume were created. In the first experiment 5 disturbed and undisturbed columns collected with the grass covered row were used during 5 measurement days. One 30-minute-long incubation was carried out in all measurement days. On the third day of the experiment water was added to each column to reach the initial water content. For *exp2* soil columns from the two row of different management (D and G) of the experimental site was used to study the behaviour of differently textured soils as well. All the PVC tubes were closed from beneath in the field than transported into the laboratory for further measurement. In this experiment 20-20 undisturbed soil columns were used from the treatments and the experiment lasted 13 days. On the ninth day of the experiment all columns were watered to the initial water content to evaluate the effect of soil moisture content on soil CO_2 emission.

CO_2 emission measurements

CO_2 emission measurements were carried out in climatic room under controlled circumstances (air temperature, 20 ^oC and humidity, 35 %). Soil columns were airtight closed also from above in the laboratory. Air samples were taken from the 10-cm-height headspace above the soil with air tight syringe (Supelco) directly after closure (t=0 min) and 30 minutes after and put into vacuumed tubes (Exetainer tubes, Labco ltd, UK). CO_2 concentration of the air samples was determined with gas chromatograph (Fisons 8000). Soil CO_2 emission was determined from the concentration changes during the incubation time using the following equation:

$$F = (MpV)/(6 \cdot 10^{29} \cdot kT)(c_1 - c_0)\frac{1}{A \cdot \Delta t}$$

Determination of soil physical, chemical and properties

The gross mass of soil columns (with the soil inside) was measured in all measurement day. Before the experiment all PVC tubes and caps were measured and after the experiment bulk density (Buzás, 1993) and volumetric soil water content (Buzás, 1993) were determined for each measurement day. The main nutrient elements (Gerei, 1970), soil pH value and humus content (Buzás, 1988) and water extractable organic carbon and nitrogen (WEOC, WEON) were determined by laboratory analyses. Microbial biomass carbon and nitrogen content was also determined with TOC/TN automatic analyser. Substrate induced respiration (SIR) was determined with gas chromatograph.

Results

Experiment 1 - CO_2 emission from undisturbed and disturbed soils

Figure 1 shows the emission values of undisturbed and disturbed soil columns in the five measurement days of the experiment. The figure also shows the deviation values and the statistical comparison of the values. For statistical evaluation the Mann-Whitney probe was used to detect the differences between soil respiration values of the undisturbed and disturbed soil columns. Although soil CO_2 emission of disturbed soil columns was about two times higher than of undisturbed ones during the first measurement day of the experiment (the emission value was $5.9*10^{-5}$ g CO_2 m^{-2} s^{-1} and

$9.9*10^{-5}$ g CO_2 m^{-2} s^{-1} respectively) the differences were not statistically significant according to the Mann-Whitney probe. Contradictory results were observed in the following four measurement days where soil CO_2 emission of the undisturbed soils was higher than that of the disturbed ones. CO_2 emission values measured from undisturbed soil columns were about 1.5-2 times higher than the values measured form disturbed ones, although statistical difference was only detected on the third measurement day. After evaluating the whole dataset with the Mann-Whitney statistics, emission of disturbed and undisturbed soil columns are significantly different at the level of significance of 10% (p=0,0816).

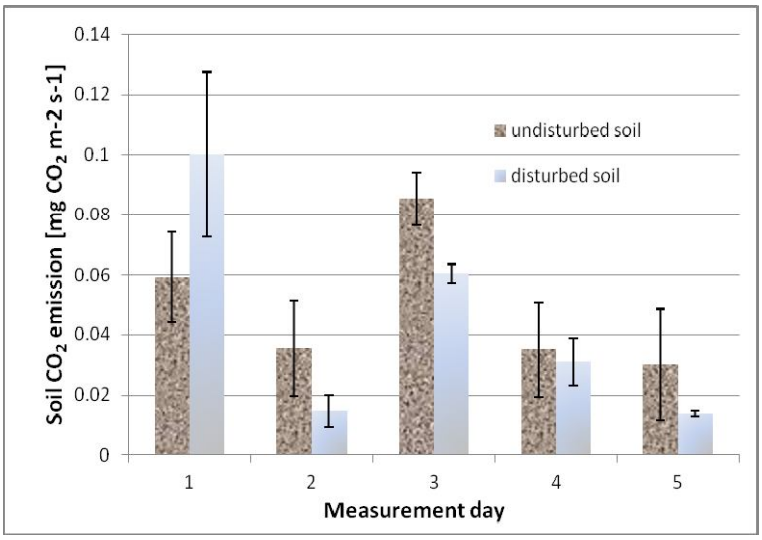

Figure 1. Soil CO_2 emission of the disturbed and undisturbed soil columns during the 5 measurement days of the experiment

Contradiction between the results of the first and the following measurement days can be explained with method of the preparation of the samples. Soil of the disturbed columns had undisturbed structure till arriving into the laboratory, since these samples were collected on the same way in the field like the undisturbed ones. During the experimental set up the soil of these columns was taken out from the PVC tubes and columns with disturbed structure were created. However, the soil went through on disturbance during preparation of the columns when taking soil out and laying the soil and stuffing it into the PVC tubes for a given volume. During these procedures soil comes to oxygen rich state which enhances the activity of aerobic microbes.
A similar phenomenon can be observed when CO_2 emission increases with order of magnitudes right after tillage application due to soil disturbance causing oxygen rich state. Then, after a few days the emission rate decreases and returns to the same level as before the tillage. During the experiment, favourable states were established for the aerobic microbes in the disturbed soil. However, in the undisturbed columns the original soil structure remained and no extra oxygen was available for microbes, except on the first measurement day when soil sample was taken. From the second measurement day the emission of undisturbed soil columns was higher which means that soil disturbance has a direct effect on soil CO_2 emission.

Experiment 2 – Relationship between soil water potential values and soil CO_2 emission rates
Determination of the relationship between soil water potential values and soil CO_2 emission has more solid physical background than seeking for relationship between soil water content and soil CO_2 emission. Measurements carried out at similar water potential values calculated on mass base make difficult to compare the results, obtained for soils of different textural classes, because the same mass-based soil water content value in e.g. sandy and clay soils reflects totally different energetic status of

water in soil. For this experiment undisturbed soil columns originating from the two rows of the plantation with different management was set to certain soil water retention values in five replicates.
Figure 2. shows the Van-Genuchten functions fitted to water retention data measured from undisturbed soil samples (100 cm^3). The four different functions represent two different depth (0-5cm and 5-10cm) in the two investigated rows (D, G). For fitting the pF curves the RET_C program (RET_C, Version 6.x, University of California, Riverside) was used. The figure shows the differences in volumetric soil water content belonging to the characteristic soil water retention values.

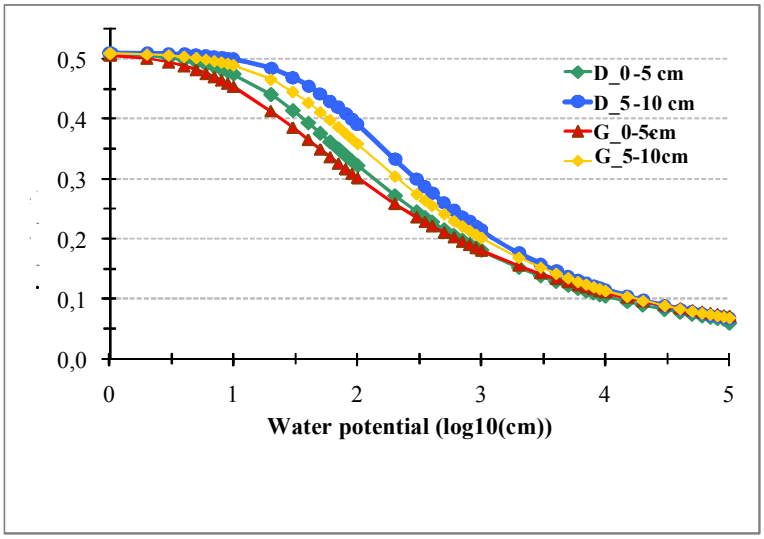

Figure 2. Van Genuchten functions (m=1-1/n) fitted to measured water retention values for soils from two different depth in the regularly disked (D) and grass-covered (G) rows of the peach plantation

Soil properties (like soil temperature and soil microbial activity) affecting soil CO_2 emission do not depend on absolute soil water content but on the energetic status of water in the soil. This means that the size distribution of pores which are filled with water strongly affect soil CO_2 emission. Soil water potential can describe best the energetic state of water in soil since the same soil water content values do not belong to the same water potential values in different soils. It is so in soils with similar mechanical composition but with different texture as it can be seen in Table 1. Actually this means that the same soil water content values can occur at totally different water-air ratio, hence soil microbial community also has different circumstances.
In the second experiment relationship between soil water potential and soil CO_2 emission could be evaluated. On the base of previous estimations we tried to set four different water potential values - pF2.0, pF2.3, pF3.2, pF3.4 in the soil columns with adding water to them. After the experiment, bulk density and the real water potential values were determined for all measurement days. The water potential values varied in a wider range; between pF1.0 and pF4.6.
Figure 3 shows the soil CO_2 emission as a function of soil water potential in two selected measurement day (7^{th} and 10^{th}) in both treatments. Sommers et al. (1981), Orchard and Cook (1983) and Andrén and Paustian (1987) also reported a linear relationship between soil water potential and soil CO_2 emission in the optimal soil water potential range, due to the logarithmical coherence in soil water potential. Other scientists (Davidson et al., 1998, Howard & Howard, 1993) described this relationship with exponential function. We fitted both the linear and the exponential model for soil water potential - soil CO_2 emission relationship, and concluded that the linear relationship describes better the relation between the two studied soil properties. So in our case, in the range of the studied soil water potential values a linear coherence can be detected between the two studied soil properties. In the measurement days showed in Figure 3. R2 values are 0.54 and 0.43in the D and 0.56 and 0.49 in the G rows, respectively.

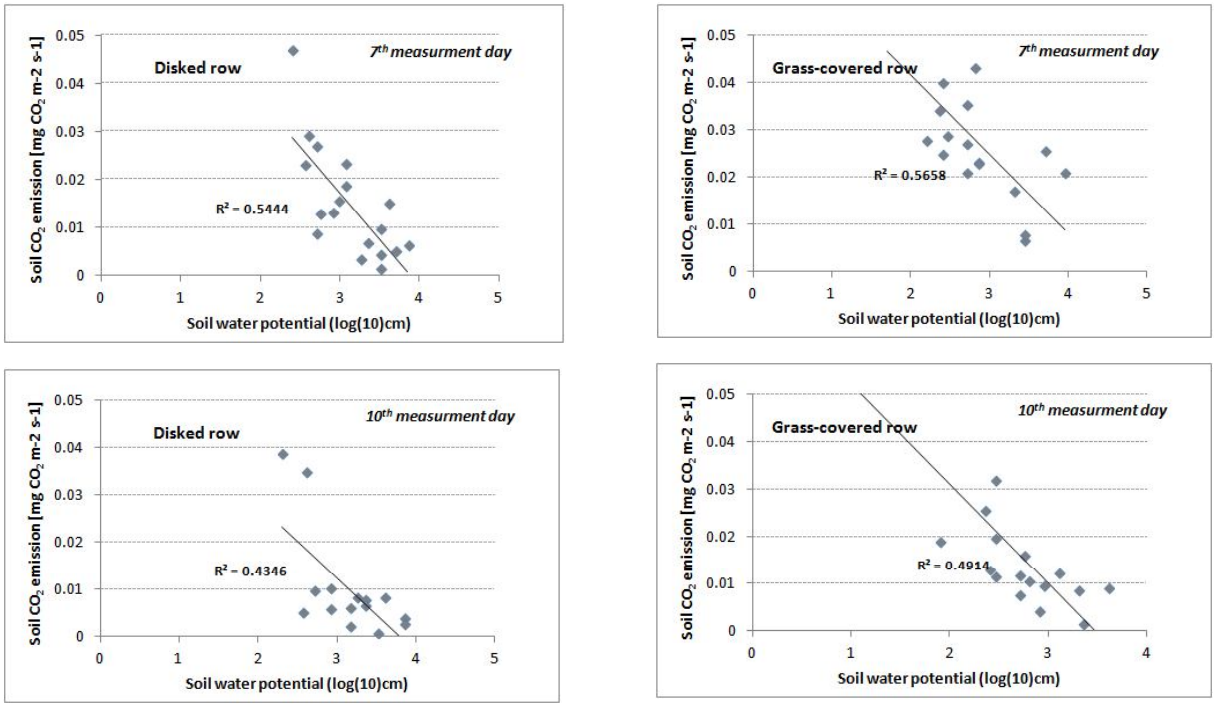

Figure 3. Coherences between soil water potential values and soil CO_2 emission determined from soil columns from the two studied treatments on selected measurement days

The deterministic coefficient (R2) values showing the closeness of coherences between soil emission and soil water potential and the significance level of correlation coefficient (R) are shown in Table 2. The agreement between the two studied soil properties was stronger on eight measurement days of the 11. Results of the first measurement day were eliminated from evaluation since on this day the transportation and preparation of samples caused elevated CO_2 emission. Coherence between soil water potential and soil CO_2 emission values were not so tight on the first measurement days than later in case of samples originating from the grass-covered row. Soil samples from the grass-covered row were more sensitive to soil sampling, transportation and experimental set up than samples from disked row. On the 9th measurement day when water was added to the samples to reach the initial water potential values an extremely bad coherence (R2= 0.022 and 0.028) was observed because of the disturbance of the equilibrium state.

Table 2. Relationship between soil water potential and CO_2 emission in case of soil samples form Vác.

Treatments	D		G	
Measurement Day	r^2	p	r^2	p
2	0,361	0,006	0,3625	0,008
3	0,489	0,002	0,1693	0,090
4	0,400	0,005	0,4675	0,0012
5	0,410	0,002	0,4200	0,005
6	0,609	0,000	0,3229	0,014
7	0,5444	0,007	0,5658	0,001
8	0,112	0,460	0,5036	0,001
9	0,288	0,022	0,0228	0,567
10	0,4346	0,004	0,4914	0,002
11	0,248	0,119	0,5022	0,002
12	0,317	0,058	0,3905	0,005

Conclusions

From the results of the first experiment, we can conclude that use of undisturbed soil samples plays an important role in studying soil carbon dioxide emission under laboratory circumstances. Hence, disturbance of soil structure, which causes oxygen rich state in the soil should be avoided. With the use of undisturbed samples, the structure-dependent soil properties influencing soil CO_2 emission directly (such as pore size distribution, bulk density, structure of the microbial community, microbial activity and the rate of evaporation) remain in their original state. Soil sampling, soil transportation and preparation work, such as measuring and watering the samples are all procedures that can significantly influence the result of the first measurement day, which must be taken into consideration by the evaluation of the results.
With the second experiment setup we tested a new laboratory method where the coherence between soil water potential values and soil CO_2 emission was studied. The coherence was stronger almost in all measurement days in case of those samples, which were collected from the grass-covered row. This statement verifies that the soil CO_2 emission values are in stronger relationship with soil water potential values in case of undisturbed soil structure than of disturbed ones. With such a laboratory analyses soils with the same mechanical compound but with different soil structure can be compared.

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