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The Effect of Pre-Incubation Duration of Soil-Biochar Model Mixtures On the Results of Determination the Intensity of Substrate-Induced Respiration (Methodological Aspects of Study).

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ABSTRACT

Was studied in laboratory experiments the effect of short term (3 days) and long term (95 and 187 days) pre-incubation of soil-biochar model mixes (ratio 20:1) in optimal conditions for the microorganisms on the results of definition the intensity of substrate induced respiration. For experiments 10 biochar samples were used. The biochars were produced from the woody and herbaceous materials under different pyrolysis conditions. Is revealed the heterogeneous influence of biochar by direction and dynamics on the rate of substrate induced respiration depending on the pre-incubation time of model mixtures. It is shown that the prolonged pre-incubation of biochar with soil material under optimal levels of moisture and temperature is a prerequisite for the correct formulation of laboratory experiments and provides a reliable assessment of the impact of biochar on a soil respiration. We can recommend the duration of pre-incubation 3-5 months.
Keywords: biochar, soil substrate-induced respiration, laboratory experiment.

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INTRODUCTION

Theoretical and methodological aspects of the definition the rate of a basal and substrate induced respiration (**SIR**) of soil microorganisms are considered in many works [1-6], but a united universally accepted methodology for determining the SIR still does not exist. The method of assessment of soil microbial activity by SIR values is based on adding in the soil sample a readily available carbon (glucose) which causes a burst of soil microorganisms activity [1]. When determining of SIR plays the important role the concentration of added glucose solution which should be sufficient to not limit the respiratory response of soil microorganisms pool but not to cause an inhibitory effect [1-3]. In paper [4] shows that when the glucose content in the range of 1-2 to 10 mg/g (according to N.D. Ananyeva et al is 2-15 mg/g [5]) the SIR of soil reaches its maximum and has a constant value. The maximum concentration of glucose in the experiments does not exceed 20 mg/g of soil [7].

In the original method of SIR determining [1] is proposed to use a fresh soil samples with natural moisture and the addition of the glucose in powder form. This approach allows us to obtain data that most full reflect the natural state of the soil microbial community. But, as is known, the results of SIR determination depends on the soil moisture and the introduction of dry glucose powder can not provide its uniform contact with the entire soil mass [3].

The results of SIR determination also dependent from the incubation time of substrate with glucose. In paper [4] recommended to incubate during 0.5-2.5 hours. Blagodatskaya E. and Kuzyakov Y. [8] showed that the transition from the potentially active microorganisms to the active state occurs in several minutes to several hours and shift from dormant to active state takes from several hours to several days. The use of kinetic methods do not allow to estimate the contribution of dormant microorganisms in soil respiration [2], yet seems preferable the use of longer incubation with glucose during 3-5 hours, but not exceeding 6 hours [5].

In recent works on the SIR study practice the different approaches to pre- incubation of the soil material as well as means to add of glucose for maximum stimulation of soil microorganisms. In papers [3,6] proposed the preliminary incubation of samples with a moisture content of 50-60% of the maximum field capacity during 1-2 day and estimated the introduction of glucose solution which provides the final moisture content of 60-65% of the maximum field capacity and glucose concentration of 0.5-20 mg/g. In paper [9] to evaluate of the activity phenol peroxidase and phenoloxidase was carried out the pre-incubation of soil material with a moisture content of 60% of the maximum field capacity during 7 day. On the basis of study by SIR method of the relationship between the carbon content of soil microbial biomass from the pre-incubation conditions N.D. Ananieva et al [5] recommend before adding of glucose carry out the incubation of soil at moisture 60% of the maximum field capacity during 7 day.

In other words, the pre-incubation procedure (even for the fresh soil samples) in optimal conditions is regarded as a necessary procedure that allows to bring the soil biota to a certain standard state. On the other hand, the pre-incubation under optimal conditions is the traditional staging technique of laboratory experiments to study the effect of different factors on soil biological activity. In recent years has increased the interest in laboratory incubation experiments in the aspect of studying the priming effect (PE) of the biochar adding to the soil. The duration of the model experiments in the study by various authors is very different. The incubation experiment with biochar and fresh organic matter (**OM**) was performed during 600 days [10]. The maximum incubation period used in work [11] is 3.2 years. However, most studies have focused on laboratory experiments and the incubation duration ranging from several days to several months [12]. In papers CO_2 emissions from soil-biochar mixture evaluated after 2 and 4 weeks [13], during 60 days [14] , 28-52 days [15] and 90 days[16].

The purpose of this paper is to evaluate the effect of the short-term and long-term pre-incubation of the soil-biochar model mixtures on the results of definition of intensity SIR.

OBJECTS AND METHODS

Characteristic of the biochar properties. For experiments 10 biochar samples were used. The biochars were produced from the woody and herbaceous materials under different pyrolysis conditions and provided by Dr. M.R. Bayan (Department of Agriculture and Environmental Sciences. Lincoln University in Missouri, USA)

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[17]. The determination of total carbon and nitrogen content in biochars was performed on the instrument vario MICRO cube (Fa Elementar). The ash content was determined by calcination at 1000°C [18]. For the total characteristic of the OM pool stability the method of stepwise oxidation proposed by Chan et al [19] was used. The stepwise oxidation method was modified and adapted by authors [20] considering of biochar properties. The determination of pH was carried out by potentiometric method [18] at ratio of biochar: water 1:12.5. Table 1 presented the biochar characteristic used in the experiment. Data demonstrate that biochars are very different on the quality organic component, ash element content and chemical properties.

Table 1. The total biochar characteristic

Plant biomass used for pyrolysis			Pine	Cedar	Oak	Miscanthus	Willow	Corn stover	Switchgrass	Willow	Corn stover	Switchgrass
Pyrolysis temperature			400-600°C							< 400°C		
Total content	C	% to the weight of charcoal	84.4	86.3	78.2	77.6	79.6	66.3	71.2	77.3	57.2	63.1
	N		0.167	0.292	0.400	0.564	0.278	0.863	0.658	0.228	0.902	0.470
	Ash		2.7	1.0	2.8	10.9	6.8	19.9	13.9	2.7	13.5	8.3
The content of the fractions with different oxidability [18]	Fr.1		0.84	0.34	0.75	0.50	0.54	0.63	1.46	1.09	5.44	3.69
	Fr.2		2.82	3.95	2.91	3.28	3.69	2.04	5.52	8.95	19.32	8.86
	Fr.3		5.37	11.21	3.59	3.92	6.82	5.62	5.20	6.15	4.28	8.23
	Fr.4		75.41	70.76	70.94	69.94	68.54	58.02	58.99	61.11	28.15	42.28
pH			8.0	7.9	8.7	9.6	9.3	9.3	9.9	8.6	7.0	5.5

Setting of the model experiment. For the model experiment the sample of humus horizon from Luvisoil selected in June under the broadleaf forest was used. After removal of roots the soil in the initial state of moisture was sieved through 2 mm diameter. 40 g of soil was mixed with 2 g of biochar that sieved through 1 mm then moisture was adgusted to 55% of the maximum field capacity and thoroughly stirred. Petri dishes with model mixtures are stored in a thermostat with periodic ventilate to provide free access of oxygen. The initial moisture of model mixtures maintained gravimetrically.

The determination of SIR speed performed in triplicate. Before SIR determining the model mixtures thoroughly stirred, sample with weight of 2.5 g was selected in small portions from different areas and placed in a flacon of 20 ml with hermetically sealed rubber stopper. The contents of the flacon thoroughly mixed. Then with doser was added the estimated amount of glucose solution with the calculated concentration so that the final moisture content of the material corresponded to 65% of the maximum field capacity and glucose content in the flacon reached to10 mg in 1 g of mix. Flacons were stored in the thermostat during 4 hours at t=25°C. Then using the syringe sampled the gas phase. The determination of CO_2 in the flacon was performed by gas chromatography Clarus 580 (PerkinElmer) with a katharometer as a detector. The intensity of SIR value expressed in micrograms C-CO_2 released by 1g model mixture during 1h. SIR is determined by 3, 95 and 187 days. For the statistical evaluation of the results was used of variance analysis with estimate of Fisher least significant difference (**LSD**). The calculations were performed in the program STATISTICA 8.0.

RESULTS

The results of the SIR intensity depending on the duration of pre-incubation of model mixes soil-biochar are presented in Fig.1.

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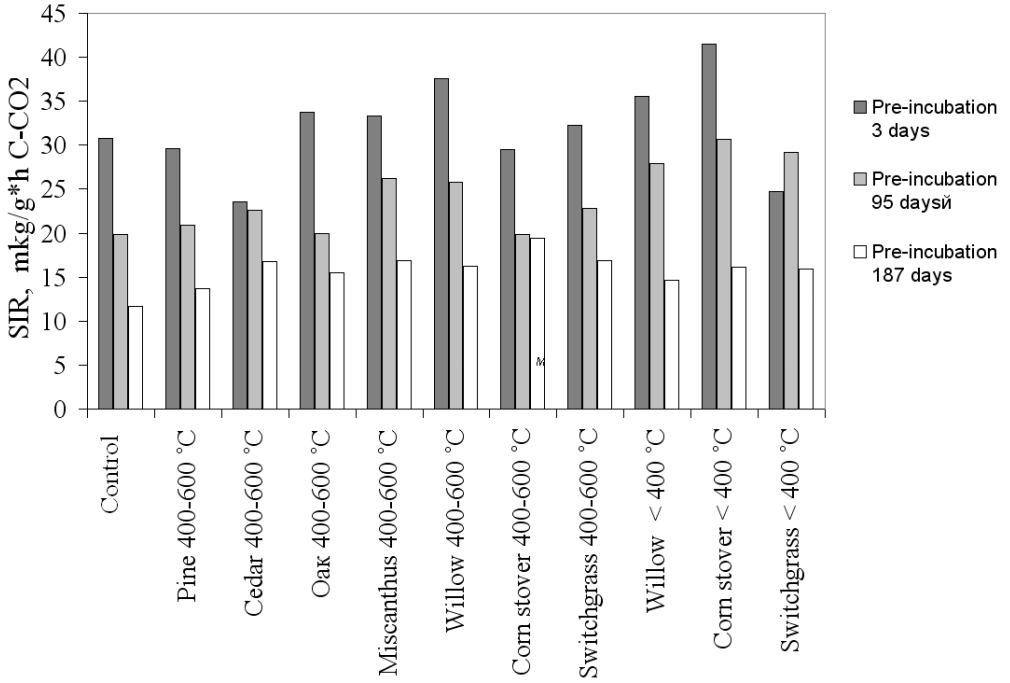

Fig.1. Effect of the pre-incubation duration (3, 95 and 187 days) on SIR.

With increasing of pre-incubation duration in control and in all variants (except for variant Switchgrass < 400°C) there is a gradual decrease in the SIR intensity that can be attributed of the decrease of available OM content in soil and biochar. Laboratory experiment [21] demonstrated that the using fresh biochar the CO_2 emission becomes 3.10 times higher than when using of biochar stationed long time in contact with the soil. Fig.2 illustrates the difference of SIR intensity of variants with control at different durations of experiments.

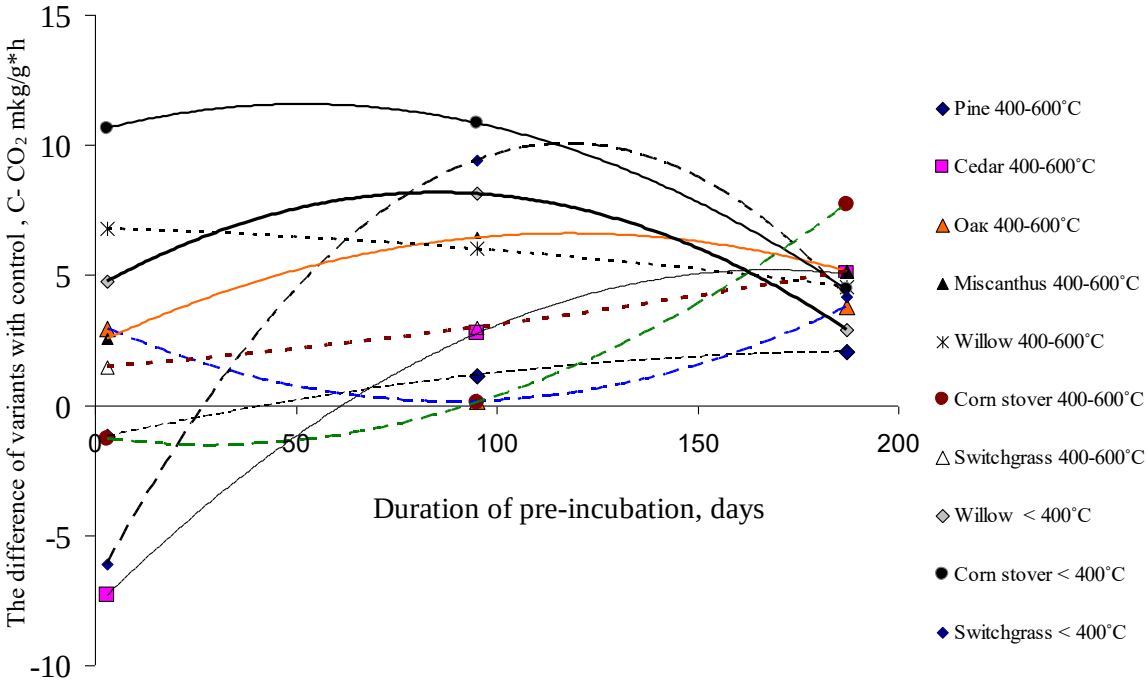

Fig.2. The difference of SIR intensity of variants with control (data approximated by polynomial curves of the second order)

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At short-term pre-incubation (3 days) the model mixtures is observed both to decrease the SIR intensity compared to the control and to increase. At long-term pre-incubation (95 and 187 days) the difference of SIR intensity in all variants compared to the control is positive. The dynamics of SIR change is multidirectional and depend on the biochar type.

Using the obtained data formed a three single-factor dispersion complex separately for each pre-incubation period. Dispersion complex includes the 11 factor levels (10 types of biochar plus control) with four replications of SIR definition. All three dispersion complex characterized by statistically significant of Fisher´s criterion (3 days – F=2.40, p=0.03; 95 days – F=4.01, p=0.00; 187 days – F=2.31, p=0.03). Results of LSD analysis are presented in table 2. The data show that at short-term pre-incubation of model mixtures observed only one the statistically significant difference of SIR intensity with control (Corn stover, < 400°C). This sample characterized by maximum content of easy and moderately oxidized OM. At pre-incubation during 95 days detected 5 statistically significant differences, during 187 days – all variants of the experiment (except Pine 400-600°C and Willow <400°C). For the variant of Pine that was produced in the pyrolysis temperature range 400-600°C observed almost the linear increase of difference with the control from negative value to positive which not becomes statistically significant. For the variant of Willow (<400°C) observed sharp decrease the SIR intensity and the difference wit control becomes statistically insignificant.

Table 2. The estimation results of LSD of SIR of the model experiments variants with control

The duration of pre-incubation	LSD	Biochar types									
		Pine	Cedar	Oak	Miscanthus	Willow	Corn stover	Switchgrass	Willow	Corn stover	Switchgrass
		400-600°C							<400°C		
3 days	The difference with control	-1.21	-7.28	2.96	2.56	6.79	-1.28	1.46	4.76	10.65	-6.09
	p-value	0.80	0.14	0.54	0.60	0.17	0.79	0.76	0.33	0.03	0.21
95 days	The difference with control	1.14	2.76	0.17	6.44	6.01	0.10	2.98	8.13	10.84	9.43
	p-value	0.69	0.33	0.95	0.03	0.04	0.97	0.30	0.01	0.00	0.00
187 days	The difference with control	2.04	5.06	3.82	5.16	4.56	7.70	5.13	2.92	4.45	4.18
	p-value	0.28	0.01	0.05	0.01	0.02	0.00	0.01	0.12	0.02	0.03

DISCUSSION

The absence the statistically significant difference in 9 variants of laboratory experiment with added biochar compared with the control at 3 days pre-incubation is due to high variability of the results of determining the SIR value in replications. Biochar are highly porous material which greatly retained a hierarchical porous fabric structure of the original plant material [17]. At short-term pre-incubation the soil-biochar model mixture, apparently, constitute the heterogeneous mixture in which do not have time to establish any equilibrium between the soil material, highly porous biochar and soil biota. Then the high variability may be due to both the inability to take representative samples for incubation with glucose and to lack of uniform response of all microorganisms pool on adding of glucose.

Long-term pre-incubation results in the equilibrium establishment in this complex system that provides homogeneous mixtures and reliable determination of SIR value. However, too long pre-incubation can reduce the reliability of SIR assessment due to the general decrease in respiration intensity as seen in the variant of Willow (<400°C).

The direction of the effect of biochar as the total soil respiration and the PE remains open to discussion. Positive PE observed in papers [13,22], negative – in papers [11,16]. Some authors have noted the absence of significant effect of the introduction of biochar on the soil respiration [15,23]. According to Maestrini et al .[12] the positive PE occur in a short time and more intensely while the negative PE occurs only after a long period of contact of pyrogenic material with the soil and with less intensity. Still debatable about the mechanisms of the biochar effect on CO_2 emissions from soils [12,24].

We have identified the heterogeneous effect of the introduction of various biochars and the different nature of the SIR values change depending on the duration of pre-incubation. This result is quite integrates into current understanding of the nature of the effect of biochar on the soil respiration intensity.

SUMMARY

This research shows that pre-incubation biochar with soil under optimal for biota conditions is a necessary condition to the correct formulation of the model laboratory experiments to evaluate the effect of biochar on the SIR value. We can recommend the duration of pre-incubation 3-5 months, at which there is the maximum difference of experiment variants (with the introduction of the pyrogenic material) with control.

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