BIOCHEMICAL PROCESSES IN CHERNOZEM SOIL UNDER DIFFERENT FERTILIZATION SYSTEMS

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Abstract. The aim of this research was to assess how biochemical processes driven by microbial activity and extracellular enzymes have developed in the long-term under farming system with different fertilizer and crop types. It was concluded that the crop types had a more considerable impact on the soil biochemical processes compared to long-term Org or mixed Min+Org fertilizers amendments.

Keywords: soil, fertilization, enzymatic activity, organic N mineralization

1. Introduction

The intensification of agriculture in the 20th century has caused several environmental problems [1]. High N fertilizer rates have increased nitrate leaching and N_2O emissions from cropping systems [2]. The intensive soil cultivation of arable land has lead to a loss of soil C, thereby contributing to anthropogenic CO_2 emission [3]. These issues spurred research interest in less intensive agricultural management practices, and their potential to reverse some of modern agriculture's negative side effects [4]. We are to increase our awareness of how soil management affects soil fertility (e.g. nutrient cycling, soil structure and water holding capacity).

Since mineralization of soil organic substrates and the release of nutrients and elements are due to the heterotrophic activity of microbial decomposer compartment, this subsystem of terrestrial ecosystems gained importance [5]. The impact of management practices on the flow of C and N trough ecosystems is largely mediated trough the soil microbial community. The C derived from fresh or native soil organic matter that is used by microbes is either mineralized to CO_2 or it is put to anabolic use in production of biomass (new or maintenance) or egested as cellular metabolites (e.g. enzyme production) [6]. Soil microbes produce extracellular enzymes that mineralize organic matter and release carbon and nutrients in forms that can be assimilated. The current understanding is that mineralization of soil organic matter is governed by several concurrent processes: 1) destabilization via oxidation/hydrolysis, desorption and diffusion and 2) the size, community composition and metabolic activity of the microbial biomass [7-8]. It is thought that substrates must pass through the dissolved phase of organic C pool to reach and pass through microbial membranes [9].

Microbial biomass carbon (C_{mic}) and related parameters such as microbial quotient (C_{mic}:C_{org}), basal soil respiration (C-CO_2 rate), and metabolic quotient (qCO_2) are widely used with the objective of understanding of microbial responses to various soil management practices [5, 10-11]. These parameters were specified by Nannipieri et al. [12] as the general parameters of soil biochemical properties (directly related to microbial activity) and proposed as eco-physiological indicators of biological soil quality [5].

The aim of this research was to evaluate the intensity of certain soil biochemical processes (e.g. soil organic C mineralization) at Organic and mixed Mineral+Organic fertilization of typical chernozem in crop rotation dynamics (for 6 years) by use of eco-physiological indicators of biological soil quality: microbial biomass carbon, basal soil respiration, as well as, microbial and metabolic quotients.

2. Experimental

Soil sampling was performed from a long-term field crop experiment, which has been established in 1971 at the Balti steppe and are supported by Research Center "Selectia" (Balti, 140 km North of Chisinau) [13-14]. The soil is classified as a typical chernozem (black) soil (silt loam) with C_{org} reach horizon up to 92 cm. Soil organic matter (SOM) content initially was 4.65% (correspondingly, C_{org} constituted 2.70%) in 0-20 cm layer. The pH value – 6.6-7.1 (water) and 6.2 (salt solution). Total N constituted 0.24-0.26%; P – 0.12-0.13%; K – 1.2-1.4%. Two treatments representing organic (Org) and mixed mineral-organic (Min+Org) fertilization were selected for comparative research of soil biochemical properties, because of both are known to be able to maintain the SOM. But both provide different increases of crop productivity in frames of studied crop rotation [15]. Soil samples were taken June 14-16, 2010 (at active crop growth phase) by an auger from the top layer (0-20 cm) of arable field plots. Samples were taken from each of 4 replicates per treatment by combining 5 soil cores inside of each replicate, in total 48 samples. After removing vegetal rests and stones soil was passed through 2 mm sieve. Samples were stored at 4°C no longer than one month necessary for set of biochemical analysis. The aliquots of air-dried soil samples were used for chemical parameters determination.

Microbial biomass carbon (C_{mic}) assay was conducted by use of rehydratation method [16]. Soil samples (2 replicates till 5g for each of treatment) were oven dried at 65-70°C for 24 h, resulting in disruption of the microbial cell wall permeability. Repeated rehydratation of dry soil samples with 0.5 M K_2SO_4 at a ratio 1:2 (w/v) resulted in

microbial cell destruction and release of microbial carbon into solution. An additional 2 replicates of 5g fresh soil samples were placed in refrigerator to serve as controls which were treated in the same way. K_2SO_4–extractable organic C concentrations in the dried and fresh soil samples were simultaneously measured using dichromate oxidation. The aliquote 1.6 ml of filtered soil extract was carefully mixed in tube with 2.4 ml of dichromate solution: 1.28 g $K_2Cr_2O_7$ in 400 ml of deoinized water is dissolved in 2 L of H_2SO_4 (d=1.84 g/cm^3). The mixture was incubated at 140^{o}C for 20 min. The optical density after cooling is measured at 340 nm against of blank mixture of reagents without soil salt extract. The amount of carbon in the samples was calculated by the following formulae: $C_d = (OD_d - OD_b) \cdot V / k_l \cdot a$, and $C_f = (OD_f - OD_b) \cdot V / k_l \cdot a$, where OD_d and OD_f are the optical densities of dried and fresh samples, respectively; OD_b is the optical density of blank probe with salt solution instead of salt extract; V represents the volume of salt extract, ml; a - is the the weight of soil sample, g; and k_l is the coefficient for transfer from optical dencity to carbon concenration according to calibration curve with glucose. Biomass C (μg C per g oven dry soil) was calculated from the expression $B_c = (C_d - C_f) / k_c$, where $(C_d - C_f)$ is the difference of C measured in dried and fresh sand samples, μg C; k_c (the portion of cell components released in solution after drying-rehydratation procedure) was 0.25 [16].

Basal soil respiration (C-CO_2 elimination) was determined by adopted method proposed by Isermeyer [17]. Briefly, soil (25-50 g of dry soil, adjusted to water content 40% WHC was weighted at the bottom of 1L glass jars containing two vessels with 10 ml of distilled water for air humidifying and 20 mL of 1 M NaOH for CO_2 trapping. The jars were sealed (air-tight) and incubated at 21^{o}C in the dark for 7-14 d. CO_2 released during soil incubation was trapped in NaOH and determined by titrimetric analysis. Before analysis 0.5 M $BaCl_2$ was added to the NaOH solution to remove carbonates. Residual NaOH was titrated with 0.1 M HCl in the presence of phenolphtalein indicator. The soil respiration was recorded as μg C-CO_2 g^{-1} dry soil h^{-1} at 21^{o}C as the average rate during the whole 7-14 days incubation.

Metabolic quotient (qCO_2) or the quantity C-CO_2 produced per unit of microbial biomass C per unit of time was calculated as a ratio $C\text{-}CO_2 : C_{mic}$ and was expressed in mg C-CO_2 g^{-1} C_{mic} h^{-1} [18].

Total organic carbon (C_{org}) was assayed using air-dried soil samples by wet oxidation with dichromate in an acid medium and evaluation of the excess of dichromate according to the method of Tiurin [19]. The value of total C_{org} is recorded as % of dry soil mass.

Microbial quotient was calculated as a ratio $C_{mic}:C_{org}$ and expressed in % of total organic C [20].

Statistical analysis. Two-way ANOVA (StatSoft STATISTICA 7.0) was conducted with fertilizer and crop type as fixed factors. Differences between means within a crop types for each investigated parameter were identified using paired t-tests.

3. Results and Discussion

Total organic carbon (C_{org}) of typical chernozem soil from Balti steppe under long-term agricultural use with 6-field crop rotation and two fertilization systems was ranged, between 2,36%-2,59% and 2,40%-2,63%, respectively, for Org and Min+Org fertilization systems (table 1). According to two-way ANOVAs data, both independent factors the fertilization system and the crop types significantly ($P < 0.05$) influence the principal component of soil fertility (table 2). Mean values of total organic C were significant higher in soil amended by mixed Min+Org fertilizers and revealed the significant differences depending on cultivated crop type. The soil under winter wheat was characterized by the least values of C_{org} at both fertilization systems, though it follows after mixture of vetch + oats in crop chain.

Microbial biomass carbon (C_{mic}) serves the index of soil microbiological and biochemical potential. The size of microbial biomass carbon in typical chernozem soil cultivated with six different crops was influenced significantly ($P < 0.05$) by the crop type and the kind of investigated fertilizers, with the evident tendency of increase at mixed Min+Org fertilizers amendment (tables 1-2). It could be explained by bigger amount and accessibility of mineral nutrients for soil microorganisms at sum action of Min+Org fertilizers. Of six crops cultivated in studied crop rotation the soils under winter wheat and sugar beet characterized with significantly ($P < 0.05$) lower size of microbial biomass despite of adequate soil fertilization. It seems in contradiction with results of other researchers [21-23], which reported that the identity of the plant species did not influence the soil microbial biomass. However, it was shown that though soil bacterial and fungal biomass did not differ between soils of different plant species the microbial community structures did, due to the quality of rhizosphere carbon [24]. The idea that plant species may have specific effects on the carbon flow into soil microorganisms was confirmed by Ladygina and Hedlund [21] when regarding the active carbon allocation from a plant to the microorganisms. These data allow the assuming that winter wheat's and sugar beet's root exudation (organic rhizodeposition) could allocate less carbon into microbial biomass in comparison to the other four crops in studied crop rotation.

Microbial quotient (C_{mic} : C_{org}) gives the insight into the capability of a soil to support the microbial growth [25], that is, it reflects the soil carbon available for growth [20]. Thus, it is expected that soils with better quality will have higher microbial quotient [10, 20]. According to results of this study (table 1) the two used fertilization systems (Org vs. Min+Org) insignificantly differed by the available C resource for microbial growth, but crop types did (table 2). Typical chernozem cultivated with winter wheat and followed sugar beet demonstrated the lowest microbial quotient

(0.7-0.8%), the sequence of crops spring barley-sunflower-vetch+oats characterized by higher values (0.9-1.0 %), but the soil under corn for grain placed in the middle of crop chain was the bend point (0.8-0.9 %).

Table 1

Eco-physiological indicators of soil quality reflecting the intensity of soil biochemical processes

Parameter	Microbial biomass carbon, C_{mic}	Microbial quotient, $C_{mic} : C_{org}$	Basal soil respiration, C-CO_2 rate	Metabolic quotient, qCO_2,	Total organic carbon, C_{org},
Units of measurement	μg C g^{-1} dry soil	%	μg C-CO_2 g^{-1} soil h^{-1} at 21°C	mg C-CO_2 g^{-1} C_{mic} h^{-1}	%
Crop type			**Organic fertilization system**		
Winter wheat	176 ± 8 [a]	0.75 ± 0.02	0.56 ± 0.14	3.2 ± 0.74	2.36 ± 0.04
Sugar beet	177 ± 23	0.69 ± 0.09	0.61 ± 0.08	3.5 ± 0.80	2.59 ± 0.04
Corn for grain	217 ± 24	0.88 ± 0.10	0.77 ± 0.08	3.6 ± 0.25	2.46 ± 0.03
Spring barley	240 ± 11	1.00 ± 0.07	0.35 ± 0.16	1.5 ± 0.65	2.40 ± 0.20
Sunflower	236 ± 5	0.96 ± 0.03	0.60 ± 0.16	2.5 ± 0.66	2.45 ± 0.05
Vetch+oats	228 ± 31	0.89 ± 0.10	0.56 ± 0.17	2.6 ± 1.00	2.55 ± 0.10
			Mineral+Organic fertilization system		
Winter wheat	182 ± 18	0.76 ± 0.08	0.43 ± 0.13	2.4 ± 0.80	2.40 ± 0.04
Sugar beet	197 ± 8	0.75 ± 0.03	0.25 ± 0.02	1.3 ± 0.13	2.63 ± 0.02
Corn for grain	220 ± 10	0.84 ± 0.05	0.52 ± 0.13	2.4 ± 0.69	2.60 ± 0.04
Spring barley	254 ± 5	0.99 ± 0.02	0.48 ± 0.11	1.9 ± 0.46	2.57 ± 0.03
Sunflower	257 ± 14	0.99 ± 0.06	0.68 ± 0.05	2.6 ± 0.26	2.60 ± 0.03
Vetch+oats	242 ± 34	0.96 ± 0.13	0.69 ± 0.11	2.9 ± 0.48	2.53 ± 0.03

[a] Mean ± S.D. (standard deviation, σ), n=4;

Table 2

Summarized results of two-way ANOVAs for soil general biochemical parameters

Dependent variables	Independent variables [a]	d.f. [b]	F[c]	P-value[d]
Microbial biomass carbon (C_{mic})	Fertilization system	1	6.24	0.017*
	Crop types	5	20.79	<0.001***
	Interaction	5	0.32	0.89
Microbial quotient ($C_{mic} : C_{org}$)	Fertilization system	1	0.90	0.35
	Crop types	5	20.21	<0.001***
	Interaction	5	0.66	0.66
Basal soil respiration (*C-CO_2 rate*)	Fertilization system	1	0.88	0.35
	Crop types	5	4.61	0.002**
	Interaction	5	2.98	0.023*
Metabolic quotient	Fertilization system	1	0.88	0.35
(*qCO2 =C-CO_2 : C_{mic}*)	Crop types	5	4.61	0.002**
	Interaction	5	2.98	0.024*
Total organic carbon (*C_{org}*)	Fertilization system	1	15.91	<0.001***
(0-20 cm)	Crop types	5	7.95	<0.001***
	Interaction	5	2.21	0.07

[a] Fertilization system (Min+Org vs Org) and crop types (six cereal and row crops in 6-years crop rotation) were the independent variables.

[b] degree of freedom – the number of given elements (e.g. two fertilization systems or six crops) inside of independent variables minus 1;

[c] F-test or Fisher's criteria;

[d] confidence level. P values: *** = P<0.001; ** = 0.001<P<0.01; * = 0.01<P<0.05

Basal soil respiration (C-CO_2 rate) serves as the indicator of metabolic activity of soil microorganisms or the content of organic carbon potentially mineralizable up to CO_2. The CO_2 emission from the soil to the atmosphere is the main cause of soil C loss [26] and it provides an early indication of soil C level when changes in organic C

due to management practices are not detectable over a short period [27-28]. According to two-way ANOVAs analysis data (table 2) the fertilization systems revealed no significant influence upon C-CO_2 rate. But differences between means within a crop types identified using paired t-tests were significant for microbial communities from soils under winter wheat, sugar beet, corn for grain, and spring barley. The most evident differences were observed for microbial communities from soils of Org plots cultivated with corn for grain and followed spring barley, respectively, with the most enhanced and the most reduced levels of CO_2 elimination. At mixed Min+Org amendment the soil cropped with sugar beet characterized by the least quantity of CO_2 elimination, but the soil under sunflower and followed vetch + oats – by the biggest intensity of respiration. Thus, the soil respiration was significantly higher in soil under crop chain the winter wheat – sugar beet – corn for grain at Org fertilizers amendment in comparison to Min+Org system. It was found, the nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied [29]. The possible explanation of enhanced CO_2 evolution from soil, cropped with corn for grain, may be the existence of significant deficiency of mobile N. Conform to general N-regulation processes by the content of soil accessible N [30], the decomposition of soil organic carbon substances, containing simultaneously and organic N, can be initiated and the elimination of CO_2 is enhanced. It was hypothesized that organic cropping systems would reduce soil CO_2 emission and increase C storage compared to conventional cropping systems [31]. Last cited researchers have found that the soil CO_2 emission rate at peak times in the Org system was higher than the conventional (Conv) that is mineral fertilization system. However, even if the cropping systems had a temporary impact on the rate of soil CO_2 emissions, the soil C output calculated as the average of cumulative CO_2 emission over the 3-year period did not show significant differences between the Org and Conv systems. On the other hand, according to last researchers, the C input in the Org system was higher than in the Conv (9.46 Mg C ha^{-1} vs. 5.57 Mg C ha^{-1}) as well as the C input/output ratio (1.10 vs. 0.72) [31]. The 3-year average of C_{org} content and C stock was higher in the Org than in the Conv system. Still, are needed to verify if C limitation for soil microbial growth and nitrogen limitation for crop growth in the organic system could hinder soil C accumulation over a longer period.

Metabolic quotient (qCO_2) was offered for the quantification of environmental effects on the microbial communities in soils [9]. The parameter qCO_2 indicates the efficiency by which soil microorganisms use C-resources in the soil, and it is expected that stressed soils will provide higher qCO_2 values than less-stressed soils [32]. The same, it reflects the microbial requirements of maintenance energy [20]. The results of our research show two certain crop chains (table 1). First one: spring barley-sunflower-vetch+oats were not affected by fertilizers, second – winter wheat-sugar beet-corn for grain had lower qCO_2 values at Min+Org than at Org fertilization. It could be explained by better supply of microorganisms and crops with mobile N at Min+Org, while at Org farming it may be as was noticed by Mancinelli et al [31] the temporary impact on the rate of soil CO_2 emissions due to use of organic substances which simultaneously contain C and N elements. In general, the metabolic quotient was not influenced significantly by fertilization system ($P > 0.05$) (table 2). The qCO_2 values of typical chernozem (soil pH 6.6-7.1) under 6 crops (Northern Moldova) ranged between 1.5-3.6 mg CO_2-C g^{-1} C_{mic} h^{-1} (mean=2.8) and 1.7-2.9 mg CO_2-C g^{-1} C_{mic} h^{-1} (mean=2.3), respectively, at Org and Min+Org fertilization, and were lower in comparison to the mean values 3.5 and 3.6 mg CO_2-C g^{-1} C_{mic} h^{-1}, published by Trasar-Cepeda et al [33] for 40 climax soils and 45 cropped soils (respectively, soil pH 4.29 and 5.83) in Galicia (NW Spain). It is known under acidic conditions the qCO_2 is elevated since maintenance energy requirements of microbes are higher [20].

In summary, having in mind the interlinkage between the soil biotic component and biogeochemical cycling this research has followed the approach proposed by T.-H Anderson [5, 20] to use the eco-physiological indicators to estimate that one of soil management practices would be more or less detrimental than another, that is, Min+Org fertilization system versus Org one, only. It was published [20], that the C_{mic} : C_{org} ratio of agricultural and forest soils at neutral pH is very similar and in the range between 2.0 and 4.4% C_{mic} of total C_{org},, depending on nutrient status and soil management. The metabolic quotient qCO_2 ranged between 0.5 and 2.0 mg C-CO_2 g^{-1} C_{mic} h^{-1} in neutral soils. Values below 2.0 for the C_{mic} : C_{org} ratio or above 2.0 for the qCO_2 could be considered as critical for soils with a neutral soil pH. The evaluation of our results on typical chernozem soil from North Moldova steppe under long-term agricultural use with the most protective fertilization systems (Org and Min+Org) and crop rotation has shown the C_{mic} : C_{org} ratio twice and more below 2.0 and the mean qCO_2 values above 2.0. It means that Org fertilization system still doesn't make it possible to avoid the loss of organic C in arable typical chernozem. These data coincide with another published results, that soil C_{org} accumulation declines in long-term experiments (>50 yr) with farm manure applications as a new equilibrium is approached [34]. The both Org and Min+Org fertilization systems equally provides the soil carbon available for growth according to C_{mic} : C_{org} ratio, but last one revealed lower level of qCO_2 values. It could mean the mitigation of the negative consequences of long-term mineral fertilization by organic fertilizers.

4. Conclusion

The crop types had a more considerable impact on the soil microbial biomass and community biochemical activity compared to long-term Organic or mixed Mineral+Organic fertilizers amendments. The chain of crops: winter wheat – sugar beet – corn for grain revealed a strong influence on soil microbial communities resulted in higher metabolic

quotient (qCO_2) at Org system of fertilization. It could demonstrate a less efficiency of soil organic carbon use may be because of a more acute need for accessible N. Next chain of three crops: spring barley – sunflower – vetch+oats revealed higher microbial quotient (C_{mic} : C_{org} ratio) and least qCO_2 values. The organic fertilizer is able to mitigate the negative consequences of long-term mineral fertilization.

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6. References

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