ECO-PHYSIOLOGICAL INDICATORS OF BIOLOGICAL QUALITY OF CALCAREOUS CHERNOZEM AFTER LONG-TERM AGRICULTURAL USE

Ecaterina EMNOVA, Anna HANGANU, Vasilii GNIDIUC*

Institute of Genetics and Plant Physiology
**Educational Experiment Station "Chetrosu", Moldavian Agricultural State University*

Practica de lucrare a solului (cu şi fără întoarcerea brazdelor) a manifestat cel mai puternic efect asupra parametrilor biologici ai cernoziomului carbonatic din sud-estul Moldovei. Lucrarea solului fără întoarcerea brazdelor în combinație cu administrarea îngrăşămintelor verzi (sideratelor)+NPK rezultă în cantitatea mai mare de C_{mic}, cele mai mici valori ale respirației microbiene a solului şi ale coeficientului metabolic (qCO_2). Utilizarea multianuală a erbicidelor nu a influențat parametrii microbieni. Cu toate acestea, cernoziomul carbonatic după un termen lung (42 de ani) în exploatare agricolă cu asolament, sistem de lucrare a solului protectiv (ploscorezul), amendament de îngrăşăminte organice şi minerale, de erbicide în doze minime încă pierde în mod constant substanța organică din sol. Mărimea biomasei microbiene este aproape de optim, dar se demonstrează activitatea de mineralizare excesivă a substanței organice şi o viteză sporită de eliminare a CO_2.

Introduction

Soil quality is the capacity of soil to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health [1-3]. Terms of soil quality and soil health may be used synonymously, however first term is generally associated with soil's fitness for a specific use, and second term is used in a sense to indicate the capacity of soil to function as a vital living system [4]. The soil quality encompasses three basic components: biological, chemical and physical properties while soil health is determined by ecological characteristics [3]. By this reason, soil quality is considered the broader concept than soil health [5]. Soil quality is conceptualized as a major linkage between the strategies for agricultural conservation management practices and achievements of the major goals of sustainable agriculture [6]. The assessment of soil quality or health, and direction of change with time, is considered as the primary indicator of sustainable land management [3].

Soil microorganisms play important roles in soil quality and plant productivity. Soil microbial biomass is an important parameter of ecological tests, since microorganisms mediate critical ecosystem carbon and nutrient cycles. Soil respiration is one of the oldest and still the most frequently used parameter for quantifying microbial metabolic activity in soil [7]. Soil microorganisms divert more energy from growth into maintenance as stress increases and thus the ratio of basal soil respiration to microbial biomass (the metabolic quotient qCO_2) can be a much more sensitive indicator of stress [8-9]. Having in mind the interlinkage between the soil biotic component and biogeochemical cycling, Anderson and Domsch [10-11] have proposed the use of eco-physiological indicators (microbial biomass carbon, basal soil respiration, microbial and metabolic quotients) to estimate the soil management practices.

Chernozem soil occupies 70% of total Moldovan land territory [12], arable land consists of 1842.6 thousand ha or 54.4% [13]. Soil degradation is the global problem, but it is especially severe in developing countries, where natural resources are under great stress, and resource-poor farmers cannot to afford the input required for restoration of degraded soils and ecosystems [14]. During many years soil research has concentrated on soil chemical and physical factors, with comparative neglect of biological factors. Consequently, there was relatively limited understanding of how best to capitalize on the dynamics and potentials of soil biology so as to enhance the regenerative capacity of soil systems for agriculture. Biological estimation of the quality of Moldavian chernozem is now the focus of research for soil microbiologists and biochemists [15-18]. The Educational Experiment Station (EES) "Chetrosu" of State Agricultural University of Moldova was established in 1949 on calcareous chernozem in South-East of Moldova (Anenii Noi area, village Chetrosu, 30 km distance from Chisinau). The long-term field experiment since 1969 reflecting the different kinds of intensive agricultural soil management practices as a combination of elements of conventional and organic cropping systems is supported for 42 years. The two soil fertilization systems used for interval 1989-2003 had to be stopped owing to the financial difficulties, but the synthetic herbicide use is continued so far.

The objective of this research was to study the effect of the long-term moldboard tillage (plow) versus moldboardless (subsurface) tillage (paraplow), long-term use of weed control agrichemicals (herbicide) for corn cultivation versus mechanical weeding, and aftereffect of fertilization systems (green manure+NPK versus cattle manure) on some soil general biochemical parameters related to microbial activity, reflecting the biological quality of calcareous chernozem soil placed in South-East Moldova.

Materials and methods

The site has a systematic block design of 5-field crop rotation. Individual plots measured 55 m^2 (5.5m x 10 m) and designed in 4 replicates. The field crops are comprised of a pea (*Pisum sativum*) - winter wheat (*Triticum durum*) - corn for grains (*Zea mays L.*) (1) - corn for grains (2) – alfalfa (*Medicago sativa)*. In 2011 the field was cultivated with corn for grains treated by herbicides as always. The three experimental factors used since 1989 include: A. Tillage - moldboard tillage (plow) vs moldboardless tillage (paraplow). B. Agrichemicals - long-term use of weed control chemicals (herbicide) for corn protection vs mechanical weeding (control). C. Fertilization - post-action of previously (1989-2003) used fertilization systems (green manure+$N_{120}P_{120}K_{120}$ vs cattle manure (60 t/ha). The arable layer of calcareous chernozem initially contained 3.68% of soil organic matter (SOM, the former term "humus") [19]. The mean values of SOM in 0-20 cm layer for 1984-1993 years constituted 2.80-2.88% (correspondingly, C_{org} =1.62-1.67%). The soil solution revealed a slightly alkaline reaction. *Soil sampling* was performed September 10, 2011 (before the corn harvest) from the top layer (0-20 cm) of arable field plots. Samples were taken from each of 3 replicates per treatment by combining 5 soil probes inside of each replicate, in total 24 samples. After removing vegetal rests and stones soil was passed through 2 mm sieve. Samples were stored at 4^{o}C no longer than one month.

Microbial biomass carbon (C_{mic}) assay was conducted by use of rehydratation method [20] Soil samples (2 replicates of 10 g) were oven dried at 65^{o}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. The 2 replicates of 10 g fresh soil samples were placed in refrigerator to serve as control. Salt–extractable organic C concentrations in the dried and fresh 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 is measured at 340 nm against of blank mixture of reagents without soil salt extract. The amount of carbon in the soil extracts was calculated according to [20]. Biomass C is recorded as µg C per g oven dry soil.

Basal soil respiration (C-CO_2 elimination rate) was determined by adopted method proposed by Isermeyer [21]. 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 and incubated at 21^{o}C in the dark for 14 d. CO_2 released during soil incubation was trapped in NaOH and determined by titrimetric analysis. 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 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-CO_2 : C_{mic}* and was expressed in mg C-CO_2 g^{-1} C_{mic} h^{-1} [22]. *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 [23]. The value of total C_{org} is recorded as % of dry soil mass. *Soil organic matter (SOM*) content was calculated by multiplication of C_{org} values on 1.724 [23]. *Microbial quotient* was calculated as a ratio *C_{mic}: C_{org}* and its value is expressed in % microbial C inside organic C [10]. Analysis of variances (ANOVA) (StatSoft STATISTICA 7.0) and paired t-tests (Microsoft Office 2010, Excel) were applied to evaluate differences between soil treatments.

Results and discussion

Total organic carbon (C_{org}) of calcareous chernozem soil from South-East of Moldova under long-term agricultural use was ranged, between 1.40%-1.55% and 1.51%-1.67%, respectively, for moldboard and moldboardless tillaged soils (table 1), what adequately corresponds to soil organic matter (SOM or humus) content 2.41%-2.67% and 2.61-2.88%. These data confirm that the loss of SOM in the majority treatments of investigated soil continues with time in comparison to initial 3.68% in 1989 and 2.80-2.88% in 1993.

According to three-way ANOVA analysis, two of three investigated factors - Tillage and Agrichemicals - significantly ($P < 0.05$) influence the level of C_{org} and SOM – the principal component of soil fertility (table 2). The impact of fertilization systems (green manure+NPK versus cattle manure) was insignificant, although the only combination of moldboardless tillage and cattle manure amendment maintains the values of SOM and C_{org}, respectively, 2.88% and 1.67%, similar observed in 1993 ones.

Table 1

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

Parameter		Microbial biomass carbon, C_{mic}	Microbial quotient, $C_{mic} : C_{org}$	Basal soil respiration, $C\text{-}CO_2$ rate	Metabolic quotient, qCO_2	Total organic carbon, [a]C_{org}
Units of measurement		μg C g^{-1} dry soil	%	μg $C\text{-}CO_2$ g^{-1} soil h^{-1} at 21°C	mg $C\text{-}CO_2$ g^{-1} C_{mic} h^{-1}	%
Treatments		**Moldboard (conventional) tillage (plow)**				
Green manure +NPK	control	[b]253 ± 15	1.68 ± 0.12	2.08 ± 0.58	8.4 ± 1.1	1.51 ± 0.04
	herbicide	252 ± 18	1.73 ± 0.17	2.55 ± 1.10	10.1 ± 2.8	1.46 ± 0.04
Cattle manure	control	266 ± 20	1.72 ± 0.08	2.41 ± 0.66	9.2 ± 3.1	**1.55** ± 0.06
	herbicide	239 ± 22	1.72 ± 0.23	2.26 ± 0.46	9.6 ± 2.6	1.40 ± 0.12
		Moldboardless (subsurface) tillage (paraplow)				
Green manure +NPK	control	274 ± 5	1.76 ± 0.11	1.86 ± 0.48	6.8 ± 1.8	1.55 ± 0.08
	herbicide	294 ± 36	1.96 ± 0.37	1.83 ± 0.19	6.3 ± 1.1	1.51 ± 0.13
Cattle manure	control	295 ± 16	1.76 ± 0.05	3.86 ± 0.53	13.2 ± 2.3	**1.67** ± 0.08
	herbicide	284 ± 28	1.88 ± 0.23	3.44 ± 0.51	12.2 ± 2.7	1.51 ± 0.04

[a]Mean values of soil organic matter (SOM), a former term "humus", of calcareous chernozem for ten years (1984-1993) constituted 2.80-2.88% or, correspondingly, C_{org} constituted 1.62-1.67% in 0-20 cm layer; [b]Mean ± S.D. (standard deviation, σ), n=3.

Microbial biomass carbon (C_{mic}) presents the important labile component of soil owing to high sensibility to any ecological stresses. According to our data neither fertilizers no herbicides use had no significant effect ($P > 0.05$) on the size of the microbial biomass in calcareous chernozem (table 1-2). But third factor – moldboardless tillage promotes the bigger amount of C_{mic}. Mean values of C_{mic} ranged between 239-266 μg C g^{-1} dry soil for calcareous chernozem subjected to moldboard tillage (plowing) versus 274-295 μg C g^{-1} dry soil - to moldboardless tillage (paraplow). It could be explained by the revealed bigger amounts of C_{org} in moldboardless tillaged soil. Thus, reduced tillage practices provide a more plant residues in soil top layer that results in increase C_{org} and other parameters related to soil quality.

Microbial quotient ($C_{mic} : C_{org}$) reflects the soil carbon available for growth and soils with better quality should have a higher microbial quotient [10]. According to results of this study (tables 1-2) all kinds of intensive agricultural soil management practices used in frames of investigated long-term experiment (for 42 years) did not affect the microbial quotient. The calcareous chernozem demonstrated the microbial quotient $C_{mic} : C_{org}$ ranged between 1.68% and 1.96%, with the tendency of a more efficiency at moldboardless tillage.

Basal soil respiration (BSR or $C\text{-}CO_2$ elimination rate) indicates the metabolic activity of soil microorganisms**.** According to three-way ANOVA analysis data (table 2) only the fertilization systems revealed significant influence upon microbial $C\text{-}CO_2$ elimination rate. But despite of insignificant affect of tillage practices, ANOVA analysis demonstrate the significant ($P<0.001$) interaction between Tillage*Fertilization factors. The most evident differences were observed for microbial communities from soils with moldboardless tillage (paraplow). Mean values of $C\text{-}CO_2$ rate ranged between 1.83-1.86 μg $C\text{-}CO_2$ g^{-1} soil h^{-1} at 21°C for calcareous chernozem fertilized by green manure+NPK versus 3.44-3.86 $C\text{-}CO_2$ g^{-1} soil h^{-1} at 21°C – by cattle manure (table 1). The moldboard tillage (plowing) levels the differences in soil fertilization systems and results in more similar microbial activity ranged between 2.08-2.55 µg $C\text{-}CO_2$ g^{-1} soil $h^{-}1$ and 2.26-2.41 µg $C\text{-}CO_2$ g^{-1} soil h^{-1} , respectively, for green manure+NPK versus cattle manure. Thus, the soil respiration or microbial activity was significantly higher in soil under moldboardless tillage and fertilized by cattle manure. Contrary expectations, long-term use (at least for last 22 years) of a variety of herbicides in the cultivation of corn for grains in the crop rotation did not affect the activity of soil microbial communities.

Table 2

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

Dependent variables	**Independent variables**	**F**[a]	**P-value**[b]
Microbial biomass carbon (C_{mic})	Tillage	14.53	0.0015**
	Fertilization	0.11	0.74
	Agrichemicals	0.28	0.60
Total organic carbon (C_{org}) (0-20 cm)	Tillage	6.62	0.020*
	Fertilization	0.59	0.46
	Agrichemicals	9.15	0.008**
Microbial quotient ($C_{mic} : C_{org}$)	Tillage	2.64	0.12
	Fertilization	0.03	0.86
	Agrichemicals	1.37	0.26
Basal soil respiration ($C\text{-}CO_2$ *rate*)	Tillage	3.52	0.07
	Fertilization	16.97	0.0008***
	Agrichemicals	0.04	0.85
	Tillage*Fertilization	16.79	0.0008***
Metabolic quotient ($qCO2 = C\text{-}CO_2 : C_{mic}$)	Tillage	0.12	0.73
	Fertilization	11,15	0.004**
	Agrichemicals	0.03	0.87
	Tillage*Fertilization	10.03	0.006**

a F-test or Fisher's criteria;
b confidence level. *** = P<0.001; ** = 0.001<P<0.01; * = 0.01<P<0.05

Metabolic quotient (***qCO_2*** or the quantity $C\text{-}CO_2$ produced per unit of microbial biomass C per unit of time) indicates the efficiency by which soil microorganisms use C-resources in the soil, including the microbial requirements of maintenance energy [10]. It is expected that stressed soils will provide higher qCO_2 values than less-stressed soils [5]. In similar way to BSR parameter, metabolic quotient according to three-way ANOVA (table 2) was significantly influenced only by the fertilization systems ($P < 0.01$), and demonstrated the dependence on significant ($P<0.01$) interaction between Tillage*Fertilization factors. For soils with moldboardless tillage (paraplow) the mean values of $C\text{-}CO_2$ rate ranged between 6.3-6.8 µg $C\text{-}CO_2$ g^{-1} soil h^{-1} at 21°C for chernozem fertilized by green manure+NPK versus 12.2-13.2 $C\text{-}CO_2$ g^{-1} soil h^{-1} at 21°C - fertilized by cattle manure (table 1). The soils under moldboard tillage (plowing) revealed less differences in qCO_2 parameters caused by fertilization systems and results in more similar values of microbial respiration per units of microbial biomass ranged between 8.4-10.1 µg $C\text{-}CO_2$ g^{-1} soil $h^{-}1$ and 9.2-9.6 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}, respectively, for green manure+NPK versus cattle manure. Thus, the soil specific respiration qCO_2 was also significantly higher in soil under moldboardless tillage (paraplow) and fertilized by cattle manure. Long-term use of different herbicides in the cultivation of corn for grains in crop rotation did not affect the efficiency of C-resources use by soil microorganisms.

Lastly, of three investigated factors the soil tillage practice revealed the strongest effect on microbiological parameters of calcareous chernozem of South-East Moldova. The moldboardless tillage (paraplow) results in higher amount of C_{mic}, that is, microbial biomass growth. The more favorable aftereffect of used green manure +NPK fertilizers also was observed for calcareous chernozem under moldboardless tillage. The same, the basal soil respiration ($C\text{-}CO_2$ rate) and metabolic quotient (qCO_2) parameters showed the lowest values. The multiannual herbicide use under field concentrations did not influence the microbiological parameters. Still, the calcareous chernozem after long-term (42 years) agricultural use with crop rotation, protective moldboardless tillage, and soil amendment by organic and mineral fertilizers, herbicides is steadily losing the soil organic matter.

As to biological quality estimation of soil, according to criteria proposed by Anderson [10], 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 $C\text{-}CO_2$ 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 [10]. Unfortunately, so far the correction of these criteria for soils with alkaline pH values is absent.

The evaluation of our results on calcareous chernozem soil from South-East Moldova under long-term agricultural use has shown the C_{mic}: C_{org} ratio in the range between 1.68% and 1.96% that is close to optimal minimum 2.0%. But the mean qCO_2 values in the range between 8.4 and 13.2 mg $C\text{-}CO_2$ g^{-1} C_{mic} h^{-1} were 4-6 times above optimal maximum 2.0. It can be the reasonable explanation of the loss of C_{org} from calcareous chernozem after long-term agricultural use. The highest values of BSR and qCO_2 parameters were found for chernozem under protective tillage practice (paraplow) and fertilized with the cattle manure. But it may be the temporary impact of the manure on the rate of soil CO_2 emissions, due to use by soil microorganisms and growing crops of organic substances which simultaneously contain C and N elements. Some another researchers [24] also have found that the soil CO_2 emission rate at peak times in the organic fertilization system was higher than under the conventional 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 organic and conventional systems [24].

Conclusions

The calcareous chernozem of South-East Moldova after long-term (42 years) agricultural use with crop rotation, protective moldboardless tillage practice, and soil amendment by organic and mineral fertilizers, herbicides is steadily losing the soil organic matter. While the size of the microbial biomass is close to optimal, but it shows an enhanced mineralization activity of the microbial decomposer compartment in relation to soil organic matter, judging by the increased CO_2 evolution. The soil tillage practice revealed the strongest effect on microbiological parameters of calcareous chernozem. The moldboardless tillage results in the higher amount of C_{mic}, the more favorable aftereffect of green manure + NPK fertilizers with the lowest values of the basal soil respiration ($C\text{-}CO_2$ rate) and metabolic quotient (qCO_2) parameters. The multiannual herbicide use under field concentrations did not influence the microbiological parameters.

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