BIOLOGICAL INDICES OF SOIL ORGANIC MATTER IN LONG TERM FERTILIZATION EXPERIMENT

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ABSTRACT

Dynamics of labile fractions of soil organic matter under 36-years of application of mineral and organic fertilizer in Chernozem (*Mollisol*) in Ukraine were studied. "Light" fractions of SOM between various mineral and organic fertilization treatments were studied. Amount of total organic C increased in manured treatments; while amount of total N didn't change under any of the treatments. Labile N was correlated to soil mineral N, MBC and MBN, LFN and LFC, while labile C correlated to the total organic C, LFC and LFN. The pattern of mineralization and accumulation of SOM suggested that OC dynamics more related to long-term substrate addition, while N dynamics better reflects short-term substrate addition. Application of mineral fertilizer alone accelerated mineralization of SOM, especially of "light" fraction, while partial or complete replacement of inorganic by organic fertilizers has a significant impact on soil microbial community and soil capability to supply plants with nutrients for longer period.

Keywords: labile SOM, "light" fraction, microbial biomass, mineralization.

INTRODUCTION

Quantity and quality of SOM and N availability are key indices, which maintain soil quality and sustain ecosystem productivity. The transformations between different forms of N are most important links of N cycle (Moreira et al., 2011). Less than 25% of SOM that are readily decomposable or labile fractions (Gregorich et al., 1994) maintain N and C cycle in soil, providing nutrients to microorganisms and plants. In our research the fractions of SOM included mineralizable organic C and N (C_0 and N_0), mainly from "light" fraction OM and microbial biomass C and N. "Light" fraction organic matter (LFOM) is the main source of easily available nutrients for plants and microorganisms. This fraction consists mainly of plant residues, small animals and microorganisms adhering to plant-derived particulate matter at various stages of decomposition that serves as a readily decomposable substrate for soil microorganisms and also as a short-term reservoir of plant nutrients. Operationally defined fractions such as C and N mineralized under controlled conditions and “light” fraction organic matter proved to be good indicators of labile SOM because it affects nutrient dynamics within single growing season and organic matter content under contrasting soil managements (Karbozova-Saljnikov et al., 2004).

Because total amounts of C and N usually not always sensitive to different land use system (Gregorich et al., 1994; Karbozova-Saljnikov et al., 2004), in order to establish sustainable soil management system under different mineral and organic fertilization system the biologically active fractions of SOM were studied: mineralizable organic C and N, microbial biomass C and N as well as “light” fraction organic matter as a main source of easily available nutrients for plants and microorganisms. Soil microbial biomass is an early indicator of changes in soil quality that result from different land management practices and is closely linked to soil organic matter decomposition (Sugihara et al., 2010). Various N and C pools were examined to get deeper insight to the processes of N mineralization/immobilization under the long-term application of different rates of mineral and organic fertilizers. Because manure plays important role in sustaining sufficient amount of soil humus while effect of mineral fertilizer on SOM varies depending on land use, soil type and climatic conditions (Chang et al., 2007) the main objective of the study was revealing an agronomic impact of fertilization and manure application on soil organic matter characteristic, with particular emphasis on the labile fractions of SOM in Chernozems in Ukraine under ling-term (36 years) field experiment.

MATERIALS AND METHODS

Site description

The field works were carried out at long-term experimental site in Ukraine. The studied soil is *Argiudolls* in Soil Taxonomy (Soil Taxonomy, USDA 1999). Soil was collected from surface 0-20 cm from three field replicates, where each sample was composed of five sub-samples. The studied field experiment had been lasting since 1964 on a base of 10-year crop rotation system: clover, wheat, sugar beet, corn for grain, peas, wheat, corn for silage, sugar beet, barley+clover. The studied treatments were: 1) control (CON, no fertilization); 2) low-rate mineral N fertilization (M1: 45 kg ha^{-1} per year); 3) high rate mineral N fertilization (M3: 135 kg ha^{-1} per year); 4) manure application (O: 67,5 kg N ha^{-1} $year^{-1}$); 5) combination of low rates of mineral N fertilizer and manure (MO1: 22,5+22,5 kg ha^{-1} $year^{-1}$); and 6) combination of high rate of mineral N and high rate of manure (MO3: 67,5+67,5 kg ha^{-1} $year^{-1}$) (Table 1). Soil sampling was performed on the middle of 4^{th} cycle of the rotation on clover phase, which received manure about 19 months before. One ton of cattle manure contained approximately 5 kg of N (Kharin 1993), therefore, total N applied with fertilizer and manure in the treatments was presented as kg N per ha per year.

Analytical methods

Total content of C and N (TC and TN), soil mineral N (IMN), potentially mineralizable N (N_0) and C (C_0), microbial biomass N and C (MBC and MBN); "light" fraction organic matter (LFOM) dry weight, "light" fraction N and C (LFN and LFC) were studied.

Total C and N were determined by dry-combustion method using NC auto-analyzer (Sumika NC-800-13N). Organic C was measured by acidification of organic C (Nelson and Sommers 1996). Soil mineral nitrogen (IMN) was analyzed on colorimeter (Shimadzu Spektra MAX-190). Nitrogen and carbon mineralization potentials were determined via sequential incubation procedure in laboratory conditions under the controlled moisture and temperature environment for 2-, 4-, 6-, 8-, 10-weeks as described by Janzen (1992).
Potentially mineralizable nitrogen (N_0) and potentially mineralizable carbon (C_0) were obtained after fitting the data of mineralized N to the first order kinetic model (SPSS Inc. 2007): $N\mathrm{min}=N_0*(1-\exp(-k*t))$, where, *N*min is an experimental value of mineralized N (or C) at a given time (*t*) that was plotted to fit the equation, N_0 is a value potentially mineralizable N (or C) that was calculated after fitting the curve, *k* is the mineralization rate constant.

Microbial biomass carbon (MBC) and nitrogen (MBN) were obtained by the chloroform-fumigation-extraction method on field-moist samples (Jenkinson and Powlson 1976) and biomass N was determined by colorimetric method and bioimass C by dissolved organic analyzer (TOC-analyzer) (Nelson and Sommers 1996).

"Light" fraction organic matter (LFOM) was separated by density separation using reagent grade NaI solution adjusted to 1.8 g cm-3 (Spycher et al., 1983; Janzen et al., 1992). 10g of soil was suspended in 40 ml of NaI solution (sp.gr. = 1,7) and the soil dispersed for 30 seconds using a Virtis homogenizer. "After centrifugation, the floating material, i.e., the "light" fraction was transferred directly to a vacuum filtration unit. The LFOM was then washed (three aliquots of 10 ml 0.01M CaCl2 followed by three aliquots of distilled water), dried at 70oC for 15h and weighed.

Table 1. Fertilization and manure application design from 36-years field experiment, kg N/ha

Treatment/fertilization		Fertilizer, kg ha^{-1}	10-year crop rotation										
			Clover, sampled	Wheat	Sugar beet	Corn	Peas	Wheat	Corn	Wheat	Sugar beet	Barley + clover	Total N applied per rotation
Control	CON		0	0	0	0	0	0	0	0	0	0	0
Low mineral	M1 NPK†	$(NH_4)_2SO_4$	0	45	90	50	10	45	50	45	90	25	450 kg N ha^{-1}
High mineral	M3 NPK	$(NH_4)_2SO_4$	50	135	180	200	60	135	200	135	180	75	1350 kg N ha^{-1}
Organic	O	manure††	0	0	225	0	0	0	225	0	225	0	675 kg N ha^{-1}
Low mineral and low organic	MO1	$(NH_4)_2SO_4$	0	22.5	30	50	0	22.5	22.5	22.5	30	25	225+225 kg N ha^{-1}
		manure	0	0	75	0	0	0	75	0	75	0	
High mineral and high organic	MO3	$(NH_4)_2SO_4$	0	67.5	90	150	20	67.5	75	67.5	90	47.5	675+675 kg N ha^{-1}
		manure	0	0	225	0	0	0	225	0	225	0	

† NPK signifies nitrogen, phosphorous and potassium fertilizer

†† One ton of straw cattle manure contains 5 kg of N. In the table the amount of N applied with manure is given.

The residue was re-suspended and the procedure was repeated to ensure complete collection of the LF. The composite LF was finely ground and analyzed for N and C concentrations.

All variables were subject to one-way analyses of variance to determine the significance of treatment effects (SPSS 1998). Where significant treatment effects were observed ($P < 0.05$), the LSD analyses was performed to permit separation of means. Nitrogen and carbon mineralization potentials were obtained via fitting the data into the exponential-rise model using the SigmaPlot software (SPSS 2007).

RESULTS AND DISCUSSION

The data on organic carbon and mineral nitrogen and labile N (PMN) as well as microbial biomass C and N for the experiment were presented in detail in the paper Saljnikov et al. (2005). In this paper, we focus on the labile C (PMC), "light" organic matter fraction and crop yield.

Generally, the content of OC after 36 years significantly changed only under manure fertilization (treatment O). Content of total N was not statistically different in any of the treatments. Initial mineral nitrogen (IMN) was distributed not proportionally to the amount of applied N. The highest content of IMN was obtained in O and MO3 treatments, followed by M3, MO1, M1 and CON treatments. The treatments O and MO3 showed greater N mineralization potentials. Despite the highest rates of N applied in the M3, the amount of mineralized N in M3 was significantly lower than in MO3 and O treatments. The mineralization pattern of carbon was identical and highly correlated to the nitrogen mineralization pattern (r= 0.89**). The lowest C_0 was observed in control treatment and the highest in MO3. The amount of C_0 in M3 and MO3 treatments, where equally high doses of N were applied was significantly different as indicated by different letters in Table 2.

Table 2. Labile pools of SOM and their indices in fertilizer and manure amended Chernozem soils

Treatments	IMN†	N_0	*kn*	C_0	*kc*	C_0/ N_0
	mg kg^{-1} soil	mg kg^{-1} soil		mg kg^{-1} soil		
Con	8.91a††	75.61a	0.0187	818.70a	0.0192	10.83
M1	6.89a	84.64a	0.0154	934.07b	0.0170	11.04
M3	16.25b	78.50a	0.0128	1098.44b	0.0132	13.99
O	17.86b	96.44b	0.0160	1060.37b	0.0198	10.99
MO1	15.47b	88.17a	0.0063	878.86b	0.0149	9.97
MO3	22.49c	151.92c	0.0062	1433.80c	0.0118	9.44

† IMN – initial mineral N; *kn* – N mineralization rate constant; *kc* - C mineralization rate constant; N_0 and C_0 is an amount of potentially mineralizable N and C

†† different letters denote significant difference at $P < 0.02$

The amount of C_0 in M3 (135 kg N from fertilizer) and O (67,5 kg N from manure) were nearly similar, in spite that in M3 the amount of applied N was 2 times higher. Mineralization rate constant (*k*) varied significantly (P < 0.05) among the treatments, and were similarly distributed for N and C. The highest mineralization rate was observed in control and manure (O) treatments, followed by M1 and M3 treatments. The lowest *k* value was observed in MO3 treatment (for N and C 0.0062 and 0.0118, respectively).

"Light" fraction organic matter (LFOM) was highly responsive to the imposed management practices, accounting for 4.21, 4.04 and 4.15 mg g^{-1} soil for the control, M1 and M3 treatments, respectively, and 6.09, 5.71, 6.25 mg g^{-1} soil for the O, MO1 and MO3 treatments, respectively (Table 3). Nitrogen and carbon of "light" fraction OM (LFN and LFC) were highly and only correlated with labile nitrogen and carbon.

Table 3. Soil N pools and their share in fertilizer and manure amended Chernozem soils

Treatments	MBN†	MBC	MBC/MBN	LFOM	LFN	LFC	Contribution to total soil N, %	
	mg kg^{-1} soil			mg g^{-1} soil	mg g^{-1} LFOM		MBN	N_0
Con	33.30a††	458.51a	13.78	4.211a	n/d	n/d	1.88	4.28
M1	35.50a	586.17b	16.53	4.037a	13.515a	225.120a	1.99	4.76
M3	21.30a	531.18b	24.97	4.150a	14.559a	220.405a	1.12	4.12
O	85.30b	566.48b	6.64	6.092b	13.589a	218.757a	4.47	5.05
MO1	71.00b	585.36b	8.19	5.707b	10.523a	174.157a	3.83	4.76
MO3	70.10b	676.90c	9.72	6.247b	12.915a	233.491a	3.76	8.15

† MBN-microbial biomass N; MBC-microbial biomass C; LFOM – "light" fraction organic matter on dry weight; LFN – "light" fraction N; LFC – "light" fraction C;
†† the different letters denote significant difference at P < 0.05

Endelman et al. (2010) reported, higher mineralization quotient observed in the treatments with higher doses of combined application of chemical fertilizer and manure suggests higher efficiency of utilization and conservation of organic matter under the specific nutrient management.

The quantity of "light" fraction organic matter (LFOM) influenced the amount of soil mineral N (0.74** at $P = 0.01$), content of total N and organic carbon, while the LFN and LFC significantly influenced only on the amount of labile N and C (Table 3). The significantly lower amount of LFOM in CON, M1 and M3 treatments compared with O, MO1 and MO3 confirms that straw based cattle manure consists of large amount of LFOM that is easily accessible to microbial attack. This confirms the hypothesis that manure can supply nutrient for longer period.

The lowest MBN in M3 treatment means that less N was immobilized into the microbial cells. The reduction observed in the microbial biomass N concentration for the M3 soil could be attributed to a greater rate of nitrification and less immobilization what is confirmed by high microbial C/N ratio in M3 treatment (Table 3) (Chang et al., 2007).

Also, the lowest MBN in M3 treatment, which received the high amount of N with fertilizer, might be a result of lysing of microorganisms in this treatment due to lack of organic substrate (Lee and Jose 2003).

The significantly (more than twice) higher MBN in the treatments receiving manure is directly correlated with the content of "light" fraction organic matter what is confirmed by high positive correlation coefficients (0.89** at $P = 0.01$) (Table 4).

Table 4. Average three years crop yields in mineral and organic fertilizer application treatments

Treatments†	Clover	Wheat	Sugar beet	Corn	Peas	Barley
	yield t ha^{-1}					
Control	6,66a††	4.01a	37.4a	4.88a	3.25a	3.48a
M3	7.81b	5.20b	51.3b	6.15b	3.79ab	4.20b
O	7.09ab	4.74b	48.0b	6.11b	3.33a	3.86a
MO1	7.73b	5.07b	54.2b	6.40b	4.21b	4.65b
MO3	7.70b	5.25b	51.7b	6.81b	3.98b	4.94b

†Data on M1 treatment is not available
†† different letter denote significance at P<0.05

The significantly higher amount of MBN in the manured treatments can be due to the direct uptake of organic N by soil microorganisms (Geisseler et al., 2009). A high microbial quotient generally implies presence of easily available C pool that sustains a large microbial community (Nilsson et al., 2005).

Table 4. Pearson correlation coefficients for soil organic matter parameters

	TN†	OC	MBN	MBC	IMN	LFOM	LFN	LFC	N_0
OC	.673**								
MBN	.540*	.369							
MBC	.267	-.060	.637**						
IMN	.607**	.283	.832**	.854**					
LFOM	.706**	.554*	.890**	.468	.739**				
LFN	.494	.492	.045	-.147	.032	.360			
LFC	.453	.028	.265	.315	.413	.326	.616*		
N_0	.632**	.421	.625**	.573*	.782**	.652**	.531*	.772**	
C_0	.568*	.477*	.322	-.022	.230	.518*	.788**	.591*	.890**

** Correlation is significant at the 0.01 level
 * Correlation is significant at the 0.05 level
† TN –total N; OC-organic C; MBN- microbial biomass N; MBC-microbial biomass C; IMN-initial mineral N; LFOM – "light" fraction organic matter on dry weight; LFN – "light" fraction N; LFC – "light" fraction C; N_0-potentially mineralizable N; C_0-potentially mineralizable C.

The C/N ratio of microbial biomass was significantly different between fertilizer and manure amendments. These big differences in the C/N ratio reflect different directions of mineralization/immobilization processes in soils provoked by application of different sources of N and plant/microorganism competition for N.

Higher MB C/N ratios in CON, M1 and M3 (C/N=14, 17 and 25, respectively), indicates that N availability is greatly limited in soils (Sugihara et al., 2010; Joergensen and Emmerling 2006). Similar to findings of Deng et al. (2000) nitrogen pools were closely associated with organic C. Mineral N and N_0 were closely associated with the LFOM as the main source of N and C and with MBC and MBN as the mediators of the mineralization and immobilization processes in soil.

It is well known that changes in microbial biomass in soil correspond to the changes in the availability of decomposable substrate (or labile C). So, the manure addition in this study provided the soil microbes with more labile C and N, increasing the microbial biomass suggesting that more N was immobilized into microbial cells, which is confirmed by lower C/N ratio of biomass in the O, MO1 and MO3 treatments compared with CON, M1 and M3 treatments (Table 4). This suggestion is also confirmed by stronger correlation of MBC and MBN with C_0 (0.867** and 0.838** for MBN and MBC), than with N_0 (0.634* and 0.525* for MBN and MBC), while mineral fertilizer had no positive effect on microbial biomass.

CONCLUSIONS

Application of mineral fertilizer alone accelerated mineralization of SOM, especially of "light" fraction, while partial or complete replacement of inorganic by organic fertilizers has a significant impact on soil microbial community and soil capability to supply plants with nutrients for longer period. Combined application of mineral and organic nitrogen supports both mineralization and immobilization processes, which sustains crop productivity as well as soil fertility. Application of manure increased amount of microbial biomass N for more than 50%. Continuous application of manure is important to maintain its residual effect, which supports both microorganisms and plants, while N from mineral fertilizer is important for better utilization of added SOM. The results can contribute in maintaining sustainable crop production and controlling SOM mineralization processes in Chernozems of Ukraine.

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