CORRELATIONS BETWEEN SOIL ORGANIC CARBON PROPERTIES AND SOIL MICROORGANISM INDICES

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Abstract

The connection between soil organic matter (SOM) and microorganisms has been investigated for a long time. On a global scale the microbial biomass (MB) in the soil is mainly influenced by the SOM content. Among other activities, microorganisms take part in the decomposition and formation of SOM. In the present research, soils from a long-term field trial involving organic and inorganic N fertilization (IOSDV, Keszthely, Hungary) were used to investigate the relationship between the amount and quality of SOM and the amount and activity of MB. The short-term (MB) and long-term (SOM) effects of fertilization on soils were also investigated. The quantity of microorganisms (microbial biomass carbon – MBC or C_{mic}) was determined using the chloroform-fumigation extraction method, while their activity was measured by means of fluorescein-diacetate (FDA) hydrolysis. The quality of SOM was measured using the E4/E6 method.

The determination of MBC and soil organic carbon (SOC or C_{org}) revealed that the C_{mic}/C_{org} ratio differed in the various treatments and was affected by organic amendments but not by the inorganic N fertilization dose. The C_{mic}/C_{org} ratio was the highest in treatments involving crop residue incorporation and the lowest in the unamended treatments. Similarly, the amount and activity of MB did not depend on the dose of inorganic N fertilization but was influenced by organic amendments. The results between the C_{mic}/C_{org} ratio and the amount and activity of microorganisms showed a correlation.

Keywords: soil organic carbon (SOC), E4/E6 method, microbial biomass carbon (MBC), fluorescein diacetate (FDA) hydrolysing activity, long-term fertilization experiment

Összefoglalás

A talajok szervesanyag-tartalma és a talajban élő mikroorganizmusok közötti kapcsolatok hosszú ideje kutatások tárgya. Globálisan a talajban élő mikrobák mennyiségét alapvetően meghatározza a szervesanyag-tartalom. A mikroorganizmusok, számos más aktivitásuk mellett, a szervesanyagok lebontásában és létrehozásában egyaránt részt vesznek. Kutatásainkban a Keszthely melletti IOSDV szerves és szervetlen N trágyázási tartamkísérlet talajait felhasználva vizsgáltuk a szervesanyag minősége, illetve mennyisége és a mikroorganizmusok mennyisége, illetve aktivitása közti összefüggéseket. A trágyázás talajra gyakorolt rövid- (mikrobiális biomassza) és hosszútávú (szervesanyag tartalom) hatásait szintén vizsgáltuk. A mikroorganizmusok mennyiségét szerves széntartalmuk alapján, kloroform fumigációs-extrakciós módszerrel, aktivitásukat fluoreszcein-diacetát (FDA) hidrolízis mérésével határoztuk meg. A szervesanyagok minőségét a humuszminőség vizsgálatára kidolgozott E4/E6 módszer felhasználásával jellemeztük.

A mikrobiális szén és a talaj összes szerves szén arányát kifejező C_{mic}/C_{org} hányadost vizsgálva megállapítottuk, hogy a különböző kezelésekben értéke nem állandó, változását nem a N-műtrágyázás adagja, hanem a szervesanyag kiegészítés befolyásolja. A C_{mic}/C_{org}

hányadost a növényi maradvány alászántásos kezelésekben volt a legmagasabb és a szerves kiegészítést nem kapott kezelésekben a legalacsonyabb. Hasonlóképpen a mikrobiális biomassza és aktivitása nem függött az N-műtrágyakezelés dózisától, de a szervesanyag kiegészítéstől igen. A C_{mic}/C_{org} arány korrelált a mikroorganizmusok mennyiségével és aktivitásával.

Introduction

Soil organic matter (SOM) sustains many key soil functions by providing energy, substrates and biological diversity to support biological activity, which affects soil aggregation, water infiltration and nutrient availability (Reeves, 1997). Recently, SOM has received growing attention from a new aspect. The global soil organic carbon (SOC or C_{org}) pool is approximately twice the size of the atmospheric pool (Lal, 2004); hence, a small change in the C loss from soils exerts a significant influence on the carbon dioxide concentration of the atmosphere (Smith et al., 2008). Soil C sequestration plays an important role in mitigating climate change (Lal, 2004; Smith et al., 2008); therefore, it is essential to understand the mechanisms underlying SOC turnover and dynamics (Tan et al., 2014).

Besides the amount of SOM, its quality is also important. Various physical and chemical methods exist for the determination of SOM or humus quality. Physical methods include ultraviolet visible (UV VIS) spectroscopy, Fourier transformed infrared (FT IR) spectroscopy, “steady-state” fluorescence spectroscopy and ^{13}C nuclear magnetic resonance (NMR) (Enev et al., 2014, Filep et al. 2016). Chemical methods are usually based on the use of various extractants, such as dilute NaOH solution or hot water. The E4/E6 method which determines the ratio of optical densities of dilute, aqueous humic and fulvic acid solutions measured at

465 and 665 nm, is frequently used to characterize humus quality. A lower E4/E6 ratio means a larger proportion of higher molecular weight humic substances (HS) containing more condensed aromatic rings (Kononova, 1966). Recently, Nadi (2012) published E4/E6 ratios for different soils in Hungary. This method was also used to characterize humus quality in the present experiment. From the theoretical point of view, more soluble, less complex HS can be more readily utilized for microorganisms as an energy source than less soluble, more complex HS. Therefore, increasing the proportion of less complex HS (higher E4/E6 ratio) can be expected to increase the microbial biomass and activity.

Although soil organisms comprise <1% of the total mass of a soil, they have a vital role in many soil processes: mineralization, recycling nutrients, humus formation, improving soil structure, promoting plant growth, controlling pests and diseases, etc. The amount of soil microorganisms (bacteria plus fungi) is usually measured by some form of chloroform fumigation method, expressed in carbon units and referred to as microbial biomass carbon (C_{mic}). In forest and grassland soils, C_{mic} is estimated to be <5.0% of C_{org}, but in agricultural soils it is usually less than 2.5% (Kallenbach and Grandy, 2011). Despite its small size, the soil MB is known to play a fundamental role in soil organic matter dynamics. Further, MB has a short turnover time and is highly sensitive to soil environmental conditions and disturbances, which makes it a useful index for diagnosing early changes in soil C (Sparling, 1992).

SOM and MB are in complex relation. SOM is mainly plant-derived and microbially processed. There is wide agreement that the amount of total soil organic matter determines the amount of microbial biomass in the soil and that there is a linear correlation between them on a local or global scale (Paul, 2007, Fierer et al., 2009; Kallenbach and Grandy, 2011; Ussiri and Lal, 2013). The data used in these meta-analyses and reviews were collected from experiments in different locations, climate zones and soil types. MB is tightly coupled both to the quantity and quality of C inputs and to SOM concentrations (Kallenbach and Grandy, 2011). Microbial responses to organic C inputs also depend on other biotic or abiotic factors, such as soil texture, crop rotation, management and changes in soil pH, etc., so the simplified notion, hat more organic matter input into soil means greater C_{org} content is misleading. In certain circumstances the input of organic matter increases the decomposition of SOM, which is known as the priming effect (Kuzyakov et al., 2000; Kuzyakov, 2010). The ratio of decomposition and humification depends on the C:N or C:N:P ratios of microbial biomass, SOM and organic matter input (Cleveland and Liptzin, 2007).

In soils, only a small part of the microbial biomass is active, usually about 2–3% or even less (Blagodatsky et al, 2000; Blagodatskaya and Kuzyakov, 2013). The rate of ecologically important processes, such as organic matter decomposition or the transformations of nutrients depends to a large extent on the active fraction of the microbial biomass (Blagodatsky et al., 2000). The activity of soil microorganisms can be determined by measuring soil respiration (carbon dioxide emission) or the activity of any enzyme. The measurement of FDA hydrolysing enzyme activity was suggested by Schnürer and Rosswall (1982) for determining total hydrolytic activity of the soil. This is now one of the most widely accepted methods both in Hungary and internationally (Adam and Duncan, 2001; Villányi et al., 2006; Bíró et al., 2014).

The direct quantification of changes in SOM content is greatly hindered by the high degree of spatial heterogeneity in soils and the relative imprecision of methods for quantifying soil carbon stocks (Paterson et al., 2009). Long-term field trials allow investigations on the effects of fertilizers and different management systems on C_{org}, C_{mic} and their ratio. Many long-term fertilization trials were primarily set up to study the impact of fertilizers on crop production, but researchers are taking advantage of these well-documented experiments to study soil microbial communities. In a meta-analysis, based on the data of 107 datasets from 64 long-term trials, Geisseler and Scow (2014) found that the long-term fertilization of agricultural soils led to increased C_{mic} content, which is to be caused by the associated increases in C_{org} due to the higher crop productivity. Across all the datasets, the C_{mic}/C_{org} ratio was not significantly affected by fertilization. Increased C_{mic} content was observed in fertilized soils compared with that observed in unmanaged ecosystems, where C_{mic} often decreased as a result of N input (Geisseler and Scow, 2014). The C_{mic}/C_{org} ratio has been thoroughly investigated, as it has relevance in many models describing carbon flows in soil. From the eco-physiological point of view, the C_{mic}/C_{org} ratio reflects the carbon available for microbial growth (Anderson, 2003).

In the present research, the amount and activity of soil MB and the amount and quality of SOM were investigated in the IOSDV long-term fertilization experiment (near Keszthely, Hungary) set up to study the effects of organic and inorganic N fertilization. Since IOSDV was set up in 1983 different SOM levels have developed in the different plots, making it possible to study soils of a single type (Eutric Cambisol) with different SOM concentrations, while the climate, cultivation method, the plant species and plant protection methods were the same. The short- and long-term effects of mineral fertilization and organic amendments on the soils were also investigated.

To reveal detailed relationships between SOM and MB, measurements were made on the amount (C_{org}) and quality (E4/E6) of SOM and on the amount (C_{mic}) and FDA hydrolysing activity of MB, after which the C_{mic}/C_{org} ratio was calculated. Answers were sought to the following questions:

1. Do mineral fertilization and organic amendments have any significant effect on the measured (C_{org}, C_{mic}, FDA activity, E4/E6) and calculated (C_{mic}/C_{org}) soil parameters?
2. Is there a close significant correlation between C_{mic} and C_{org}?
3. Which factors effect on C_{mic}/C_{org} ratio if this ratio has changed?
4. Does a higher ratio of more soluble, less complex humic substances (HS) in the soil increase the amount and activity of microorganisms?

Materials and methods

The International Long-term Fertilization Experiment (IOSDV) is located in Keszthely, Hungary (46° 45' N, 17° 14 E) at 115 m above sea level. This area has moderate rainfall and mild temperature conditions. The annual precipitation is 683 mm and the mean temperature over the 1901-2000 100 year long period is 10.51 °C when averaged. The soil type at the experiment location is Ramann's brown forest soil (Eutric Cambisol – WRB, 2014) with sandy loam texture. At the start of the experiment it was poor in organic carbon (~1.0% C_{org}), with a medium amount of available K (AL-K_2O ~ 135 mg/kg), available P-content was low (AL-P_2O_5 ~ 20 mg/kg). The pH_{KCl} varied between pH 6.8-7.0 (Kismányoky and Balázs, 1996).

The IOSDV is a bifactorial field experiment set up in 1983 with a cereal crop rotation in a strip block design. The crop rotation consists of maize, winter wheat and winter barley, sown in three replications with a plot size of 48 m^2. The factors in the experiment are increasing rates of N fertilizer and three kinds of organic amendments. I: inorganic fertilizer only (control); F: I+35 t ha^{-1} farmyard manure every 3rd year before sowing maize; S: I+stalk, straw and green manure (GM) incorporation. Green manure is applied as oilseed radish (*Raphanus* *sativus* var. Oleiformis) grown only once in the rotation after barley as a second crop before maize. In case of straw incorporation 10 kg N was added per 1 t of straw (Kismányoky and Balázs, 1996).

As basic fertilization every plot (including the N control) was given 100 kg ha^{-1} P_2O_5 and K_2O mineral fertilizer, while N was applied at five rates (N1, N2, N3, N4, N5), with 0-70-140-210-280 kg ha^{-1} for maize, and 0-50-100 (50+50)-150 (50+50-50)-200 (100+50+50) kg ha^{-1} for wheat.

Microbial biomass carbon (C_{mic}) contents were measured using the fumigation extraction method, according to Vance et al. (1987). The soil samples for MB (and for all other) measurements were taken from a depth of 0-20 cm at the following sampling times:

- 23rd May 2014, after maize germinated, when the plants were in phenophases 14-16 on the BBCH scale (Lancashire et al., 1991);
- 9th September 2014, before the maize harvest;
- 30th April 2015, when the wheat was in phenophase on the 37 on the Zadock (1974) scale;
- 27th October 2015, before the sowing of winter barley;
- 13th April 2016, when the barley was in phenophase 47 on the Zadock (1974) scale (head in the boot);
- 29th October 2016 after the barley harvest.

The determination of soil organic carbon content (C_{org}) was carried out according to the Hungarian standard (MSZ 08-0452:1980) in 2013. As C_{org} changes very slowly and no further measurements were made, these C_{org} values were used in all the comparisons. The C_{mic}/C_{org} ratio was calculated by dividing C_{mic} by C_{org}.

Measurements of FDA hydrolysing activity were carried out according to Alef and Nannipieri (1998) in autumn 2015 and spring and autumn 2016 by incubating 0.5 g wet soil was incubated with 20 ml buffered FDA solution for 3 h at 37 °C, under continuous shaking. The enzymatic reaction was stopped by the addition of 20 ml acetone, and the concentration of fluorescein was determined as absorbance at 490 nm (Hitachi U-1100 spectrophotometer). Enzyme activities were expressed as the amount of product released per hour and per gram of dried soil.

In the E4/E6 method for measuring humus quality, 1.25 g soil was dissolved in 50 ml 0.5% NaOH solution and mixed thoroughly. Following decantation, absorbance was measured at 465 and 665 nm (Hitachi U-1100 spectrophotometer). The ratio of the two absorbance values is the E4/E6 ratio. The measurements of humus quality were carried out in autumn 2014, spring 2015 and autumn 2016.

After sampling, the soil samples were stored in open nylon packs in a refrigerator (~4 °C) for at most 6 weeks prior to analysis.

The statistical evaluation of the experimental data involving univariate variance analysis, Duncan test and correlation analysis was done with SPSS Student Version 15.0 and Microsoft Excel.

Results

270 soil samples were analysed during the three years of the experiments. The statistical properties of the measured C_{org}, C_{mic}, E4/E6 and FDA data are shown in Table 1 (range, average, median and standard deviation). Only the C_{mic} values were measured twice a year (spring, autumn).

Table 1. Statistical properties of the measured soil parameters. N: number of data

Soil parameter	Range	Lowest value	Highest value	Mean	Median	Standard deviation	N
C_{org} (%)	0.39	1.03	1.42	1.19	1.15	0.10	45
E4/E6	6.70	2.91	9.61	6.26	6.29	1.44	90
C_{mic} (mg C kg^{-1} soil)	730.3	15.4	754.7	217.7	182.8	121.6	270
FDA (µg fluorescein g^{-1} soil h^{-1})	60.3	0.5	60.8	32.9	34.1	12.9	135

Univariate ANOVA was used to analyse the effects of three different factors (N fertilizer dose, organic amendment, year) on the measured soil parameters (Table 2). Interactions between the factors are also shown in Table 2. The N fertilizer dose had a significant effect on both the C_{org} and the grain yield (data not shown). The type of organic amendment influenced all the measured soil parameters significantly, as well as the grain yield (data not shown). The year also has a significant effect on all soil parameters except FDA hydrolysing activity.

Table 2. Effects of three different factors on the measured soil parameters. The significance levels are the results of univariate ANOVA.

Factors	C_{mic}	FDA	C_{org}	E4/E6	C_{mic}/C_{org}
Dose of N fertilizer (D)	0.594	0.422	0.003	0.220	0.418
Organic amendment (A)	0.000	0.000	0.000	0.001	0.000
Year (Y)	0.000	0.005	-	0.000	0.000
D*A	0.387	0.985	0.155	0.781	0.382
D*Y	0.145	0.975	-	0.635	0.117
A*Y	0.000	0.025	-	0.000	0.000
D*A*Y	0.382	0.999	-	0.786	0.346

Tables 3 and 4 show the mean values recorded in the different N fertilizer and organic amendment treatments and the results of the Duncan tests.

Table 3. Effect of the N fertilizer dose on the measured parameters. Letters after the mean values denote the results of Duncan tests.

Dose of N-fertilizer	C_{mic} (mg C kg^{-1} soil)	FDA (µg fluorescein g^{-1} soil h^{-1})	C_{org} (%)	E4/E6	C_{mic}/C_{org} (%)
N0	222.6 a	29.6 a	1.16 a	6.40 a	1.94 a
N1	228.7 a	31.1 a	1.20 b	6.36 a	1.91 a
N2	210.6 a	33.2 a	1.21 b	6.44 a	1.75 a
N3	217.4 a	36.0 a	1.18 ab	6.23 a	1.84 a
N4	209.0 a	34.4 a	1.19 ab	5.88 a	1.77 a

Table 4. Effect of organic amendments on the measured parameters. Letters after the mean values denote the results of Duncan tests.

Type of organic amendment	C_{mic} (mg C kg^{-1} soil)	FDA (µg fluorescein g^{-1} soil h^{-1})	C_{org} (%)	E4/E6	C_{mic}/C_{org} (%)
None	164.9 a	25.1 a	1.13 a	6.52 a	1.47 a
Farmyard manure	220.3 b	35.5 b	1.29 b	6.44 a	1.71 b
Crop residues incorporation	267.7 c	37.9 b	1.14 a	5.81 b	2.34 c

Correlations between the soil parameters were investigated using Pearson correlation analysis. The correlation coefficients and significance levels between the measured and calculated soil parameters are shown in Table 5. The correlation coefficients were not significant for C_{org} but were usually significant for C_{mic} and the C_{mic}/C_{org} ratio.

The results of correlation analysis showed that an increase in the E4/E6 ratio was correlated with a decrease in both the amount (C_{mic}) and activity (FDA) of soil microbial biomass (Table 5). An increase in the E4/E6 ratio thus led to lower humus quality.

Table 5. Correlation coefficients and significance levels between the measured and calculated soil parameters (C_{mic}/C_{org}, C_{mic}, C_{org}, E4/E6 and FDA).

	C_{mic}/C_{org}	C_{mic}	C_{org}	E4/E6	FDA
C_{mic}/C_{org}	1	0.989**	-0.064	-0.348**	0.263**
C_{mic}		1	0.075	-0.343**	0.268**
C_{org}			1	0.058	0.056
E4/E6				1	0.082
FDA					1

**: correlation significant at the 0.01 level.

Discussion

The results indicated that C_{org} was significantly affected both by the dose of N fertilizer and by organic amendments. These findings are in accordance with the results of other authors (Rees et al., 2001; Geisseler and Scow, 2014). One surprising result of the statistical analyses was that C_{org} did not correlate with any of the investigated soil parameters (humus quality, C_{mic}, C_{mic}/C_{org} and FDA). SOM has a dominant role in almost all soil properties, including the amount and activity of MB, but the present results suggest that the relationship is not linear. The lack of a correlation between C_{org} and C_{mic} could be the combined effect of various factors, such as the narrow range of C_{org}, the quantity and quality of other organic inputs into soil and the year effect.

In the last 32 years of the IOSDV experiment, different organic matter concentrations developed in the variously treated plots, but the range was narrow, with values varying between 1.03 and 1.42% C_{org} (Table 1). The quality of the organic matter showed greater variation, ranging (in a single soil type) from 4.8 to 9.6. E4/E6 ratios recently published by Nadi (2012) ranged from 2.1 to 13.5 for different Hungarian soils. The N fertilizer had no direct effect on the humus quality, but crop residue incorporation increased it (lower E4/E6 value means better humus quality). Unlike crop residue incorporation, the addition of farmyard manure had no significant effect on humus quality compared to the control. This finding emphasizes the importance of crop residue management (Chen et al., 2014, Nicholson et al., 2014). The amount of incorporated crop residue was dependent on the N fertilizer dose, so it had an indirect effect on humus quality.

The total amount of microbial biomass is usually relatively small, 50-2000 mg C kg^{-1} soil The soil microbial parameters, C_{mic} and FDA values were in the range normally found in arable soils (Blagodatskaya and Kuzyakov, 2013). The FDA values (average 32.2 mg fluorescein kg^{-1} soil h^{-1}) were within the range of 21-56 mg fluorescein kg^{-1} soil h^{-1}, reported by Adam and Duncan (2001). The dose of N fertilizer had no significant effect on the microbial parameters. The type of organic amendments influenced the quantity and activity of microbial biomass, which exhibited the highest values in the case of crop residue incorporation. These results were in accordance with those reported by Kautz et al. (2004), based on similar treatments in an IOSDV trial located in Berlin-Dahlem, Germany. This could be due to the fact that crop residue incorporation was carried out every year, while organic manure was only added every third year (in autumn 2013, deep ploughing).

FDA hydrolysing activity and C_{mic} together reflect the ratio of active and dormant microbes in the soil. Only a tiny portion of the total microbial biomass maintains an active state in soil without an input of easily available substrates, while a large proportion of living cells are inactive, dormant (Prosser et al., 2007; Blagodatskaya and Kuzyakov, 2013). In the present experiment these two values correlated with each other, so the treatments did not change the active/dormant microorganism ratio.

The C_{mic}/C_{org} ratio of agricultural and forest soils at neutral pH is very similar, in the range of 2.0 to 4.4% C_{mic} to total C_{org}, depending on the nutrient status and soil management (Anderson, 2003). The values calculated in the present work (1.47-2.34%) were within this range. Anderson and Domsch (2010) analysed over 100 plots with a long-term management history (at least 15 years to approximate a quasi-equilibrium state) from 26 sites at different locations in Europe, and found that the mean C_{mic}/C_{org} for crop rotation soils was 2.9, a value somewhat higher than that found in the present experiment, where the type of organic amendment and the year had a significant effect on C_{mic}/C_{org} ratio. Both farmyard manure and crop residue incorporation increased the C_{mic}/C_{org} ratio compared to the unamended treatments. A high C_{mic}/C_{org} ratio is indicative of the accumulation of labile C in the soil, forming a favourable environment for microbial growth, whereas a low ratio is usually closely linked to organic matter of poor quality (Cheng et al., 2013). It was reported by Anderson and Domsch (2010) that the pH had a pronounced effect on the C_{mic}/C_{org} ratio, so differences in pH between the IOSDV plots might explain the differences in C_{mic}/C_{org} ratio, together with the organic matter input into the soil. In the present experiments the pH values were not measured. However, such measurements are planned in a future step, to provide a better elucidation of the results.

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