

Soil metabolic activity profiles of the organic and conventional land use at Martonvásár

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Abstract: These days, increasing attention has been paid to understand the relationship between different farming systems and soil microbiological processes supporting sustainable land use. Soil microbiota have been considered as a priority component in organic land managements and sustaining soil health in long term. This statement should be supported by appropriately selected indicators - physiological properties or more precisely the metabolic activity profile of the soil microbes. A recently developed method, MicroResp™ gives a promising characterization of the catabolic activity pattern of the soil microbial communities. The goal of this study was to compare the catabolic profile of soil microbial communities of organic and conventional land management in a long-term experiment at Martonvásár (Hungary) from two consecutive years by two samplings (autumn and spring). MicroResp™ with 22 different substrates were used to characterize the catabolic activity patterns of these soils. Multivariate statistical analysis revealed a significant difference between the catabolic activity profiles of the soil microbial communities of the two management systems. Considering the soil chemical parameters, the $AL-P_2O_5-K_2O$, NO_3^-N were responsible for the divergence of the two farming systems according to the principal component analysis (PCA). The biotic (crop) and abiotic (EC, humus content, pH) parameters could affect not only the rate of soil respiration but the catabolic activity profiles as well. Organic farming increased the catabolic activity of soil microbes.

Keywords: organic, conventional, farming system, MicroResp

Introduction

Nowadays organic farming has a great significance because fertile soil supply the crops with essential nutrients thereby contribute to an active and diverse soil microorganisms community. Soil microbes have crucial role in ecosystem functions and in the sustainability of soil resources (Allison and Martiny 2008). Evaluation of physicochemical soil properties and determination of the soluble nutrient content available for plants is complicated because these factors has a very complex interaction with each other. It is largely depends on the efficient utilization of agricultural sources (Garbisu et al. 2011; Mäder et al. 2002).

The type of agricultural land management can have a remarkable impact on the activity of soil microbial communities. The organic land managements with green manure application and crop rotation can have an important influence on the soil microbial communities (Ge et al. 2013).

The MicroResp™ method is one of the CLPP (community level physiological profiles)

techniques (Campbell et al. 2003). This is based on the microbial utilization of different carbon sources. This method is widely used to indicate the activity in soil microbial communities (Bárány et al. 2014). It is a suitable method which can illustrate the quality of the agricultural farming systems and the degree of the fertility of soil (Romaniuk et al. 2011). The advantage is the rapid microbial identification of the total activity of the soil microorganisms (Chapman et al. 2007). Moreover MicroResp™ is an appropriate tool to detect the differences between the management practices because the metabolic fingerprints could discriminate between the different land management methods (Campbell et al. 2003; Ge et al. 2013). By adding 23 different substrates (simple sugars, amino acids and carboxylic acids) to the soil samples it is possible to get a catabolic fingerprint of the soil microbial community because the individual species of soil bacteria could have distinct capabilities to respire to the substrates (Mucsi et al. 2017). In this paper MicroResp™ method was tested on soil samples from two

farming systems under different vegetation types where soils were treated with fertilizer at conventional land managements and green manure at organic plots.

The activity of the soil microbial community is mainly influenced by the composition of plant's root exudates in the rhizosphere. In general the soil microorganisms utilize of the photosynthetic assimilates from the host plant including sugars (Bais et al. 2006).

Gunina and Kuzyakov (2015) showed that the application of glucose resulted in higher raise of the soil microbial activity in organic and conventional farming system. Sugars account about 52% of the organic substances of the root exudates of pea. The main sugar of root exudates is glucose with 50%, while fructose and saccharose have a lower contribution of 23% for both. Exudation of sugars could increase microbial activity and biomass in soil which later raises the available nitrogen for crop. The carboxylic acids could decrease the pH in the rhizosphere, the amino acids could mobilize micronutrients (Strickland et al. 2015).

Our goal was to compare the soil catabolic activity in different seasons (autumn and spring) under both organic and conventional land management systems. Further, our aim was to find relationship between the soil physicochemical parameters and catabolic activity results. We presumed that the organic land management will increase the activity of the soil microorganisms and will alter the community structure, which might result in a difference of the catabolic profiles of the samples from the two management systems.

Material and methods

Study area and soil sampling

The area is characterized by temperate climate, the annual precipitation was 308.6 mm and 405.3 mm, the mean annual air temperature was 11 °C and 12 °C in 2011 and 2012, respectively (<http://www.metnet.hu>).

The soil samples were collected in November of 2011 and May of 2012 from the upper 20 cm of the organic and conventional plots of a 15-year-long experiment, in the Centre for Agricultural Research, Martonvásár (Hungary) with a calcic chernozem soil (FAO 1998) (Table 1) with a loam soil texture. The GPS coordinates were the following: 47° 18' 38" N, 18° 46' 45" E. The soil samples were air-dried, ground and sieved through a 2 mm mesh for physical and chemical analysis, the other part of the samples were stored at + 4 °C for microbiological analyses.

The main soil physical and chemical properties

The soil texture was characterised by pipetting method, determining three fractions, sand, silt and clay. The humus content (%) was calculated from soil organic C measured by wet digestion and back titration. The total salt content was calculated from the electric conductivity (EC 2.5) of soil:water (1:2.5) suspensions. The pH_{H_2O} and pH_{CaCl_2} (0.01N $CaCl_2$) values, the NH_4-N ($mg\ kg^{-1}$), NO_3-N ($mg\ kg^{-1}$), the total N (%), C% values were measured. The ammonium-lactate (AL)-soluble nutrient content (Ca (m/m %), K_2O ($mg\ kg^{-1}$), Na ($mg\ kg^{-1}$), P_2O_5 ($mg\ kg^{-1}$) were determined. The above values were measured according to the Hungarian soil standards (Buzás 1988; Buzás 1993).

Table 1. Crops and fertilization in organic (OF) and conventional (CF) farming systems at Martonvásár

Years	OF		CF	
	crop	fertilization	crop	fertilization
2011	peas	green manure (peas)	spring wheat	250 $kg\ ha^{-1}$ NPK (0:10:24%)
2012	cereal	no	corn	300 $kg\ ha^{-1}$ NPK (15:15:15 %) 270 $kg\ ha^{-1}$ N (39%)

Legend: Complex NPK fertilizer was used with 15-15-15% active agent. 15% N:10 % ammonium nitrogen + 5 % urea N; 15 % P_2O_5 :P content 6.2 %; 15 % K_2O :K content 12.5 %, N fertilizer (calcium ammonium nitrate fertilizer (CAN)) was used which contain 27 % N, 5 % Ca (7 % calcium oxide) and 3 % Mg (5 % magnesium oxide).

Catabolic activity pattern of soil microbial communities by MicroRespTM, statistical analyses

MicroRespTM technique was used to evaluate the catabolic activity pattern of the microbial community of soils. It is based on the colorimetric detection of CO₂ evolved from the soil after addition of substrate solutions (Campbell et al. 2003). 23 different substrates (simple sugars, amino acids and carboxylic acids) and ultrapure distilled water (control) in four replications were distributed to each plate. Four plates were used for OF and four for CF samples, within the plates each substrates were tested in 4-4 replicates. The following substrates were used: D-galactose (Gal), trehalose (Tre), L-arabinose (Ara), D-glucose (Glc) and D-fructose (Fru) in 80 mg ml⁻¹, citric acid (Cit), DL-malic acid (Mal), Na-succinate (Suc), L-alanine (Ala) and L-lysine (Lys) in 40 mg ml⁻¹, L-glutamin (Gln) in 20 mg ml⁻¹, L-arginine (Arg), 3,4-dihydroxybenzoic acid (Dhb) and L-glutamic acid (Glu) in 12 mg ml⁻¹. Myo-inositol (Ino), D-xylose (Xyl), D-mannitol (Mat), D-mannose (Man), D-sorbitol (Sor), L-rhamnose (Rha) 80 mg ml⁻¹, L-asparagin-monohydrate (Asn) 20 mg ml⁻¹, D-gluconic-acid-potassium (Gla), L-ascorbic acid (Asa) 40 mg ml⁻¹. The pH of the substrate solutions was adjusted to 6.5 by 1N NaOH or HCl solutions. The plates were read before and after 6 h of incubation at 25 °C in dark with a plate reader (Anthos 2010, Biochrom, Cambridge, UK) at 570 nm for colorimetric detection. Then respiration rates were calculated from the normalized % CO₂ data after 6 h incubation period (Szili-Kovács et al. 2011).

Significant differences in the soil chemical, physical properties (n=12) between the OF and CF types were tested by two-sample tests. Similarity Percentage (SIMPER) test with Bray Curtis dissimilarities was used for statistical analysis to identify which physical-chemical parameters and substrates had the largest contribution to the average dissimilarity between the two different land use systems. MicroRespTM method was evaluated with n=4 soil samples per sites with four replicates. Ascorbic acid substrate resulted a remarkably high respiration rate at all samples, therefore it was excluded from the further statistical analyses. Principal component analysis (PCA) was used to compare the main soil chemical parameters and also the catabolic activity profile data between the two type of land managements. PCA was calculated by using the correlation matrix with disregard groups, and Bootstrap N parameters of 4. These statistics were made by Past3 software package (Hammer et al. 2001).

Results*Main soil physical and chemical properties*

The soil texture was classified as loam according to particle size distributions of soil samples (Table 2). The area was not salty based on the electric conductivity (EC) of the saturation extract (< 2 mS cm⁻¹) value and slightly alkaline (Table 3). The most important first three parameters for the autumn and spring soil samples were AL-P₂O₅, AL-K₂O and NO₃⁻-N on the basis of SIMPER test. According to the ammonium-lactate soluble

Table 2. The main physical properties of soil from organic (OF) and conventional (CF) fields of Martonvásár

Soil physical properties	Martonvásár farming system			
	Autumn		Spring	
	OF	CF	OF	CF
Sand (%) (0.05 - 2 mm)	33.74 ± 3.36	31.99 ± 4.05	32.7 ± 3.2	30.05 ± 4.55
Silt (%) (0.002 - 0.05 mm)	42.47 ± 2.45	42.74 ± 2.39	42.44 ± 2.56	43.00 ± 2.68
Clay (%) (< 0.002 mm)	23.79 ± 1.35	25.29 ± 2.22	24.86 ± 1.26	26.95 ± 2.77

Legend: Data are means ± standard deviation of the means; n = 12.

Table 3. The main chemical properties of soil from organic (OF) and conventional (CF) fields of Martonvásár

Soil chemical data	Martonvásár farming system							
	Autumn			Spring			SL among the OF's (A+S)	SL among the CF's (A+S)
	OF	CF	SL among OF and CF (A)	OF	CF	SL among OF and CF (S)		
AL-Ca (m/m %)	1.52 ± 0.62	1.23 ± 0.96	n.s.	1.62 ± 0.52	1.16 ± 0.95	n.s.	n.s.	n.s.
AL-P ₂ O ₅ (mg kg ⁻¹)	646.54 ± 130.94	569.2 ± 56.03	n.s.	737.83 ± 261.96	654.83 ± 46.10	n.s.	n.s.	***
AL-K ₂ O (mg kg ⁻¹)	539.67 ± 73.77	474.69 ± 53.05	*	605.33 ± 89.25	526.50 ± 58.21	**	**	*
AL-Na (mg kg ⁻¹)	16.38 ± 2.32	20.36 ± 3.00	**	11.19 ± 0.97	11.06 ± 3.20	n.s.	***	***
EC (mS cm ⁻¹)	0.28 ± 0.03	0.24 ± 0.03	**	0.21 ± 0.03	0.27 ± 0.05	**	***	n.s.
pH _{H2O}	7.79 ± 0.10	7.85 ± 0.12	n.s.	7.92 ± 0.14	7.75 ± 0.16	**	***	**
pH _{CaCl2}	7.91 ± 0.07	7.85 ± 0.07	n.s.	7.85 ± 0.10	7.71 ± 0.12	**	n.s.	***
NH ₄ ⁺ -N (mg kg ⁻¹)	4.95 ± 1.18	4.04 ± 0.93	n.s.	4.94 ± 0.67	8.84 ± 4.64	**	***	**
NO ₃ ⁻ -N (mg kg ⁻¹)	37.12 ± 10.56	23.14 ± 8.83	**	5.24 ± 0.98	40.24 ± 17.86	***	***	**
Total N (%)	0.20 ± 0.01	0.17 ± 0.01	***	0.19 ± 0.00	0.17 ± 0.01	***	***	n.s.
Humus content (%)	2.90 ± 0.20	2.51 ± 0.18	***	2.98 ± 0.26	2.66 ± 0.24	**	n.s.	*

Legends: Data are means ± standard deviation of the means; n = 12. SL= significance level. Significant differences among the different land managements with two-sample tests, p* = < 0.05; p** = < 0.01; p*** = < 0.001; n.s. = not significant between OF and CF). EC = electric conductivity, Total N = total nitrogen, AL= ammonium-lactate (AL)-soluble nutrient content, A = autumn, S = spring.

nutrient content, the AL-Ca content of soils were not differ significantly between OF and CF in both seasons. AL-P₂O₅ content of soils were not significantly different only between spring and autumn CF data with significantly higher value of the former. The AL-K₂O content of the soil from OF management was significantly higher in both seasons compared to the CF. The AL-Na content of the soils in the spring samples were not differ significantly between in organic and conventional managed plots, while in autumn samples the AL-Na content of soils of OF were significantly lower than that of the CF. The soil

NH₄⁺-N content between OF and CF were not differ significantly in autumn. However in spring the NH₄⁺-N level of soil was significantly higher in CF. Soil NO₃⁻-N was significantly higher at OF than at CF in the autumn samples, while it was significantly higher at CF in spring. The soil total N as well as humus content were significantly higher in OF in both seasons.

Catabolic activity pattern of soil microbial communities

The three most active substrates were Mal, Cit and Glc (Figure 1) for autumn and Glc, Fru,

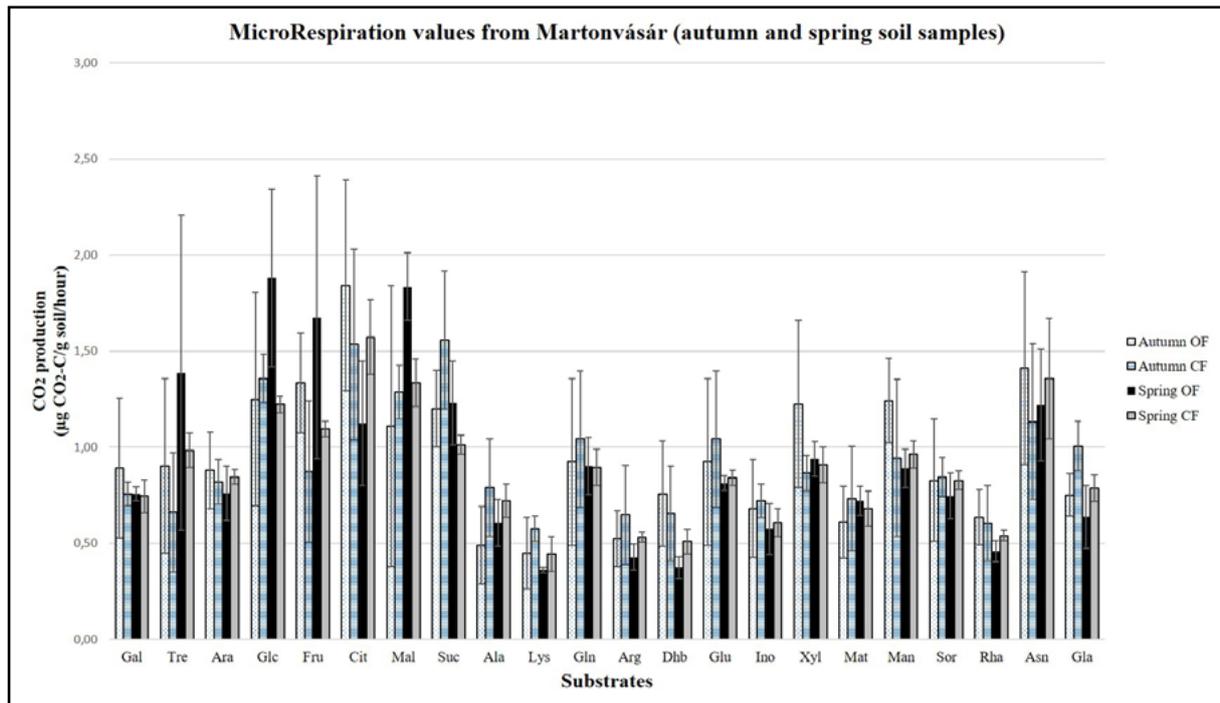


Figure 1. Mean catabolic response for 22 substrate sources in two land management systems, organic (OF) and conventional (CF) in autumn and spring by MicroResp™.

Legends: Error bars = standard deviation; Gal = D-galactose, Tre = trehalose, Ara = L-arabinose, Glc = D-glucose, Fru = D-fructose, Cit = citric acid, Mal = DL-malic acid, Suc = Na-succinate, Ala = L-alanine, Lys = L-lysine, Gln = L-glutamin, Arg = L-arginine, Dhb = 3,4 dihydroxybenzoic acid, Glu = L-glutamic acid, Ino = Myo-inositol, Xyl = D-xylose, Mat = D-mannitol, Man = D-mannose, Sor = D-sorbitol, Rha = L-rhamnose, Asn = L-asparaginmonohydrate, Gla = D-gluconic-acid-potassium.)

Mal for spring sampling originated from OF and CF. The malic acid and glucose were the most utilized substrates in both years and farming systems. The most differentiating substrates between OF and CF were glucose, fructose, malic acid, gluconic acid and citric acid ($p < 0.05$) according to the SIMPER test. The soil catabolic response to citrate substrate was significantly higher ($p < 0.05$) in CF than OF, while to malic acid, glucose and fructose it was significantly higher in OF than CF.

Principal component analysis (PCA) partially separated the soil samples to their own group according to OF and CF. Humus and total nitrogen correlate with each other (Figure 2). This PCA result was related to the Table 3, where the amounts of nitrate and ammonia were doubled in the conventional land management in spring while the total nitrogen and humus remained similar.

The PCA of the catabolic activity patterns resulted a clear separation of the OF samples

from CF in spring while they were not separated significantly in autumn (Figure 3).

Discussion

The influence of organic and conventional land managements on the main soil physical - chemical and microbial properties have been studied earlier (Monokrousos et al. 2006; Santoyo et al. 2017; Girvan et al. 2003; Clark et al. 1998). Nearly all of the soil chemical parameters studied varied significantly depending on the season and soil managements (Table 1). In our experiment, the soil AL-Ca content was the only one among the soil chemical parameters which resulted no significant difference between OF and CF. AL-soluble nutrient content and the available N of soils showed significant difference between OF and CF (Table 3). Marinari et al. (2006) also found significantly better soil nutritional conditions and enhanced soil microbial activity in OF managed soils in central Italy, after seven years of organic management. They used

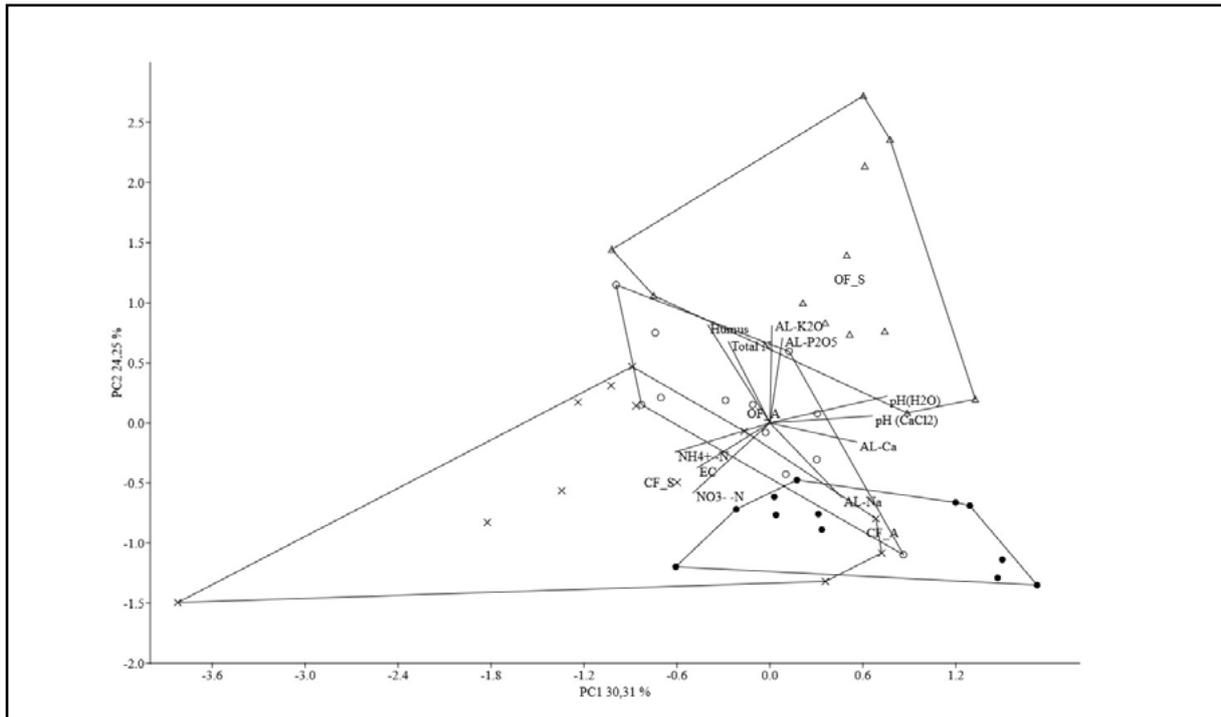


Figure 2. PCA analyses between the main soil chemical parameters according to OF and CF land managements from autumn and spring.
 Legends: OF = organic farming system, S = spring, A = autumn, AL = ammonium-lactate soluble nutrient content, EC = electric conductivity.

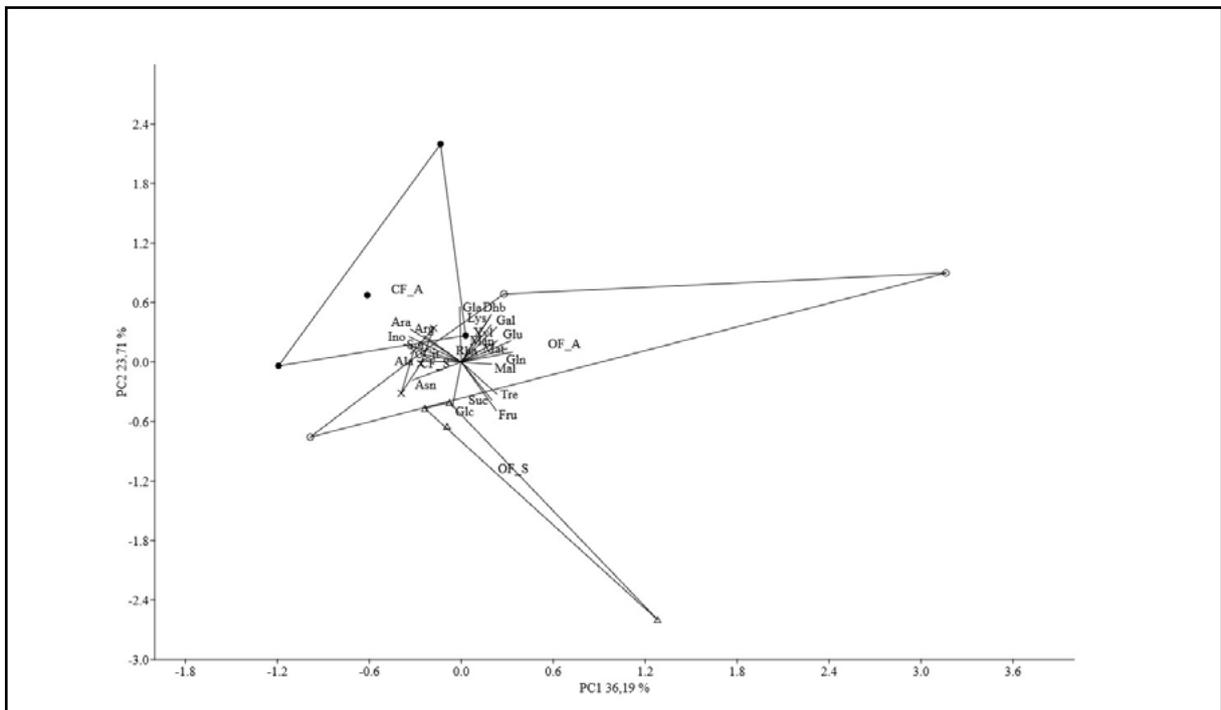


Figure 3. PCA analyses between the catabolic activity of OF and CF soils from both seasons.
 Legends: OF = organic farming system, S = spring, A = autumn, Gal = D-galactose, Tre = trehalose, Ara = L-arabinose, Glc = D-glucose, Fru = D-fructose, Cit = citric acid, Mal = DL-malic acid, Suc = Na-succinate, Ala = L-alanine, Lys = L-lysine, Gln = L-glutamin, Arg = L-arginine, Dhb = 3.4 dihydroxybenzoic acid, Glu = L-glutamic acid, Ino = Myo-inositol, Xyl = D-xylose, Mat = D-mannitol, Man = D-mannose, Sor = D-sorbitol, Rha = L-rhamnose, Asn = L-asparagin-monohydrate, Gla = D-gluconic-acid-potassium.)

composted poultry manure and green manure for OF and N, P fertilizer for CF. These values were mostly significantly higher in soils from the spring period. Indirectly, the season may affect the chemical parameters of the soil through fertilization, green manure and crop rotation (Meleora et al. 2006).

The EC, humus content, pH and the crops could be established affecting the rate of CO₂ evolution and the catabolic profiles. The metabolic respiration response is a useful method to classify the soil microbial communities. The results (Figure 1) showed that the catabolic activity of soil microbial communities was larger in OF land managements compared to CF. Gunapala and Scow (1998) got similar results in California, in loamy soil. They used mineral fertilizer for CF and green, -turkey manure for OF. Similarly to our results, Tautges et al. (2016) found that the substrate utilization was significantly higher in organic sites in wheat/pea crop rotations on OF and CF sites using EcoPlates™. Our results showed that OF and CF land managements also affect the soil substrate utilizing pattern. Glucose substrate resulted the most active catabolic activity in the spring at OF ($1.88 \pm 0.46 \mu\text{g CO}_2 - \text{C g soil}^{-1} \text{ hour}^{-1}$) however lysine was the least active substrate in the spring OF soil ($0.36 \pm 0.02 \mu\text{g CO}_2 - \text{C g soil}^{-1} \text{ hour}^{-1}$).

The MicroResp™ method is suitable for separating the soil microbes of the two different cultivated areas of Martonvásár according to their catabolic activity. PCA results (Figure 3) showed that the different farming systems were partially separated by season and cultivation. In autumn, the most responsive substrates were Mal, Cit, Glc, while in spring Glc, Fru, Mal acids and sugars. These substrates were the most responsive from the all examined substrates. Romaniuk et al. (2011) found also that, after a discriminant analysis of the catabolic response profile data, D-glucose was one of the most important substrates differentiating organic and conventional horticultural plots.

The results of our analyses proved that the organic cultivation in spring is more sustainable compared to the conventional land management. Higher soil microbial activity would be an appropriate marker of good soil quality which has got major effect on the nutrient cycling and the growth of vegetation as well (Ge et al. 2013; Creamer et al. 2016). The activity of substrate utilizing soil bacteria was influenced by the different farming systems. Our results showed that soil microbial communities have a large metabolic potential which can be easily activated by metabolisable substrates (Gunina and Kuzyakov 2015).

Conclusion

We have concluded that organic farming practice enhanced the catabolic activity of soil microorganisms. A divided pattern of catabolic activity profiles was observed by MicroResp™ according to the autumn and spring seasons and organic and conventional farming systems. The soil AL-P₂O₅, AL-K₂O and NO₃⁻-N were correlated to differences in catabolic activity profiles. Numerous environmental factors - EC, humus content, pH and the crops could also affect the generated amount of CO₂.

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