Effects of crop production practices on soil characteristics and metabolic diversity of microbial communities under winter wheat

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Abstract. The aim of this research was to compare the effects of organic (ORG, no mineral fertilisers and pesticides) and conventional (CON, with mineral fertilisation and plant protection chemicals) crop production systems on some soil properties: soil organic matter (SOM) and particulate organic matter (POM) contents, dehydrogenase activity and metabolic microbial diversity (Biolog EcoPlateTM). Samples of Haplic Luvisol soil were collected from 23-year-old experimental fields under winter wheat from the depth layers of 0-5, 5-10, 15-20 and 30-35 cm. Soil under ORG showed higher contents of SOM, POM and POM expressed as a percentage of SOM by 16.0%, 25.6% and 23.9% respectively, in particular down to 5 cm depth, compared with the CON system. Also, dehydrogenase activity was 47.5% higher under the ORG than the CON system. The Biolog EcoPlate revealed higher metabolic microbial diversity in soil under ORG crop production system compared with CON. This result was supported by the average well colour development and Shannon's diversity index values. Significant correlations between soil quality parameters and winter wheat yield confirmed their effectiveness as indicators of soil disturbance. The results showed that the ORG system much better maintained SOM and soil fertility compared with CON.

Additional keywords: Biolog EcoPlate, management systems, microbial metabolic diversity, soil properties, *Triticum aestivum* L.

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Introduction

More attention should be paid to the introducing into broad agricultural practice of management systems that meet the principles of protection of both the soil and the agroecosystem, which would allow today's agriculture to fulfil requirements related to protection of soil quality (Doran *et al.* 1996). Conventional production systems maximise yields through intensive soil fertilisation and the use of pesticides to protect plants. Such practices often lead to deterioration of biological and physicochemical soil properties. In recent years, there has been increasing interest in and development of organic practices. The organic production system is one alternative to conventional agriculture (Marinari *et al.* 2006; Kus and Jończyk 2008; de Ponti *et al.* 2012), and stimulates the microbial activity and diversity at or near the soil surface (Gajda *et al.* 2016).

The activities in soil of various enzymes, mostly of microbial origin, determine the decomposition of soil organic matter (SOM) and nutrient cycling (Marinari *et al.* 2006). The

SOM is a key factor affecting the soil directly by supplying nutrients or indirectly modifying its physical, chemical and microbiological properties and so improving soil environment quality for microorganisms and plant growth (Liebig and Doran 1999). Particulate organic matter (POM) is a type of SOM fraction, and has been demonstrated as a useful tool in the process of orientation of research on SOM (Sequeira *et al.* 2011). The POM is the SOM fraction of size in the range 0.053–2 mm (Cambardella and Elliott 1992) – it is regarded as the most labile component of SOM and is important for chemical and microbial transformation processes of carbon (C) in soil (Baldock and Skjemstad 2000). The POM is considered to be the intermediate state between fresh, readily-decomposable organic (usually plant) remains and the more decomposed and stable SOM (humus).

Healthy ecosystems are usually characterised by high species diversity, and the Biolog EcoPlate[™] method is often used to analyse functional diversity in soil microbial communities (Furtak *et al.* 2017; Li *et al.* 2017). EcoPlate

allows testing for several substrates and enables evaluations of metabolic potential diversity of microbial populations in different environmental samples. The substrates on the EcoPlate are naturally occurring in the soil environment and some are products of exudates of plant roots (Preston-Mafham *et al.* 2002). This method provides many results on metabolism of microorganisms that correlate with other soil quality indicators (Gałązka *et al.* 2017).

The influence of agricultural techniques on the diversity of soil microorganisms is well known (Liebig and Doran 1999; Wolińska *et al.* 2017). The objective of the present study was to evaluate the effects of conventional and organic crop production systems on chosen soil characteristics: chemical (SOM and POM contents) and microbiological (dehydrogenase activity (DHA) and metabolic microbial diversity using EcoPlate).

Material and methods

Field experiment and soil sampling

This study was conducted during 2015–2017 in a long-term field experiment established in 1994 (area of each field ~1 ha) and located predominantly on Haplic Luvisol soil at the Experimental Station of Institute of Soil Science and Plant Cultivation State Research Institute (IUNG – PIB) in Osiny (Lublin Voivodeship; 51°28' N, 22°30' E), Poland. Winter wheat (*Triticum aestivum* L.) cv. Jantarka was grown in two different crop production systems: organic (ORG) and conventional (CON). The ORG included a crop rotation of potato–spring barley–grass/clover mixture (1st year) and grass/ clover mixture–winter wheat (2nd year), with grass/clover compost (30 t ha⁻¹) applied under potato. No mineral fertilisers and plant protection chemicals were used. Weed control was mainly by mechanical treatments. The CON

system was based on mouldboard ploughing inverting soil to 20 cm depth and crop rotation of winter rape-winter wheat-spring barley, and mineral fertilisation and plant protection chemicals. Organic fertilisation was limited to winter rape and winter wheat straw spread on the field surface after the fall harvest and ploughed into the soil. In the CON system, crops were grown according to the highinput recommendations generally used in Poland. The tillage treatment for each field always remained the same. Crops in this experiment were grown in different crop production systems on non-replicated fields. More information about the field experiment can be found in Kus and Jończyk (2008). The weather conditions in the experimental fields are shown in Table 1. Each year, soil samples for analysis were collected throughout the growing season, at ear formation (55–56 BBCH) and at harvest. The representative soil samples (~1500 g each) were taken from the in-row planting area from the depths of arable layer (0-5, 5-10 and 15-20 cm) and sub-arable layer (30-35 cm) randomly across each trial and transferred to the laboratory in coolers. Plant roots were carefully removed from soil before the analysis procedure.

Then, soil samples were weighed and 15 g of subsamples were dried at 105°C for 24 h to determine soil water content. Next, soil samples were sieved through a 2-mm sieve and stored at 4°C until analysis. Some soil chemical characteristics are contained in Table 2.

SOM and POM contents

The SOM content was measured with the $K_2Cr_2O_7$ – H_2SO_4 wet oxidation Tiurin method. For POM analysis, representative air-dry soil samples were dispersed with 100 mL of sodium hexametaphosphate (5 g L⁻¹) and shaken for 18 h on a reciprocal

Table 1. Monthly mean air temperature and total precipitation in the growing season of winter wheat at Osiny Experimental Station (2015–2017)

Month	Temperature (°C)				Precipitation (mm)			
	1950-2013	2014/2015	2015/2016	2016/2017	1950-2013	2014/2015	2015/2016	2016/2017
Sept.	13.3	14.7	15.3	15.6	53.2	15.5	126.0	21.0
Oct.	8.4	10.0	7.0	7.7	39.3	19.0	30.0	100.0
Nov.	3.1	4.7	5.2	3.2	39.2	30.4	47.0	45.0
Dec.	-0.9	0.6	4.0	0.8	38.5	55.3	25.0	65.0
Jan.	-2.9	0.6	-3.4	-4.6	29.0	49.7	33.0	30.2
Feb.	-2.0	0.9	3.7	-1.2	28.4	8.1	65.0	44.1
Mar.	1.9	5.2	4.3	6.0	28.1	49.0	53.0	31.9
Apr.	8.1	8.2	9.6	7.6	42.0	29.4	38.0	65.1
May	13.8	13.6	15.5	13.6	55.0	108.7	72.0	61.7
Jun.	17.1	16.8	19.8	18.1	71.0	29.2	28.0	30.9
Jul.	18.6	19.8	20.0	18.6	78.2	52.1	87.0	108.5
Aug.	17.8	22.4	18.7	19.6	67.3	4.3	42.0	96.3

Table 2. Properties of the Haplic Luvisol soil (0-20 cm layer) in 2015-2017

Mean values with different letters are significantly different (Tukey's HSD test, P < 0.05, n=3). SOM, soil organic matter; P, phosphorus; K, potassium; Mg, magnesium; ORG, organic; CON, conventional

Crop production system	SOM (g kg ⁻¹ soil)	pH H ₂ O	ł KCl	P_{Egner}	$rac{K_{Egner}}{(mg kg^{-1} soil)}$	$Mg_{Schachtschabel}$
ORG	16.5 ^a	6.6 ^a	5.7 ^a	153.9 ^b	156.3 ^b	134.3 ^a
CON	14.3 ^b	6.7 ^a	6.0 ^a	204.1 ^a	230.1 ^a	79.5 ^b

shaker at ~180 oscillations min⁻¹. Next, the dispersed soil was passed through 500- and 53- μ m sieves. Particles retained on the 53- μ m sieve corresponded to the POM fraction. The quantities of the POM fraction were measured according to Cambardella and Elliott (1992), in a modified method, in which POM content was estimated with loss-on-ignition (LOI) procedure (Schulte and Hopkins 1996), as detailed by Gajda *et al.* (2001). For calculations of the percentage of POM in total SOM, the SOM content was assessed using the LOI procedure.

DHA

The DHA was evaluated spectrophotometrically by the reduction of triphenyl tetrazolium chloride to triphenyl formazan (TPF) (Polish Standard PN–EN ISO 23753–1, 2011). The DHA values were calculated based on oven-dry (105°C) weight of soil.

Metabolic diversity of microbial communities

The Biolog EcoPlate (Biolog Inc., Hayward, CA, USA) method was used to evaluate diversity in metabolic activity of soil microorganisms. Each plate contained 31 different sole C sources and water (as a blank sample) in three replicates. The C sources distributed in 96 wells on each plate were subdivided into five groups of substrates: carbohydrates (CH), carboxylic acids (CXA), amino acids (AA), amines and amides (AMAD) and polymers (PLM) (Weber and Legge 2009). The utilisation rate of each group was measured by reduction of a redox indicator dve tetrazolium violet that changes from colourless to purple. One gram of each fresh soil sample was suspended in 99 mL of sterile water and shaken for 20 min. Next, the suspensions were incubated at 4°C for 30 min (Weber and Legge 2009; Gajda et al. 2017). After this, solutions were filtered (Bag Filter, Interscience, Paris, France). Then, 120 µL of the sample was applied to each well on EcoPlate and incubated at 25°C for 120 h. The absorbance at 590 nm was measured every 24 h with a plate reader Biolog MicroStation[™] (Biolog Inc.).

Statistical analyses

Statistical analyses were performed using Statistica ver. 10.0 software (Stat. Soft Inc., Tulsa, OK, USA) and ANOVA. The significant differences within the data were calculated according to *post-hoc* Tukey's HSD (honest significant difference) and *post-hoc* Fisher's l.s.d. (least significant difference) tests at P < 0.05. Pearson's correlation coefficients were calculated to show relationships between soil properties and winter wheat yield (WWY) at P < 0.01 and P < 0.05.

Based on the data (OD_{590}) obtained from EcoPlate after 120 h of incubation, Shannon's diversity (*H'*) index was calculated (Hill *et al.* 2003) and a heat map created (Stat. Soft Inc.). Average well colour development (AWCD) was measured as a mean of optical densities from 31 wells as given in Garland and Millis (1991).

Results

SOM and POM contents

The ORG system favoured increase in SOM content (17.0 g kg^{-1} of soil), in particular at the top soil (0–5 cm) compared with

CON (14.3 g kg⁻¹ of soil) (Fig. 1). In the sub-arable layer (30–35 cm) an inverse image of the SOM content was observed in relation to upper soil layers (0–5, 5–10 and 15–20 cm). The SOM content in sub-arable soil was 1.5 times higher in CON compared with ORG system (5.7 g kg⁻¹ of soil).

A good and sensitive parameter in monitoring changes in soil environment is POM, a biologically active fraction of SOM (Fig. 2). The POM content in top soil (0–5 cm) for ORG was 3.9 g kg^{-1} air-dry soil and was 25.6% higher than under CON. In ORG, the POM content decreased with increased soil depth, but no such relationship was observed in CON. In sub-arable soil, the POM content was 76% lower, on average, compared with upper soil layers (0–5, 5–10 and 15–20 cm).

Compared with POM, the changes in SOM content in soil under both crop production systems were difficult to detect within the three-year study (Fig. 3a, b). The POM content in soil under ORG measured in 2017 was significantly higher (twice, on average) than in 2015, whereas in the same soil under CON the POM increase was negligible (Fig. 3b). The positive effects on soil of the ORG system were also reflected in changes in the



Fig. 1. Mean values (24 replicates) of soil organic matter (SOM) under different crop production systems at different depths for years 2015–2017. Values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 24). Vertical bars represent the standard error (SE). ORG, organic; CON, conventional.



Fig. 2. Mean values (24 replicates) of particulate organic matter (POM) content in soil under different crop production systems at different depths for years 2015–2017. Values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 24). Vertical bars represent SE. ORG, organic; CON, conventional; ADS, air-dry soil.

percentage of POM in total SOM (Fig. 4). In ORG, especially in the top soil layer (0–5 cm), there was a significantly (P < 0.05) greater percentage of POM in SOM (23.9%) than in CON (20.3%).

DHA

The studied crop production systems significantly determined the soil microbial activity measured as DHA (Fig. 5). The DHA in the 0–5 cm soil layer was 47.5% higher in ORG than CON (0.16 and 0.11 mg TPF g DW soil⁻¹ 24 h⁻¹ respectively). The DHA in the sub-arable soil layer (30–35 cm) was significantly lower than in the arable layer (0–20 cm) for both ORG and CON systems, but the DHA decrease with depth was more marked for ORG than CON.

Diversity and metabolic activity of soil microbial communities

The abilities of the microbial communities in soil under ORG and CON systems to utilise specific C-substrates were analysed using EcoPlate (Figs 6–9). Optical density values at 120 h of incubation were used to assess microbial functional diversity and for statistical analyses because at that time the highest activity of soil microorganisms was recorded.



It was observed that some C substrates were utilised differently and some similarly by microorganisms inhabiting soil under the two crop production systems. Also, the rate of Csubstrate utilisation was influenced by depth in the soil profile. In the ORG system, the highest utilisation rates of C sources by microbial communities were for the CH group (34.3%) in the top soil (0-5 cm); and in the CON system, the CXA group (30.8%) in the 15–20 cm layer. In the other studied soil layers, the microbial communities utilised the CH and CXA groups at a similar rate, with no significant differences observed. The least used C sources by soil microorganisms for both crop systems was the AMAD group (5.1% in ORG and 5.6% in CON, on average), but with significant differences in the rate of utilisation between crop systems in the 0-5, 5-10 and 30-35 cm layers. Also, utilisation rate of the PLM group differed significantly in both crop systems, particularly in the 5-10 cm layer by ~2.0%. In the sub-arable layer (30-35 cm) the CXH and PLM utilisation rates showed negligible differences between the two crop systems, but the CH, AA and AMAD



Fig. 4. Particulate organic matter (POM) expressed as a percentage of soil organic matter (SOM) under different crop production systems at different depths for years 2015–2017. Values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 24). Vertical bars represent SE. ORG, organic; CON, conventional.



Fig. 3. Soil organic matter (SOM) (*a*) and particulate organic matter (POM) (*b*) contents under different crop production systems at different depths for years 2015–2017. Values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 24). Vertical bars represent SE. ORG, organic; CON, conventional.

Fig. 5. Mean values (24 replicates) of soil dehydrogenase activity (DHA) under different crop production systems at crop production systems for years 2015–2017. Values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 24). Vertical bars represent SE. ORG, organic; CON, conventional; DW, dry weight of soil.



Fig. 6. Percentage of utilisation of particular carbon substrates group (PCSG) by microbial communities influenced by crop production systems based on 120 h of incubation; CH, carbohydrates; CXA, carboxylic acids; AA, amino acids; AMAD, amines and amides; PLM, polymers; ORG, organic; CON, conventional.



Fig. 7. Average well colour development (AWCD) of metabolised substrates on EcoPlate based on data after 120 h incubation. Values with different letters are significantly different (Fisher's l.s.d. test, P < 0.05, n = 3). Vertical bars represent SE. ORG, organic; CON, conventional.

groups were utilised at significantly (P < 0.05) different rates between the systems (3.0% difference, on average).

The AWCD values showed differences in microbial metabolic activity between the two crop systems (Fig. 7). In all arable soil layers (0–5, 5–10 and 15–20 cm) the AWCD values were higher in the ORG (1.64–1.67) than the CON system (1.47–1.60), but the difference (12%) was only significant in the 5–10 cm layer. In the sub-arable layer (30–35 cm), the AWCD value was ~8% higher in the ORG than the CON system.

The H' values indicated the effects of studied crop production systems on the metabolism of soil microorganisms (Fig. 8). In general, higher values of H' characterised soil under the ORG compared with the CON system. The highest H' values were in the ORG system for the 5–10 cm layer (3.36, on average); the lowest H' values were in soil at 30–35 cm in CON (3.30).



Fig. 8. Mean values of Shannon's diversity (H') index based on date after 120 h incubation for EcoPlate. Values with different letters are significantly different (Fisher's l.s.d. test, P < 0.05, n=3). Vertical bars represent SE. ORG, organic; CON, conventional.

There were significantly (P < 0.05) higher H' values for ORG compared to CON in soil at 5–10 and 30–35 cm depths.

A heat map was generated for better visualisation of similarities and dissimilarities in C-substrate utilisation rates by soil microbial communities between the crop systems (Fig. 9).

WWY

In our studies, the WWY differed for the ORG and CON systems with 6.38 and 8.14 t ha^{-1} respectively (Table 3). The higher WWY in the CON system may be due to relatively high yield losses as a result of pests and diseases and phosphorus limitations in the ORG system (Table 2).

Correlations among soil properties

There were strong correlations (P < 0.01) between DHA and SOM (0.931) and POM (0.969) (Table 4). There were also significant (P < 0.05) positive correlations of WWY with SOM (0.783), POM (0.587), DHA (0.512) and precipitation (0.445).

Discussion

In our studies, positive effects of the ORG system on SOM content in soil were observed. Several studies have shown that SOM decreases in intensively-managed cropland soils (Liebig et al. 2004; de Ponti et al. 2012; Gajda et al. 2016). Management systems that affect SOM accumulation usually also affect the content of the labile POM fraction. Cambardella and Elliott (1992) reported that POM is preferentially depleted compared to SOM with increasing intensity of tillage. As a readily available source of soil nutrients, POM contributes to improved soil structure and is very sensitive to soil management, and therefore is frequently used as an indicator of soil quality (Liebig et al. 2004; Gajda et al. 2016). The high POM content in soil is an indicator of soil organic C and other nutrients stored in an intermediary pool, which are protected from losses and available when needed. Within our three-year study, the quantitative changes in POM content showed relatively early the direction of ongoing changes in SOM



Fig. 9. Heat maps for the carbon utilisation patterns of the substrates on EcoPlate based on the average absorbance values after 120 h incubation. ORG, organic; CON, conventional; soil depths: 1, 0–5 cm; 2, 5–10 cm; 3, 15–20 cm; 4, 30–35 cm.

Table 3. Effects of organic and conventional crop production systems
on winter wheat yield (average for 2015–2017)

Mean values with different letters are significantly different (Tukey's HSD test, P < 0.05, n = 3). ORG, organic; CON, conventional

Crop production	Yield	Number of ears per m ²	Thousand-grain
system	(t ha ⁻¹)		weight (g)
ORG	6.38 ^b	415.0 ^b	45.8 ^b
CON	8.15 ^a	497.0 ^a	48.2 ^a

Table 4. Pearson's correlation coefficients among selected soil properties, precipitation and winter wheat yield

DHA, dehydrogenase activity; PCSG, particular carbon substrate group utilised by microbial communities; SOM, soil organic matter; POM, particulate organic matter; PCP, precipitation; WWY, winter wheat yield; **P < 0.01; *P < 0.05

Related parameter		Correlation coefficients	Level of significance	
DHA	SOM	0.931**	High degree positive	
	POM	0.969**	High degree positive	
	PCSG	0.650*	Significant positive	
	PCP	0.418	Non-significant	
PCSG	SOM	0.706*	Significant positive	
	POM	0.832*	Significant positive	
WWY	DHA	0.512*	Significant positive	
	SOM	0.783*	Significant positive	
	POM	0.587*	Significant positive	
	PCSG	0.246	Non-significant	
	PCP	0.445*	Significant positive	

induced by crop production systems, while changes in SOM content were scarcely noticeable in the same period. The positive effects of the ORG system on soil were well described by changes in the percentage of POM in total SOM. The significant differences in the percentage of POM in total SOM between the two crop production systems were mostly in the surface soil (20.3%) in favour of ORG. Cambardella *et al.* (2001) reported that the share of POM ranged within 10–45% in soils of the Great Plains, USA. These results indicated that POM is important to SOM turnover and responds much faster to changes in soil caused by management than does the total SOM. Similar effects of farming practices on the content of the labile POM fraction in soil were reported by Marriott and Wander (2006) and Gajda *et al.* (2016).

Many researchers have reported that crop production systems greatly influence the activity of soil microbial communities, very often is measured in terms of DHA (Marinari et al. 2006; Karaca et al. 2011). This group of enzymes is widely used as a good indicator of changes in soil microbial activity (Wolińska et al. 2017). In our studies, the ORG system enhanced SOM and its labile fraction POM content which positively influences the abundance and activity of microbial communities, as reflected in higher DHA, compared with the CON system. Our results are supported by the findings of Järvan et al. (2014), who concluded that an organically-managed crop rotation significantly increased the number of soil microbial communities and the DHA. Also, Heidari et al. (2016) showed beneficial effects of no-tillage and organic fertilisation on enzyme activities in contrast to conventionally managed soil. The authors of most published

results have mentioned that soil microbial diversity and abundance, and soil enzymatic activity, are significantly greater for organic compared with conventional management (Karaca *et al.* 2011).

Knowledge of the diversity of soil microbial communities is crucial to understanding soil function in the ecosystem (Nannipieri and Badalucco 2003). The C-substrate utilisation pattern indicated that studied crop production systems influenced soil microorganisms, and that the ORG system created a more favourable environment for soil microbial communities and their metabolic activity compared with CON. Liu *et al.* (2007) observed that functional diversity as indicated by C-substrate utilisation patterns was considerably higher in organic than conventional management practice.

The AWCD index is very useful to characterise activity and diversity of microbial populations (Furtak *et al.* 2017). It characterises well the oxidative ability of microorganisms developed in the EcoPlate system and may serve as an indicator of microbial activity (Garland and Millis 1991). The EcoPlate results expressed as AWCD values showed some differences in the catabolic potential of microorganisms between systems of studied arable and sub-arable soil layers. That may indicate that agrotechnical treatments (e.g. ploughing) can spread microorganisms deeper into soil, in accordance with the results of Wang *et al.* (2007) and Gajda *et al.* (2016).

As is widely known, crop production systems affect crop yields by changing the soil properties (Mäder et al. 2002; Marinari et al. 2006). In this research, the ORG system resulted in a lower WWY than for the CON system. de Ponti et al. (2012), Bilsborrow et al. (2013), Švančárková and Żák (2015) and Gałązka et al. (2018) reported similar yields. An organic production system relies for its crop nutrients on natural soil fertility, whereas a conventional system relies on external inputs to maintain soil fertility. Crop yields are a result of the interaction of many overlapping factors, both related to applied agrotechnics and environment, e.g. weather conditions. Variability in meteorological conditions is a significant challenge to crop production and yield stability. Precipitation and temperature during the early vegetative and late reproductive phases of winter wheat growth explained much of the yield variation in our studied crop production systems, consistent with the results of Teasdale and Cavigelli (2017).

Although the yields in the ORG system varied considerably because of the high dependency on the weather, this system favours protection of soil quality as the main basis for crop production. Therefore, the ORG can be recommended as an alternative to the CON system.

Conclusions

Greater activity of soil microbial communities in a Haplic Luvisol soil was found under the ORG compared with CON production system. This activity was measured with dehydrogenases as well as metabolic diversity revealed by Biolog EcoPlate analysis and confirmed by AWCD and H'results. Also, higher concentrations of POM and SOM were observed in soil under the ORG, particularly in the 0–5 cm layer, compared with the CON system. The results suggest that ORG much better maintained soil fertility than the CON system, which is extremely important for the soil environment and sustainable land use as it avoids SOM losses and soil degradation processes and improves the microbial status of soil. Therefore, legume-based crop rotations in an organic crop production system, utilising organic fertilisers generated within the system are a realistic alternative to the conventional crop production system. However, further field experiments are needed to monitor changes in the soil environment and to confirm the qualitative and quantitative long-term benefits of the applied crop production systems.

Conflicts of interest

The authors declare no conflicts of interest.

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