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## Crops and Soils Research Paper

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### Abstract

Increasing crop diversity has been highly recommended because of its environmental and economic benefits. However, the impacts of crop diversity on soil properties are not well documented. Thus, the present study was conducted to assess the impacts of crop diversity on selected soil quality indicators. The cropping systems investigated here included wheat (*Triticum aestivum* L.) grown continuously for 5 years as mono-cropping (MC), and a 5-year cropping sequence [(wheat–cover crop (CC)–corn (*Zea mays* L.)–pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.)–sunflower (*Helianthus annuus* L.)]. Each crop was present every year. This study was conducted in the northern Great Plains of North America, and soil quality data were collected for 2016 and 2017. Selected soil quality indicators that include: soil pH, organic carbon (SOC), cold water-extractable C (CWC) and N (CWN), hot water-extractable C (HWC) and N (HWN), microbial biomass carbon (MBC), bulk density (BD), water retention (SWR), wet soil aggregate stability (WAS), and urease and  $\beta$ -glucoside enzyme activity were measured after the completion of 5-year rotation cycle and the following year. Crop diversity did not affect soil pH, CWC, CWN, HWC, HWN and SWR. Cropping systems that contained CC increased SOC at shallow depths compared to the systems that did not have CC. Crop diversity increased WAS, MBC, and urease and  $\beta$ -glucoside enzyme activity compared with the MC. Comparison of electrical conductivity (EC) measured in this study to the baseline values at the research site prior to the establishment of treatments revealed that crop rotation decreased EC over time. Results indicate that crop diversity can improve soil quality, thus promoting sustainable agriculture.

## Introduction

Soil degradation due to increasingly intensive farming has drawn researchers' attention in recent years (Mekonnen *et al.*, 2015; Khaledian *et al.*, 2017). It has been reported that approximately 60% of the soil ecosystem services have undergone soil degradation by varying degrees since the 1950s (Lal, 2015), with agricultural activity being one of the principal factors (Oldeman *et al.*, 2017). Consequently, taking action to minimize the loss of soil fertility and improve soil structure is a priority (Khaledian *et al.*, 2017). Crop management practices greatly influence soil properties by changing microbial activity and carbon (C) sequestration (Zuber *et al.*, 2018). Crop diversification has been treated as an implementable practice to enhance crop production, soil quality (Cook, 2017) and agricultural sustainability (Congreves *et al.*, 2015; Yadav *et al.*, 2016).

Increasing diversity cropping system has the potential to maintain or improve soil quality with the help of increased crop residue and varied root systems, thereby increasing and diversifying the microbial activity (Studdert, 2000). A number of studies have reported that crop diversity increased soil water storage, wet soil aggregate stability (WAS), plant available nutrients, C cycling and soil enzyme activity compared to less intensive crop management systems (mono-cropping or fallow) (Dick, 1984; Tiemann *et al.*, 2015; Zuber *et al.*, 2015; Nunes *et al.*, 2018). It was reported by Karlen *et al.* (2006) that crop diversity decreased soil bulk density (BD), resulting in increased crop yield when compared to the continuous corn (Smith *et al.*, 2008).

Further, crop diversity can enhance soil nitrogen (N) concentrations, which is considered the main nutrient for plant growth (Stanger and Lauer, 2008), and increase microbial biomass C (MBC), which is an important index of soil quality (McDaniel *et al.*, 2014). Crop diversity can reduce pest pathogens by breaking the pest cycle (Rusch *et al.*, 2013) and making full use of nutrients in different soil layers with different crop root systems (O'Rourke *et al.*, 2014). Crop diversity can increase soil C content, which makes a great contribution to recovering,

creating and binding soil aggregate structure (Chateil *et al.*, 2013; McDaniel *et al.*, 2014; Tiemann *et al.*, 2015). Soil organic C (SOC), one of the most important soil quality components, has a significant impact on physical, chemical and biological properties (King and Blesh, 2018). Soil property responses to crop diversity may vary due to different soil types, topography, climate, tillage intensity, irrigation and fertilizer rate (Al-Kaisi *et al.*, 2005; Gál *et al.*, 2007). Therefore, an improved understanding of soil physical, chemical and biological properties as impacted by crop diversification is important. Several studies have compared soil quality from a crop grown in crop diversity with a similar crop grown in mono-cropping (Tiemann *et al.*, 2015; Zuber *et al.*, 2015; Nunes *et al.*, 2018).

Corn (*Zea mays* L.) and soybean (*Glycine max* L.) sequence is the most common agricultural practice in many places worldwide (Hoss *et al.*, 2018). However, interest in adopting crop diversity has been increasing among producers to enhance crop production. Farming practices have changed from conventional systems to continuous cropping with a variety of cropping systems (Sindelar *et al.*, 2017). Including cover crops (CC) in a diverse system has the potential to relieve soil compaction, erosion, nutrient leaching, and alter C cycling and microbial activity, and could therefore regulate the ecosystem functioning and soil structure maintenance (Schipanski *et al.*, 2014; Lewis *et al.*, 2018). Utilizing leguminous crops in arable cropping systems was suggested as an implementable practice to enhance soil C and N sequestration in rainfed areas (Bhattacharyya *et al.*, 2009; Zhou *et al.*, 2017).

There are few studies investigating the effect of crop diversity on soil quality from different crop species within the system. Therefore, the objective of this research was to investigate the impacts of the crop diversity that are part of a rotation on some selected soil chemical, physical and biological properties. It was hypothesized that diverse cropping system would improve soil quality due to the inputs of the below and aboveground biomass, thus increasing soil C content, microbial biomass, soil enzymes activity and soil quality.

## Materials and methods

### Experimental site

This study was initiated in 2011 at the Dickinson Research Extension Center located near Dickinson, North Dakota (46°53'N, 102°49'W). Soils data for this study were collected in 2016–2017. The site consists of 18 uniform rectangular 1.74 ha (31.3 ha in total) plots. This experimental site was established to investigate the soil quality responses to the adoption of diversified cropping (DC) system under a no-till system. Soils of the study site are Vebar Series (coarse-loamy, mixed, superactive, frigid Typic Haplustolls) and Savage Series (fine, smectitic, frigid Vertic Argiustolls). To minimize the impact of soil on the data analysis, two replicates were assigned to Savage Series and the third replicate was assigned to Vebar Series for each treatment (Abagandura *et al.*, 2019). The treatments were laid out in a randomized complete block design with three replications. Additional details of the site were described in Abagandura *et al.* (2019).

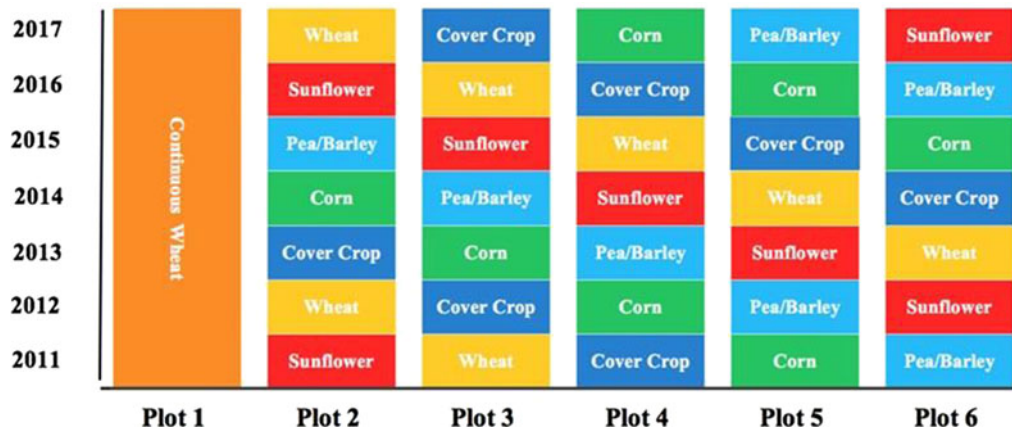
The cropping system investigated here included mono-cropping (MC) system [wheat (*Triticum aestivum* L.)] grown continuously for 5 years and 5-year crop sequences [wheat–cover crop (CC)–corn (*Z. mays* L.)–pea (*Pisum sativum* L.)/barley (*Hordeum vulgare* L.)–sunflower (*Helianthus annuus* L.)]. Wheat, sunflower and corn were used as cash crops, while

legumes (pea/barley) was used to increase nutrient and organic matter inputs, CC was used to supply nutrients for the following crop, balance the soil moisture and reduce soil erosion. Each crop was present every year. The cropping sequence over the study period is presented in Fig. 1. Cover crop species used in this study are listed in online Supplementary Table S1. Crop agronomic information, including planting time, planting population, row spacing and seeding depth is listed in Table 1. Wheat was harvested in August and the other crops were harvested between October and November. No fertilizer was applied in 2016 and 2017 according to the soil test results from North Dakota State University Laboratory, and pesticides were applied as needed to control pests. All crops were grown under rainfed conditions, and no irrigation was applied. Average minimum and maximum air temperature and total precipitation (mm) for 2016 and 2017 (online Supplementary Fig. S1) were collected from a weather station located approximately 25 km from the field (Abagandura *et al.*, 2019). Climate is between dry season and semi-arid climate, caused by low precipitation and a monthly mean temperature of –8.7°C in January and 20°C in July. Average (30-year) air temperature at the site was 6.2°C, and mean annual precipitation was 610 mm (Abagandura *et al.*, 2019).

### Sample collection and laboratory analysis

Soil samples were collected in June 2010 to measure the baseline values at the research site prior to the establishment of treatments. Although a 5-year rotation cycle was completed in 2015 (Fig. 1), yet, it was not possible to take soil samples after harvesting in 2015 due to the cold weather in the study area. When the snow melted around May 2016, soil samples were taken in June 2016 to represent the end of the 5-year rotation cycle. Soil samples were also collected when a new cycle of the 5-year rotation began in 2016. Again it was not possible to take soil samples after harvesting in 2016; therefore, soil samples were taken in June 2017 to represent the beginning of the second cycle of the 5-year rotation.

Soil samples were collected from six random spots from 0–5 cm depth in 2010 and from 0–5, 5–15, 15–30, 30–45 and 45–60 cm depths in 2016 and 2017 using a soil auger (3.2 cm diam.) from each plot. In addition, a separate soil core (5 cm i.d.) was taken from each plot for soil water retention (SWR) and BD analyses in 2016 and 2017. All the soil samples were kept in plastic zip-lock bags, transported to the lab and stored in a cold room (4°C) for further analysis. Soil samples for all depths were air dried, ground and sieved to pass through a 2 mm sieve. A total of 10 g of soil was placed in a centrifuge tube with 10 ml of distilled water (the ratio of soil to water being 1:1), the suspension was stirred with a vortex mixer for 30 s, and then a pH and EC meter (Thermo Scientific Orion, model-Orion Star A215) was used to measure the soil pH (Kalra, 1995). Electrical conductivity (EC) was measured with 1:2.5 of soil:water slurry through pH and EC meter. Total C and N were determined using the TruSpec CHN analyser (LECO Corporation, St. Joseph, MI, USA) with the dry combustion method. Inorganic C was determined for all the soil samples by reaction with hydrochloric acid (Schumacher, 2002). Soil organic C was calculated by subtracting the soil inorganic C from total C. There were not sufficient samples in 2010; therefore, the following analyses were conducted on 2016 and 2017 soil samples only.



**Fig. 1.** Colour online. Layout of the crop sequences used in this study from 2011 to 2017. The cropping systems investigated in this study included continuous wheat, and five crop sequences (wheat–cover crop–corn–pea/barley–sunflower).

Labile C and N fractions [cold water-extractable C (CWC), cold water-extractable N (CWN), hot water-extractable C (HWC), hot water-extractable N (HWN) and microbial biomass C (MBC)] were determined in this study. Labile C and N fractions (except for MBC) were extracted for 0–5 and 5–15 cm depths using cold water and hot water extraction methods (Ghani *et al.*, 2003) and analysed with the TOC-L analyser (Shimadzu Corporation, Columbia, MD, USA, model-TNM-L-ROHS). To summarize the methodology, 3 g of soil and 30 ml of distilled water were taken into 50 ml centrifuge tubes, then moved to an end-over-end shaker for 30 min at 40 rpm. The suspension was centrifuged at 3000 rpm for 25 min, and the supernatant was subsequently separated from the soil with 0.45 µm pore-size filter paper to get CWC and CWN. Thirty millilitres of distilled water was added to the leftover suspension and kept in a water bath at 80°C for 12 h, centrifuged at 3000 rpm for 25 min and filtered to get HWC and HWN. The microbial biomass C was determined for 0–5 cm depth by chloroform fumigation direct extraction method (Beck *et al.*, 1997).

For SWR measurements, a cheesecloth was fixed at the bottom of the intact cores with a rubber band, then the soil core was saturated with water for 1–3 days. Soil water retention was measured for 0–5 cm depth at seven different matric potentials; 0, –0.4, –1.0, –2.5 and –5 kPa using the tension table and –10 and –30 kPa using the ceramic pressure plate. After this, soil cores were oven dried at 105°C for at least 48 h to get the dry mass for measuring soil BD. This dry mass was divided by the known soil volume. The WAS was measured for the top two depths (0–5 and 5–15 cm) using the method described by Kemper and Rosenau (1986). Three grams of 1–2 mm air-dry soil were saturated with a cold vapour machine, then transferred to shaking slots for 5 min to get unstable aggregates, the sonicator breaking down the remaining soil particles to get the stable aggregates. Then, the soil suspension was kept in the oven to dry overnight at 105°C to get to a constant weight. The percentage of soil stable aggregates was calculated by dividing the oven dry stable aggregates by the stable and unstable aggregates weight.

To study the microbial activity response to the cropping systems, urease and β-glucosidase enzymes activity were analysed for 0–5 cm depth for both years. Urease enzymes activity was ascertained with the colorimetric determination of ammonium method described by Kandeler and Gerber (1988) using the

following equation:

$$\text{Urease activity}[\mu\text{g NH}_4\text{-N/g/h}] = (\text{NCS} - \text{NCC}) \times \text{DF} \times \text{V} \times \text{T/DW}$$

where NCS is the NH<sub>4</sub>-N concentration of the sample average (µg NH<sub>4</sub>-N/ml), NCC is the NH<sub>4</sub>-N content of the control (µg NH<sub>4</sub>-N/ml), DF is dilution factor (10), V is the volume of urea solution used (2.5 ml), T is incubation time (2 h), and DW is the dry weight of the soil taken (5 g). The β-glucosidase enzyme activity was determined with the method described by Eivazi and Tabatabai (1988) using the following equation:

$$\begin{aligned} \beta\text{-glucosidase activity}(\mu\text{g p-nitrophenol/g/h}) \\ = (\text{NCS} - \text{NCC}) \times \text{V} \times \text{T/DW} \end{aligned}$$

where NCS is the p-nitrophenol content of sample average (µg NH<sub>4</sub>-N/ml), NCC is the p-nitrophenol content of control (µg NH<sub>4</sub>-N/ml), V is the volume of p-nitrophenyl-β-D-glucosidase solution used (1 ml), T is incubation time (1 h), and DW is the dry weight of soil taken (1 g).

### Statistical analysis

Data were normal (Skewness and Kurtosis's tests) and homogeneous (Levene's test) for all the soil parameters. Data in 2016 and 2017 were analysed using the MIXED model, with replication considering as a random effect, treatments and year considering as fixed effects. Four orthogonal linear contrasts were conducted: (1) mono-cropping (MC: which refers to the continuous wheat) compared to diverse cropping system (DC: wheat in diversified cropping system, CC, corn, pea/barley, and sunflower) (MC *v.* DC); (2) MC compared to wheat in diversified cropping system present in 2016 and 2017 (DC<sub>(w)</sub>) (MC *v.* DC<sub>(w)</sub>); (3) the CC present in 2016 and 2017 (CC) compared to the non-cover crops (NCC: wheat in diversified cropping system, corn, pea/barley and sunflower) (CC *v.* NCC); and (4) the legume present in 2016 and 2017 (LG) compared to non-legume crops (NLG: wheat in diversified cropping system, CC, corn and sunflower) (LG *v.* NLG). Mean values were separated by using pairwise differences method (adjusted by Tukey) using the GLIMMIX



**Table 1.** Agronomic management information at the study site performed during 2016 and 2017 (adapted from Abagandura *et al.*, 2019)

Crop	Planting date		Population (plants/ha)	Seeding depth (cm)	Hybrid	Row spacing (cm)
	2016	2017				
Continuous wheat	9-May	3-May	3 087 500	2.54	Barlow	19.1
Wheat	9-May	3-May	3 087 500	2.54	Barlow	19.1
Sunflower	9-May	15-May	49 400	5.08	60ME80	76.2
Pea/barley	9-May	1-May	3 087 500	2.54	Mixed	19.1
Corn	21-May	9-May	49 400	5.08	Master graze BMR	76.2
Cover crops	13-Jul	15-Jun	3 730 170	2.54	13 species	19.1

procedure. To evaluate the effect of crop rotation on certain soil properties, data on 2010, 2016 and 2017 were analysed using a Student's test. Differences were considered significant at 0.05 probability. All analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

## Results

Data on soil pH under different cropping systems at 0–5, 5–15, 15–30, 30–45 and 45–60 cm depths in 2016 and 2017 are summarized in online Supplementary Table S2. Soil pH ranged from 5.2 to 7.9 in 2016 and from 5.4 to 7.9 in 2017. The effect of cropping systems on soil pH was not significant in either year (online Supplementary Table S2). Data on SOC (g/kg) under different cropping systems for the five depths in 2016 and 2017 are represented in Fig. 2. There is a significant difference for the SOC values between 2016 and 2017, especially under CC (Fig. 2). Comparing CC and NCC at the 5–15 and 15–30 cm depths in 2017 indicated that SOC was higher under CC than NCC (Fig. 2). The other cropping system did not affect SOC at any depth in 2016 and 2017 (Fig. 7). Cold water C, CWN, HWC and HWN under different cropping systems for the 0–5 and 5–15 cm depths in 2016 and 2017 are listed in Table 2. The means for these soil C and N fractions had similar performance within each contrast group (Table 2). Data on MBC (g/kg) under different cropping systems at 0–5 cm depth in 2016 and 2017 are shown in Fig. 3. Although the MBC values (regardless of the cropping system) seem to be higher in 2016 than in 2017, no significant differences ( $P \geq 0.05$ ) on MBC between years were recorded. The effects of cropping system on MBC were significant for MC *v.* DC, MC *v.* DC<sub>(w)</sub> and LG *v.* NLG contrasts in both years (Fig. 3). Comparing MC to DC and MC to DC<sub>(w)</sub> showed that the mean MBC under MC was significantly lower than that under DC and DC<sub>(w)</sub> in both years (Fig. 3). The contrast of LG *v.* NLG indicated that LG recorded significantly lower MBC than that of the NLG in 2016; however, the trend was opposite in 2017 with LG recording higher MBC than NLG (Fig. 3). Data on soil TN (g/kg) at all five depths in both years are presented in online Supplementary Table S3. Total N values range from 0.8 to 2.4 g/kg across all depths. The effect of the cropping systems was not significant at any depth in either year (online Supplementary Table S3). Thus, TN results will not be discussed any further.

Data on BD (mg/m<sup>3</sup>) under different cropping systems at five depths in 2016 and 2017 are shown in Fig. 4. The effects of the cropping systems on BD were significant only for the MC and DC<sub>(w)</sub> contrasts at 0–5 and 30–45 cm depths in 2016 (Fig. 4),

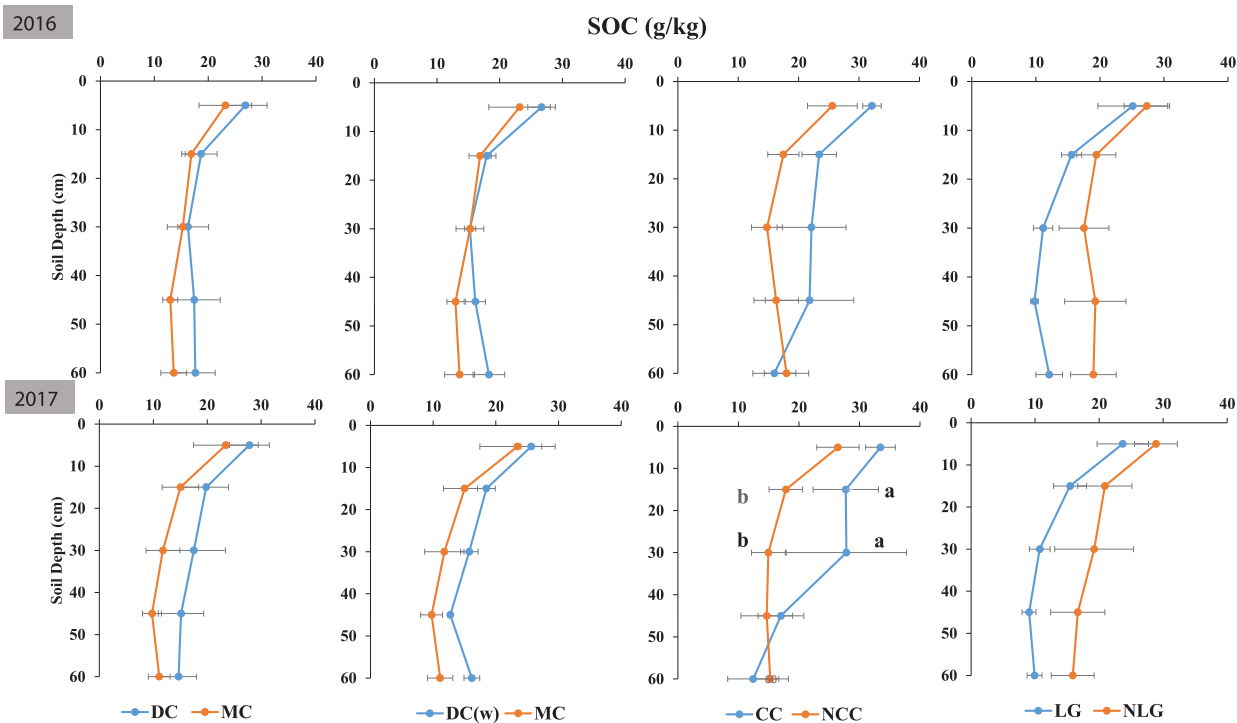
with MC recording significantly higher BD than DC<sub>(w)</sub> at these depths (Fig. 3). Data on SWR (m<sup>3</sup>/m<sup>3</sup>) under different cropping systems at 0.0, –0.4, –1.0, –2.5, –5.0, –10.0 and –30.0 kPa in 2016 and 2017 are represented in online Supplementary Fig. S2. No differences were observed on the SWR for any pressures among all comparisons of each contrast group for either year. Data on WAS (%) under different cropping systems for the 0–5 and 5–15 cm depths in 2016 and 2017 are represented in Fig. 5. No differences were observed on WAS at either depth for any contrast group treatment in 2016. However, the contrast between MC *v.* DC and MC *v.* DC<sub>(w)</sub> in 2017 showed that DC and DC<sub>(w)</sub> had higher WAS compared to MC at the 0–5 cm depth (Fig. 5).

Data on urease and  $\beta$ -glucosidase ( $\mu\text{g/g/h}$ ) under different cropping systems at 0–5 cm depth in both years are shown in Fig. 6. Regardless of the cropping system, both urease and  $\beta$ -glucosidase were lower ( $P$  values  $\geq 0.05$ ) in 2016 than in 2017 (Fig. 6). No significant differences were observed in urease among each contrast group in 2016 (Fig. 6). However, the results of MC *v.* DC and MC *v.* DC<sub>(w)</sub> contrasts in 2017 showed that DC and DC<sub>(w)</sub> had higher urease than MC (Fig. 6). The effects of cropping systems on  $\beta$ -glucosidase were significant for MC *v.* DC contrast in 2017 and for MC *v.* DC<sub>(w)</sub> contrast in 2016, with  $\beta$ -glucosidase being higher under DC compared to MC in 2017 and under DC<sub>(w)</sub> compared to MC in 2016 (Fig. 6).

Comparison of pH, EC and TN measured in 2016 and 2017 to baseline values at the research site prior to the establishment of treatments is represented in Fig. 7. Although only three parameters were included, it gave an overall picture of the soil quality changes over time. Soil pH and TN values in 2010 were similar to the average of 2016 and 2017 (Fig. 7). However, EC in 2010 was significantly higher compared to the average of 2016 and 2017 (Fig. 7).

## Discussion

The absence of significant effects due to crop diversity on soil pH in the current study (online Supplementary Table S2) may be due to the strong buffering capacity of the soil of the present study. Soils with a high amount of clay have a larger number of surface sites, and thus can hold hydrogen ions and resist changes in hydrogen ion concentration in the soil solution (Weaver *et al.*, 2004; McCauley *et al.*, 2017). Similarly, Sharma *et al.* (2018) conducted a study on silt loam soil and reported that CC with corn or soybean recorded similar soil pH as that with corn or soybean without CC over the 4 years of the experiment. Roldán *et al.* (2003) also reported that the pH of sandy loam soil did not change with or without leguminous *Vici asp.* and leguminous *P.*



**Fig. 2.** Colour online. Mean value of soil organic carbon (SOC g/kg) under different cropping systems. MC, mono-cropping (continuous wheat); DC, diversified cropping system (wheat, cover crop, corn, pea/barley and sunflower); DC<sub>(w)</sub>, wheat in diversified cropping system; CC, cover crop; NCC, no cover crop (wheat, corn, pea/barley and sunflower); LG, legume; NLG, no legume (wheat, cover crop, corn and sunflower). Different letters in the graphs at each depth indicate significant differences at that depth. Horizontal bars indicate standard errors of the means.

*vulgaris*. However, Zuber *et al.* (2015) reported that soil pH after 15–20 years of rotation was significantly different from that of the MC for clay loam soil.

Soil organic C is an essential indicator of soil quality and fertility (Lal, 2015; Fu *et al.*, 2017). Crop diversity is considered one of the management practices that can best increase SOC by increasing soil aggregate, soil moisture, nutrient cycling and biological activities (Studdert, 2000; Karlen *et al.*, 2006; Aller *et al.*, 2017). The large increments in SOC from 2016 to 2017 may be due to the increase in  $\beta$ -glucosidase activity in the latter compared to the former (Fig. 6), which can enhance C storage (Adetunji *et al.*, 2017). Similar SOC between DC and DC<sub>(w)</sub> and MC were recorded at the time when a 5-year rotation cycle was completed and even when a new rotation cycle began afterwards (Fig. 2). Significant changes in SOC may need long-term study to be detected. Van Eerd *et al.* (2014) conducted a study for 11 years and reported no SOC differences were observed between continuous corn, continuous soybean, soybean-corn, soybean-wheat, and soybean-wheat-corn systems. Jarecki *et al.* (2018) found that corn grown with oats (*Avena sativa* L.)–alfalfa (*Medicago sativa*) in a sequence for 57 years recorded higher SOC than continuous corn. Diverse cropping systems input more crop residues than MC (Gál *et al.*, 2007), resulting in higher SOC. However, CC increased SOC compared to NCC in 2017 (Fig. 2), which may be associated with diversified microbial communities more present in CC than in NCC plots (Sainju and Singh, 1997; Dabney *et al.*, 2001; Kabir and Koide, 2002; Sarrantonio and Gallandt, 2003) and rapid decay of the CC residues compared to the other crops (Buchanan and King, 1993). This finding suggested that alternating long-term CC has the potential to increase SOC (Poeplau *et al.*, 2015). There were no noticeable SOC changes

between LG and NLG in this study (Fig. 2), which was in agreement with Drinkwater *et al.* (1998), who reported that the C storage was not quantitatively different among legume-based and conventional (corn-soybean) treatments.

Total soil C and N pools consist of the labile and stable C and N (Liu *et al.*, 2013). The labile C and N breakdown faster and active food source for microorganisms (Juan *et al.*, 2008; Ciampitti *et al.*, 2011; Uchida *et al.*, 2012). The stable C cannot be directly used by microbes, but makes a great contribution in soil aggregation, water holding capacity, the maintenance of soil structure and soil C sequestration (Li *et al.*, 2018). Labile C and N fractions are considered a sensitive index of changes in soil quality due to the fast turnover rate (Soon *et al.*, 2007). Compared with CWC and CWN, HWC and HWN have higher biodegradability rate and can supply higher nutrients and energy for plant and microbes (Gregorich *et al.*, 2003). The findings in the current study showed that all labile C and N fractions under all cropping systems had similar performance in both years (Table 2), also associated with the turnover rate of labile C and N fractions (Jandl and Sollins, 1997; Zhang *et al.*, 2017). These findings are consistent with the results of a long-term study in the USA conducted by Alhameid *et al.* (2017) who reported that DC did not impact soil C and N fractions compared to MC under similar weather conditions. Another long-term study conducted in Canada indicated that crop diversity [pea-wheat-canola (*Brassica rapa* L.)–wheat] did not affect CWC compared to continuous wheat, which was attributed to the unstable character of CWC (Soon *et al.*, 2007). A study conducted in Mexico showed that including the leguminous *P. vulgaris* CC did not make significant changes regarding the water-soluble C (Roldán *et al.*, 2003).

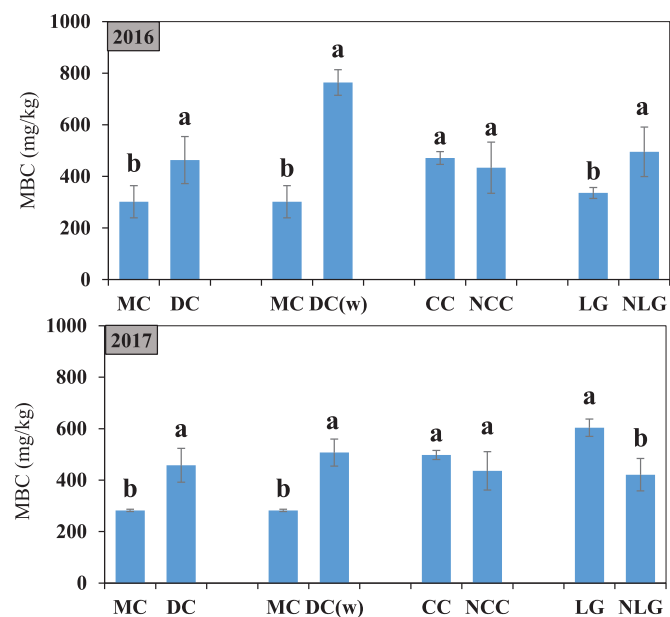
**Table 2.** Soil cold water-extractable C (CWC,  $\mu\text{g/g}$ ), cold water-extractable N (CWN,  $\mu\text{g/g}$ ), hot water-extractable C (HWC,  $\mu\text{g/g}$ ) and hot water-extractable N (HWN,  $\mu\text{g/g}$ ) at 0–5 and 5–15 cm depths under different treatments in 2016 and 2017

Treatment contrasts	CWC		CWN $\mu\text{g/g}$		HWC		HWN	
	Soil depth (cm)		0–5	5–15	0–5	5–15	0–5	5–15
	0–5	5–15						
<b>2016</b>								
MC	26.6	20.4	3.1	2.2	72.2	54.6	7.9	5.5
DC	24.9	15.0	3.4	2.0	77.3	39.4	8.4	4.0
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
MC	26.6	20.4	3.1	2.2	72.2	54.6	7.9	5.5
DC <sub>(w)</sub>	24.1	15.2	3.4	1.8	76.0	42.8	8.9	4.2
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
CC	25.9	15.9	3.2	1.6	85.2	40.4	9.0	4.1
NCC	24.7	14.8	3.4	2.1	75.3	39.1	8.2	4.0
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
LG	26.0	15.4	4.3	2.4	81.6	36.7	8.4	3.7
NLG	24.7	15.0	3.1	1.9	76.2	40.1	8.4	4.1
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
<b>2017</b>								
MC	29.0	19.4	1.5	1.0	69.8	34.9	2.9	1.3
DC	30.0	20.3	1.8	1.0	77.5	37.7	3.3	1.5
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
MC	29.0	19.4	1.5	1.0	69.8	34.9	2.9	1.3
DC <sub>(w)</sub>	33.3	19.5	1.7	1.0	69.7	36.9	2.5	1.4
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
CC	29.5	23.5	1.8	1.0	86.2	45.7	4.0	2.0
NCC	30.2	19.5	1.7	0.9	75.3	35.6	3.1	1.4
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS
LG	29.6	19.3	1.9	0.9	71.6	33.6	3.3	1.4
NLG	30.1	20.6	1.7	1.0	79.0	38.7	3.3	1.6
<i>P</i> value	NS	NS	NS	NS	NS	NS	NS	NS

MC, mono-cropping (continuous wheat); DC, diverse cropping system (average of wheat, cover crop, corn, pea/barley and sunflower); DC<sub>(w)</sub>, wheat in the diverse system; CC, cover crop; NCC, average of wheat, corn, pea/barley and sunflower; LG, legume; NLG, average of wheat, cover crop, corn and sunflower.

Microbial biomass C is an important living component of soil labile C, which directly involves crop nutrient supply and cycling (Moore *et al.*, 2000; Guimarães *et al.*, 2013; Bongiorno *et al.*, 2019). Numerous studies have reported that MBC is easily affected by crop management and cultural treatments (de Moraes Sá *et al.*, 2001; Chu *et al.*, 2007; Chen *et al.*, 2009; Liang *et al.*, 2012). In the current study, MBC under MC was lower than that under DC and DC<sub>(w)</sub> at the 0–5 cm depth in both years (Fig. 3), likely because crop diversity can increase quantity and health of plant residues entering the soil, thus accelerating the growth of microorganisms. Several studies have reported that crop diversity increased MBC compared to MC (Moore *et al.*, 2000; Balota *et al.*, 2004a; Govaerts *et al.*, 2007). Cover crops can stimulate soil microbial activity and can change the amount of soil microbial biomass (Wang *et al.*, 2007; Frasier *et al.*, 2016). However, CC did not affect MBC compared to NCC in this reported study (Fig. 3). Based on

published literature, the effects of CC on MBC are not consistent because of several factors, including climate conditions, CC type, soil temperature and moisture, pH, and aeration (Finney *et al.*, 2017; Kumar *et al.*, 2018). For example, Garcia *et al.* (2013) reported that a high C content or C:N ratio in CC may inhibit soil microbial activity. Although the MBC values (regardless of the cropping system) seem to be higher in 2016 than in 2017, no significant differences in MBC between years were recorded. The reasons for higher MBC from NLG compared to LG in 2016 were not known in this study. As it was expected LG increased MBC compared to NLG in 2017, supporting the results of Loepmann *et al.* (2016) and Soon *et al.* (2007), who reported that residues from legumes have a higher N content, which can enhance MBC. The increase in microbial activity under one cropping system compared to another in this study was not always accompanied with differences in C between these systems as seen



**Fig. 3.** Colour online. Mean value of soil microbial biomass carbon (MBC mg/kg) at 0–5 cm depth under different cropping systems in 2016 and 2017. MC, mono-cropping (continuous wheat); DC, diversified cropping system (wheat, cover crop, corn, pea/barley and sunflower); DC(w), wheat in diversified cropping system; CC, cover crop; NCC, no cover crop (wheat, corn, pea/barley and sunflower); LG, legume; NLG, no legume (wheat, cover crop, corn and sunflower). Vertical bars indicate standard errors of the means. Different letters in the graphs at each depth indicate the significant differences at that depth.

in the SOC results. Increasing microbial activity can increase C and N mineralization, which may cause an increase in the loss of C into the atmosphere (Bardgett *et al.*, 2008).

Soil BD is an indispensable indicator of soil quality and productivity, which reflects the degree of soil compaction and influences the air, water and nutrient movement and retention (De Vos *et al.*, 2005; Rodríguez-Lado *et al.*, 2015; Chen *et al.*, 2018). Crop diversity is one of the most important managements that can influence BD (Calonego and Rosolem, 2008; Nascente *et al.*, 2015; Rorick and Klavivko, 2017). Crop diversity decreased BD compared to MC when a 5-year rotation cycle was completed (Fig. 4), supporting the results reported by Feng *et al.* (2011) and Shrestha *et al.* (2013). A study conducted by Garcia *et al.* (2013) recommended DC as a management practice to reduce soil BD. This reduced BD in DC is likely related to greater aggregate stability and greater residue accumulation from DC compared to MC (Kazula *et al.*, 2017). However, this decrease in BD from DC compared to MC was not observed when a new rotation cycle began afterwards (Fig. 5). Differences in weather conditions between 2016 and 2017 (Fig. 2) may result in differences in the response of BD to the cropping systems in these 2 years.

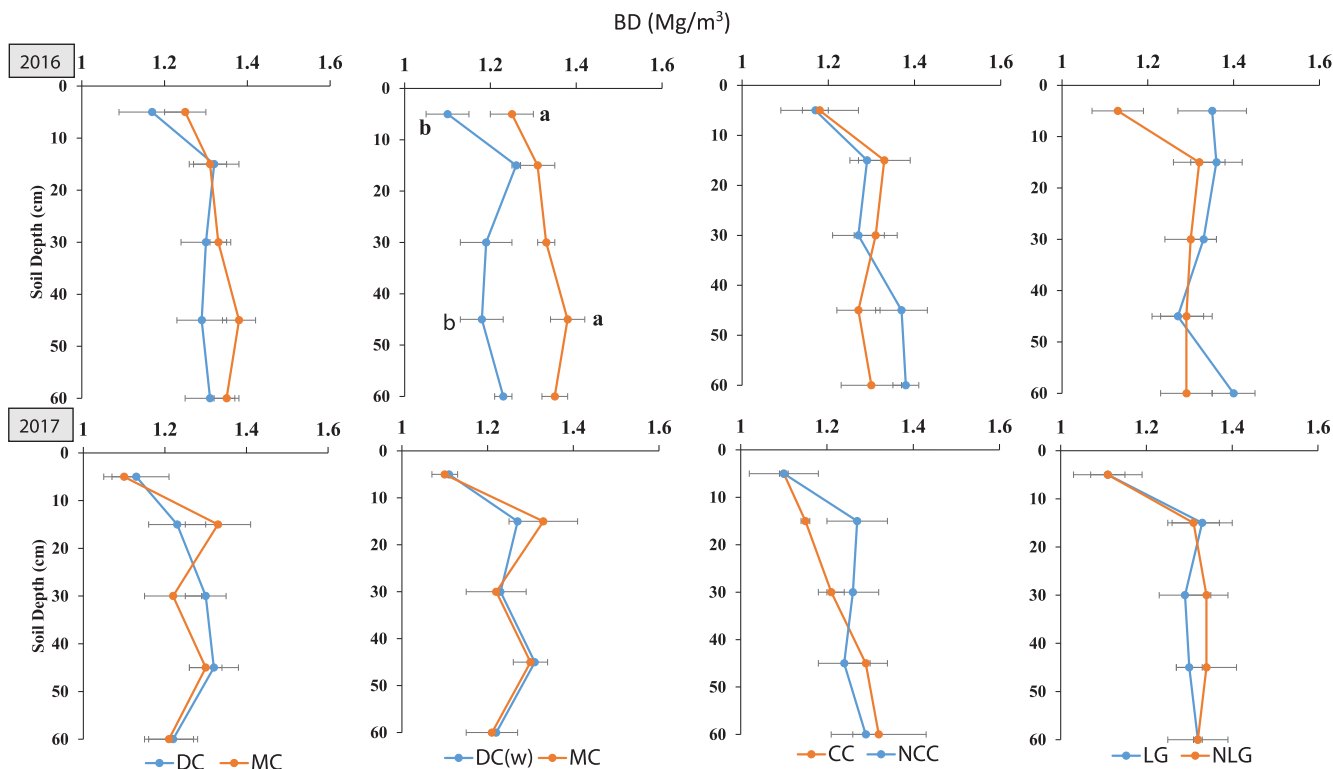
Soil water retention is an important hydraulic property associated with soil porosity; therefore, it can influence water and nutrient transport and storage and crop usage efficiency (Saxton and Rawls, 2006; Kazula *et al.*, 2017). Crop diversity may indirectly impact SWR by altering the soil porosity, soil C content and its turnover rate (McVay *et al.*, 2006). However, the findings in the current study showed that the SWR was not affected by crop diversity (online Supplementary Fig. 2S). The non-significant effect from crop diversity on SOC at the surface depth may explain the lack of effect on SWR from crop diversity. Irmak *et al.* (2018) reported that CC–corn system did not impact

SWR compared to corn without CC. However, Qi *et al.* (2011) reported that in the USA, the corn–soybean system with winter rye as a CC increased the SWR compared with the corn–soybean system without CC.

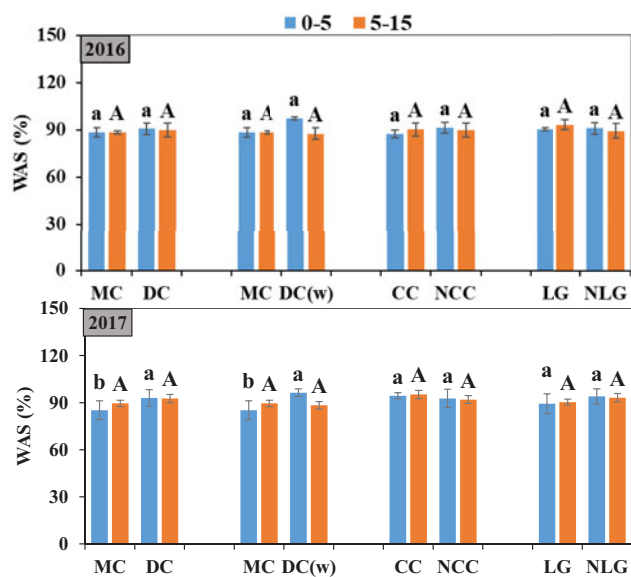
Diversifying the cropping system is considered one of the agricultural practices that can best increase soil C and ultimately improve aggregate development (Wright and Anderson, 2000; Kazula *et al.*, 2017). Soils with weak aggregate structure are highly susceptible to wind and water erosion (Williams *et al.*, 2018). The findings in the current study showed that WAS under MC and DC and DC(w) were similar when a 5-year rotation cycle was completed, but it was expected WAS under MC was lower compared to DC and DC(w) when a new rotation cycle began afterwards (Fig. 5). Increasing the underground biomass production through crop diversity can loosen soil and improve soil structure (Holeplass *et al.*, 2004). Nouwakpo *et al.* (2018) reported that soybean under corn–soybean system recorded significantly higher WAS compared to continuous soybean at 0–15 and 15–30 cm depths. Alhameid *et al.* (2017) reported that corn–soybean–wheat and corn–soybean–wheat–oat systems had significantly increased WAS compared with corn–soybean system at 0–15 cm depth. Cover crop root exudates alter the quality and quantity of SOC, biological activity and environment (Congreves *et al.*, 2015); however, CC did not affect WAS compared to NCC in this study. Rorick and Klavivko (2017) reported that corn–soybean system with CC increased the WAS compared with NCC systems at 0–10 and 10–20 cm depths after 4-year rye CC application. As discussed above, CC effects on soil properties may be influenced by climate conditions and CC type.

Compared to soil physical and chemical properties, soil biological properties such as microbial activity are easier to change (Ajwa *et al.*, 1999; Wang *et al.*, 2015). Therefore, microbiological properties such as soil enzyme activity can be used as a sensitive potential index for maintaining soil biological diversity and soil quality. Urease can promote the hydrolysis of urea in soil, thus supplying nutrients to plants (Lloyd and Sheaffe, 1973; Dharmakeerthi and Thenabadu, 2013). The  $\beta$ -glucosidase is considered a sensitive soil biological indicator which reflects short-term changes in soil quality (Melero *et al.*, 2011; Sanaullah *et al.*, 2016). Higher precipitation was received in May of 2017 compared to May of 2016 (online Supplementary Fig. S1) which caused an increase in soil moisture in June of 2017 compared to 2016 (data not showed), thus increasing decomposition rate and microbial activities, perhaps explaining why both urease and  $\beta$ -glucosidase were lower in 2016 than in 2017 in this study. Soil moisture is the major factor influencing enzyme activity as reported by Brockett *et al.* (2012).

The results reported here showed that urease activity was similar under DC and DC(w) compared to MC at the time when a 5-year rotation cycle was completed, but as it was expected, urease activity was higher under DC and DC(w) compared to MC when a new rotation cycle began afterwards (Fig. 6), due to the fact that lower diversity crops had less soil C and underground biomass inputs (Eivazi *et al.*, 2003). A study conducted by Balota *et al.* (2004b) reported that crop diversity with a high input and diversity of organic materials can directly influence the activity of microbial populations and enzymes in soils compared with MC systems. Similarly, Klose and Tabatabai (2000) reported that the urease activity was higher in 4-year oats–meadow systems as compared to continuous corn. In addition, Zhong *et al.* (2015) reported that banana–papaya (*Carica*) system had higher urease activity than banana MC.



**Fig. 4.** Colour online. Average soil bulk density (BD,  $\text{mg}/\text{m}^3$ ) at 0–5, 5–15, 15–30, 30–45 and 45–60 cm depths under different cropping systems in 2016 and 2017. MC, mono-cropping (continuous wheat); DC, diversified cropping system (wheat, cover crop, corn, pea/barley and sunflower); DC<sub>(w)</sub>, wheat in diversified cropping system; CC, cover crop; NCC, no cover crop (wheat, corn, pea/barley and sunflower); LG, legume; NLG, no legume (wheat, cover crop, corn and sunflower). Vertical bars indicate standard errors of the means. Different letters in the graphs at each depth indicate the significant differences at that depth.

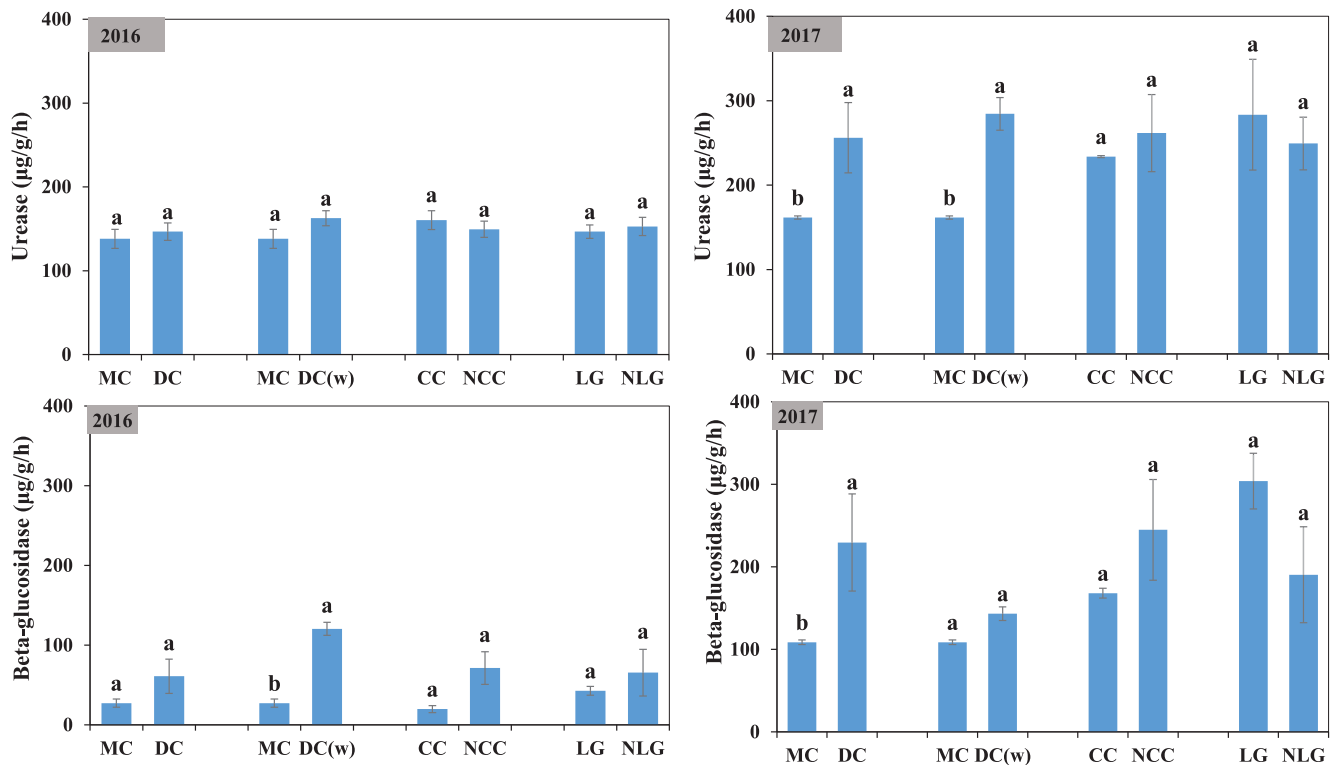


**Fig. 5.** Colour online. Average soil wet soil aggregate stability (WAS %) at 0–5 and 5–15 cm depths under different cropping systems in 2016 and 2017. MC, mono-cropping (continuous wheat); DC, diversified cropping system (wheat, cover crop, corn, pea/barley and sunflower); DC<sub>(w)</sub>, wheat in diversified cropping system; CC, cover crop; NCC, no cover crop (wheat, corn, pea/barley and sunflower); LG, legume; NLG, no legume (wheat, cover crop, corn and sunflower). Vertical bars indicate standard errors of the means. Different small letters indicate significance at 0–5 cm depth, and different capital letters indicate a significant difference at 5–15 cm depth.

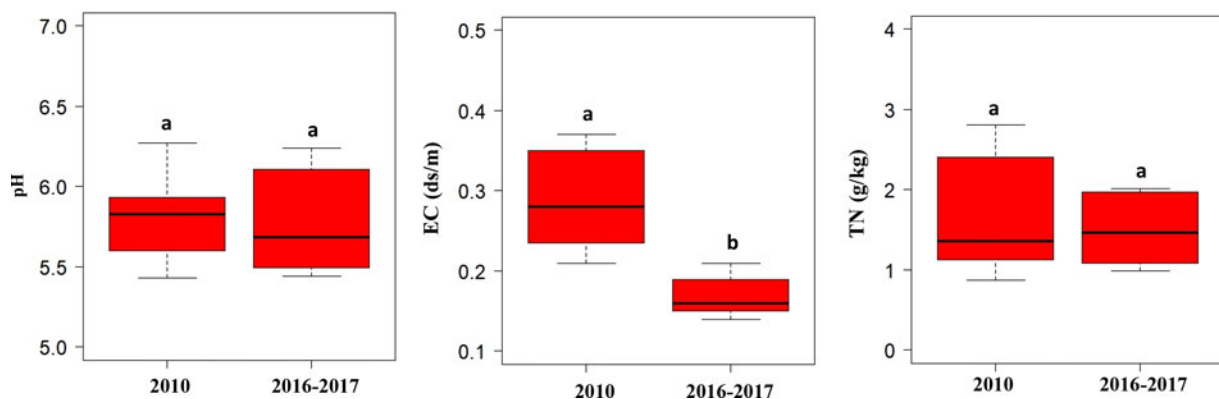
Lower  $\beta$ -glucosidase under MC compared to DC(w) at the time when a 5-year rotation cycle was completed under MC compared to DC when a new rotation cycle began afterwards (Fig. 6) can be attributed to the fact that DC can modify the soil C content and, thereby, increasing the available energy source for crop growth (Acosta-Martínez *et al.*, 2003; Qin *et al.*, 2017). Differences in weather conditions between 2016 and 2017 (Fig. 2) may result in differences in the response of  $\beta$ -glucosidase to the cropping system in these 2 years as discussed earlier. Zhang *et al.* (2014) reported that the bacterial community and  $\beta$ -glucosidase activity under corn-soybean system and continuous corn treatment were similar. The effect of CC and legumes on urease and  $\beta$ -glucosidase activity was not observed in the current study (Fig. 6). However, Mbutia *et al.* (2015) found that cropping systems including CC (vetch) significantly increased the  $\beta$ -glucosidase content compared to treatments without CC, most likely because different quantities of SOC input and microbial respiration are produced by different types of CC. Liang *et al.* (2014) found that the Austria winter pea had a significantly positive effect on  $\beta$ -glucosidase compared to NCC and other types of CC (hairy vetch and crimson). The different results between these cited studies and the current study may be attributed to the different kinds of legumes and CC utilized.

In this study, comparison of data measured to baseline values in this study at the research site prior to the establishment of treatments revealed that crop rotation did not affect pH and TN, but decreased EC over time (Fig. 7). The results of soil pH and TN were consistent with that reported by Neugschwandtner *et al.* (2020) that observed no significant





**Fig. 6.** Colour online. Average soil urease ( $\mu\text{g/g/h}$ ) and  $\beta$ -glucosidase ( $\mu\text{g/g/h}$ ) enzymes activity at 0–5 cm depth under different cropping systems in 2016 and 2017. MC, mono-cropping (continuous wheat); DC, diversified cropping system (wheat, cover crop, corn, pea/barley and sunflower); DC<sub>(w)</sub>, wheat in diversified cropping system; CC, cover crop; NCC, no cover crop (wheat, corn, pea/barley and sunflower); LG, legume; NLG, no legume (wheat, cover crop, corn and sunflower). Vertical bars indicate standard errors of the means. Different letters in the graphs at each depth indicate the significant differences at that depth.



**Fig. 7.** Colour online. Average values of soil pH, electrical conductivity (EC, ds/m) and total nitrogen (TN, g/kg) under different cropping systems for 2010, and 2016–2017 at 0–5 cm depth. Vertical bars indicate standard errors of the means. Different letters in the graphs indicate significant differences.

difference in soil pH and TN after 15 years of different crop rotation managements. However, salt levels in the soil were decreased over time. This reduction over time can be attributed to the removal of nutrients by crops (Drury *et al.*, 2004), supporting the results reported by Gura and Mnkeni (2019), who found that crop rotation had positive effects in reducing salinity levels.

## Conclusions

This study was conducted to explore the response of soil quality to crop diversity under no-till system. Soil properties were compared

between DC and MC, DC<sub>(w)</sub> and MC, CC and NCC, and LG and NLG for 2 years. The soil pH, SWR, TN, CWC, CWN, HWC and HWN were not affected by cropping systems for both years. Crop diversity improved SOC, MBC, WAS and urease and  $\beta$ -glucosidase activity, and lowered BD. Crop diversity, especially cropping systems including cover crops, can sustain soil quality by enhancing SOC and reducing soil BD, making a great contribution for agroecosystems sustainable for soils. Future studies are needed to characterize the long-term crop diversity influence on soil quality.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0021859620000994>

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**Conflict of interest.** None.

**Ethical standards.** Not applicable.

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