

Crop rotation enhances soybean yields and soil health indicators

Ikechukwu V. Agomoh^{1,2}  | Craig F. Drury¹  | Xueming Yang¹  | Lori A. Phillips¹ 
| W. Daniel Reynolds¹

¹ Agriculture and Agri-Food Canada,
Harrow, Ontario, Canada

² Agriculture and Agri-Food Canada,
Fredericton, New Brunswick, Canada

Correspondence

Craig F. Drury, Agriculture and Agri-Food
Canada, Harrow, Ontario, Canada.
Email: craig.drury@canada.ca

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Abstract

Soybean (*Glycine max* L.) is known to contribute to soil N reserves when grown in rotation with other high-value crops such as corn (*Zea mays* L.) and winter wheat (*Triticum aestivum* L.). However, continuous soybean and “short” soybean rotations (e.g., corn–soybean, wheat–soybean) may cause declining soybean yields and degrading soil health over time. In this long-term field study, we determined crop rotation effects on soybean yield and soil health indicators for a cool, humid, clay loam soil in southwestern Ontario. The study used nine soybean rotations, which included continuous soybean (S), corn–soybean (C–S), corn–soybean–soybean (C–S–S), and six rotations where winter wheat (WW) was grown with red clover (*Trifolium pratense* L.) (+RC) and without underseeded red clover (i.e., winter wheat–soybean [WW–S, WW+RC–S], corn–soybean–winter wheat [C–S–WW, C–S–WW+RC], and winter wheat–soybean–soybean [WW–S–S, WW+RC–S–S]). Ten soil health indicators during the first and second soybean phase of each rotation were measured in 2018, whereas soybean yield was measured from 2002 to 2018. Soybean yields in 2- and 3-yr rotations were 39–44% and 48–52% greater, respectively, relative to continuous soybean excluding the rotations with 2 of 3 yr of soybean (C–S–S, WW–S–S, WW+RC–S–S), which were only 22–35% greater than continuous soybean. Partial least squares regression indicated that inorganic N, particulate organic matter N, particulate organic matter C, potentially mineralizable N, total C, soil respiration rate, and water extractable organic soil C were the most important soil health indicators, explaining 34% of the total variation in soybean yields. It was concluded that soybean grown in 3-yr rotations with corn and winter wheat produced the largest soybean yields and the greatest positive impacts on soil health indicators likely owing to cereal crops enhancing C inputs into soil.

Abbreviations: CC, cover crop; IN, soil inorganic nitrogen; PLS, partial least square; PMN, potentially mineralizable soil nitrogen; POMC, carbon content of the particulate soil organic matter; POMN, nitrogen content of the particulate soil organic matter; POXC, permanganate oxidizable soil carbon; RESP, soil respiration; SOC, soil organic carbon; SOM, soil organic matter; TC, total soil carbon; TN, total soil nitrogen; VIP, variable importance in the projection; WEOC, water extractable organic soil carbon; WEON, water extractable organic soil nitrogen.

1 | INTRODUCTION

Soybean (*Glycine max* L.) is an important grain crop in many regions of the world because of its potential to produce high protein for food and feed (Orf, 2010; Pagano & Miransari, 2016). In 2018, there were 125 Tg of soybean produced in the United States alone (USDA–NASS, 2018). In Canada, it

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is estimated that over 7.3 Tg of soybean were produced in 2018, an 18% increase from 6.2 Tg in 2015, with most of the production occurring in central Canada (Statistics Canada, 2015, 2018). In 2018, approximately 2.6 and 36 million ha of agricultural land were planted to soybean in Canada and the United States, respectively (Statistics Canada, 2018; USDA–NASS, 2018). Soybean yield and production continues to increase over time mainly due to improved agronomic practices and the introduction of high yield soybean cultivars that are better able to withstand environmental stresses and disease (Grassini et al., 2014; Orf, 2010; Specht et al., 2014). The longer crop-growing period in the U.S. corn belt and central Canada may have also encouraged soybean production in these regions (Gaudin, Tolhurst et al., 2015).

The majority of U.S. and Canadian soybean is grown in 2- and 3-yr rotations with corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Gaudin, Tolhurst et al., 2015; Karlen et al., 1994) because of the profitability and soil health benefits of crop rotation (Bullock, 1992; Gaudin, Janovicek et al., 2015; Hoss et al., 2018). It is well documented that crop rotation improves soil health and resilience by increasing yields (Bullock, 1992; Karlen et al., 1994), increasing soil organic C (SOC) (Lal, 2016; McDaniel & Grandy, 2016; Poeplau & Don, 2015), improving soil structure (Campbell & Zentner, 1993; Raimbault & Vyn, 1991), enhancing nutrient availability (Copeland & Crookston, 1992), reducing buildup of plant pests and pathogens, and increasing disease-suppressing soil microbes (Krupinsky et al., 2002; Peralta et al., 2018). However, increasing frequency of soybean in crop rotations may impair crop productivity and soil health through greater disease pressure and loss of SOC. In Wisconsin, Lund et al. (1993) suggested that soybean yields might be increased when grown one year out of three in 3-yr rotations, such as corn–soybean–wheat, compared with growing it every other year in corn–soybean rotations. Crop rotations with a high frequency of soybean have decreased SOC storage and reduced macroaggregation because of low residue input by soybean (Studdert & Echeverría, 2000; Wright & Hons, 2004, 2005). As a leguminous crop, soybean derives its N supply through biological N₂ fixation as well as utilizing soil inorganic N (Córdova et al., 2019; Salvagiotti et al., 2008). Soybean residue has a low C to N ratio (relative to cereal crops), which promotes rapid residue decomposition (Halvorson & Schlegel, 2012; Martens, 2000a, 2000b; Wright & Hons, 2004). Martens (2000a, 2000b) reported lower C accumulation and sequestration in stable soil aggregates from soybean residue compared with corn and cereal residues, which the authors attributed to low C to N ratio and low phenolic acid content of soybean residue.

Because more agricultural land in central Canada and the U.S. Midwest is planted to corn and soybean relative to other field crops (Bullock, 1992; Gaudin, Janovicek et al., 2015; Hailu et al., 2015; Hoss et al., 2018), there is a substantial

Core Ideas

- Including cereal crops in rotations with soybean increases soybean yields and soil health.
- Crop rotation increased POMC 73–91%, whereas TC was increased 13–17% relative to continuous soybean.
- Compared with continuous soybean, soybean yields were 39–44% greater for 2-yr rotations with corn and wheat.
- Compared with continuous soybean, soybean yield was 48–52% greater for 3-yr rotations with corn and wheat and only 1 yr of soybean.
- Changes in soil health parameters PMN, POMN, IN, WEOC, POMC, TC, and RESP accounted for 34% variability in soybean yield.

need to determine how soybean impacts soil health using soil health indicators such as those included in the Cornell Soil Health Test (Idowu et al., 2008; Moebius-Clune et al., 2016) and Haney Test (Haney et al., 2012, 2018), and now also recommended by the Soil Health Institute (SHI, 2017). These indicators include crop yield and the following soil biochemical indicators: soil inorganic N concentration (IN), soil potentially mineralizable N concentration (PMN), soil respiration rate (RESP), permanganate oxidizable C concentration (POXC), total soil C concentration (TC), total soil N concentration (TN), water extractable organic C concentration (WEOC), water extractable organic N concentration (WEON), particulate organic matter C (POMC), and particulate organic N (POMN) content of the soil. The primary objective of this study was to determine crop rotation effects on soybean yields and soil biochemical health indicators. The study also sought to identify soil health indicators that could predict soybean yield or explain the variability in productivity in the different cropping systems. We hypothesized that soybean grown in more diverse rotations would produce greater soybean yields and better soil health than soybean grown in less diverse rotations or as a continuous crop.

2 | MATERIALS AND METHODS

2.1 | Field site

The study made use of an ongoing long-term (18 yr) field experiment (initiated in the fall of 2001) at the Honourable Eugene F. Whelan Experimental Farm, Woodslee, ON (42°13' N, 82°44' W) (Drury et al., 2008; Zhang et al., 2014). The region has a moderate humid continental climate with a mean annual air temperature of 9.9 °C and annual

precipitation of 934 mm (ECCC, 2020). The field plots were located on a poorly drained Brookston clay loam soil (mesic Typic Argiaquolls).

2.2 | Treatments

The treatments included continuous, 2-, 3-, and 4-yr crop rotations, laid out in a randomized complete block design with four replications (144 field plots, each 20 m long by 6.1 m wide); each phase of each rotation was present every year. In 2018, a subset of these treatments (48 field plots ending the cropping sequence in the soybean phase of the rotation) was selected for this study and soil samples were collected for soil health assessment from nine rotations typical to Ontario (Drury et al., 2008) that included soybean in the cropping sequence: continuous soybean (S), corn–soybean (C–S), corn–soybean–soybean (C–S–S), winter wheat–soybean with and without underseeded red clover (*Trifolium pratense* L.; WW+RC–S, WW–S), corn–soybean–winter wheat with and without underseeded red clover (C–S–WW+RC, C–S–WW), and winter wheat–soybean–soybean with and without underseeded red clover (WW+RC–S–S, WW–S–S). The average of the first and second year of soybean was used for C–S–S, WW–S–S, and WW+RC–S–S crop rotations as this average would be more important to producers than using just the first or second year of soybean in the comparison with other rotations. The 2006, 2012, and 2018 agronomic operations for soybean are summarized in Table 1, and additional detail for corn and winter wheat management can be found in Agomoh et al. (2020). A chisel plow (Glencoe Equipment) to ~18–20 cm depth was used in the fall following corn and soybean grain harvest on all crop treatments except those planted to winter wheat (wheat is planted directly into the soybean stubble) with secondary tillage in the spring with a cultivator prior to planting corn and soybean.

2.3 | Soil biochemical health indicator analysis

Ten replicate soil cores were collected from the 0-to-15-cm depth of each plot in July 2018 using a zigzag or “w” pattern as described in Agomoh et al. (2020). The 10 cores from each plot were composited, sieved to 4 mm, placed in plastic containers, and stored at 4 °C in darkness until IN and PMN were analyzed. The remaining soil biochemical indicators (i.e., RESP, POXC, TC, TN, WEOC, WEON, and POMC) were measured on subsamples after air-drying.

Soil IN (nitrate [NO₃-N] + ammonium [NH₄-N]) was determined by extracting the soil with 2 M KCl (1:5 soil/solution ratio), and the concentrations of NO₃-N and NH₄-N in the filtered extract were analyzed using the

TABLE 1 Information of the agronomic operation

Year	Date	Field operation
2006	8 May	herbicide applied to plots (Roundup 1.4 kg a.i ha ⁻¹)
	2 June	soybean planted to plots (Pioneer 92M50 RR; 486,693 seeds ha ⁻¹) using a 15" Kearney planter
	6 June	herbicide applied to plots (Dual II Magnum 1.4 kg a.i ha ⁻¹ + Sencor 0.5 kg a.i ha ⁻¹)
	6 July	herbicide applied to plots (Roundup 1.4 kg a.i ha ⁻¹)
2012	16 Oct.	soybean grain harvested with a Wintersteiger plot combine
	17 May	soybean planted to plots (Pioneer 92M91; 486,693 seeds ha ⁻¹) using a 15" Kearney planter
	22 June	herbicide applied to plots (Roundup 0.9 kg a.i ha ⁻¹ + Classic 9 g a.i ha ⁻¹)
	27 June	foliar application of Manganese 2 kg a.i ha ⁻¹ on plots to control visual Mn deficiency
2018	11 Oct.	soybean grain harvested with a Wintersteiger plot combine
	29 May, 3 July	herbicide applied to plots (Roundup 1.4 kg a.i ha ⁻¹)
	5 June	soybean planted (Pioneer P22T69R; 592,622 seeds ha ⁻¹) using a 15" Kearney planter;
		herbicide applied to plots (Dual II Magnum 1.6 kg a.i ha ⁻¹ + Sencor 0.5 kg a.i ha ⁻¹)
	12 July	foliar application of Manganese 5 L ha ⁻¹ on plots to control visual Mn deficiency
	24 July	soil sampling (0–15 cm) from all soybean plots for soil health indicators determination
	17 Oct.	soybean grain harvested with a Wintersteiger plot combine

cadmium reduction (QuickChem Method 12-107-04-1-B) and sodium salicylate (QuickChem Method 12-107-06-2-A) methods, respectively. Potentially mineralizable soil N was calculated as soil NH₄-N concentration after 7-d anaerobic incubation at 40 °C minus initial soil NH₄-N concentration (Curtin & Campbell, 2008). The nitrate and ammonium concentrations for IN and PMN determination were measured using a Lachat flow injection analyzer (Hach QuikChem 8500).

The flush of CO₂ (also known as the short-term soil heterotrophic respiration rate) was determined via aerobic incubation (22 °C) using methods modified from Hopkins (2008)

and Zibilske (1994). A 4-mm sieved, air-dried subsample of soil (100 g) was placed in a 1-L Mason jar, and wetted to 60% water-filled pore space. A 10-ml glass vial containing deionized water was then placed in the jar (to maintain 100% relative humidity), and the jar was covered with parafilm.

Immediately after 4 d of preincubation (to remove the “Birch effect”) (Birch, 1959; Hopkins, 2008), the parafilm was removed and the jars capped with lids equipped with an airtight 9-mm rubber septum. At the start of incubation ($t = 0$), gas samples from the head space of each closed jar were collected using a 24-ml syringe and injected into 12-ml pre-evacuated Exetainers (Labco). After 24 h, a second set of samples were collected and injected into a second set of Exetainers. The jars were then aerated for 15 min by removing lids, and resealed with the lids and placed back into the incubators. This sequence was repeated daily for another 3 d. The concentration of CO_2 in headspace gas was measured using a chromatograph (Varian 450) equipped with a thermal conductivity detector.

Soil POXC was estimated using the Weil et al. (2003) method, modified by Schindelbeck et al. (2016). Briefly, 2 ml of 0.2 mol L^{-1} potassium permanganate (KMnO_4) solution was added to a 50-ml centrifuge tube containing 2.5 g of soil with 18 ml deionized water. The tubes were shaken for 2 min at 120 rpm and allowed to settle for exactly 10 min, after which 0.5 ml of the supernatant was transferred to a second 50-ml centrifuge tube containing 49.5 ml of deionized water. Sample absorbance was read with a spectrophotometer (Beckman Coulter DU 640) at 550 nm. Soil permanganate oxidizable C was determined using KMnO_4 standard curves and the equation of Weil et al. (2003):

$$\text{POXC}(\text{mg kg}^{-1}\text{soil}) = [0.02 \text{ mol L}^{-1} - (m + n \times \text{Abs})] \times (9,000 \text{ mg C mol}^{-1}) \times (0.02 \text{ L solution Wt}^{-1})$$

where 0.02 mol L^{-1} is the concentration of the initial KMnO_4 solution; m and n are intercept and slope of the standard curve, respectively; Abs is the absorbance of the extracted soil; 9,000 mg is the amount of C oxidized by 1 mol of MnO_4 converting from Mn^{7+} to Mn^{2+} ; 0.02 L is the volume of KMnO_4 solution reacted; and Wt is the mass of soil (kg) used.

The WEOC and WEON were determined from the supernatant of a 5-g subsample of air-dried soil that was shaken with 50 ml of deionized water for 30 min at 120 rpm on a horizontal shaker, and then filtered through $0.45 \mu\text{m}$ (GN-6, Pall Corporation Metricel Black PES membrane disk filter). The concentrations of WEOC and total water extractable N were analyzed using a TOC-L analyzer (Shimadzu Scientific Instruments) equipped with a N analysis module (Shimadzu TNM-L). The WEON concentration was subsequently calculated as the difference between total water extractable N and inorganic N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$).

Total C and N were determined on air-dry soil that was ball milled and sieved to pass $250 \mu\text{m}$, then dry-combusted using a LECO TruMac CN analyzer (Leco Corp.). Particulate organic matter C and POMN were determined using the method of Yang et al. (2009, 2012). Specifically, 15 g air-dried soil was dispersed by shaking in 100 ml of sodium hexametaphosphate solution overnight using a rotatory shaker set at 120 rpm. The dispersed soil was then passed through a $53\text{-}\mu\text{m}$ sieve, and the retained sand fraction oven-dried at 60°C , weighed, ball milled, and analyzed as above for TC and TN. The TC was assumed to be organic C as these surface soils were free of carbonates (pH ranged from 5.8 to 6.9).

2.4 | Statistical analyses

Analysis of variance (ANOVA) was performed using the GLIMMIX procedure (SAS Institute Inc., 2018) to determine the effect of crop rotation on soybean yield and soil biochemical health indicators. The average of the first and second soybean year in the C–S–S, WW–S–S, and WW+RC–S–S rotations was used in the final ANOVA. We used the average soybean yields from 17 previous years (2002–2018), instead of the 2018 soybean yields from the nine soybean cropping treatments, to ensure detection of multiyear yield effects that could be masked in any given year (e.g., 2018) by variations in weather and field operations. The UNIVARIATE procedure (SAS Institute Inc., 2018) was used to assess each dependent variable for normality and homogeneity of variance prior to ANOVA. The normal distribution was specified in PROC GLIMMIX for yield, TN, TC, POMC, and POMN, and the lognormal distribution for RESP, PMN, POXC, IN, WEOC, and WEON. Crop rotation was modeled as a fixed factor and block (i.e., replicate) as the random effect. Treatment differences were compared at $\alpha = .05$ using the Tukey–Kramer adjustment for multiple comparisons. The correlations among the variables was explored by computing the Pearson’s correlation coefficient using the CORR procedure in SAS (SAS Institute Inc., 2018).

Partial least square (PLS) regression analysis was performed using PROC PLS in SAS (SAS Institute Inc., 2018) to identify soil biochemical health indicators (predictor or explanatory variables) that best explain the variability in soybean yield (response variable) across all the cropping systems. A subset of the soybean yield data (average of 2006, 2012, and 2018) was used for PLS regression as these treatment plots were the same for all 3 yr and correspond to the same plots that were sampled in 2018 for soil biochemical health indicators. Partial least square analysis is a versatile method for multivariate data analysis and is preferred over ordinary least square regression techniques for our data because PLS is not affected by multicollinearity arising from intercorrelation among soil properties (Mehmood et al., 2012; Ping et al., 2004). Partial

TABLE 2 Effect of rotation on soil respiration (RESP), potentially mineralizable N (PMN), permanganate oxidizable C (POXC; i.e., active C), inorganic N (IN), water extractable organic C (WEOC), water extractable organic N (WEON), total C (TC), total N (TN), soil organic C (POMC), and soil organic N (POMN) of the particulate organic matter and soybean yield

Effect ^a	RESP ^c	PMN	POXC	IN	WEOC	WEON	TC	TN	POMC	POMN	Yield ^b
	mg C kg ⁻¹ h ⁻¹										
S	0.79b	8.3d	708a	15.4a	78b	9.6a	19.7d	2.04c	2.03b	0.09b	2.3d
C-S	1.39ab	15.6abc	688a	8.8ab	116a	11.5a	21.0bcd	2.11bc	3.05ab	0.16ab	3.3ab
WW-S	1.35ab	18.7abc	656a	11.9a	106ab	11.3a	21.2abcd	2.13abc	3.22ab	0.18ab	3.2abc
WW+RC-S	1.63a	28.6a	699a	13.8a	109a	12.1a	22.4 abc	2.31a	3.88a	0.24a	3.3ab
C-S-S	1.30ab	15.1bcd	734a	14.3a	102ab	10.2a	20.9cd	2.12abc	3.27ab	0.17ab	3.1abc
C-S-WW	1.77a	25.3ab	723a	2.6b	119a	12.0a	22.8a	2.31ab	3.80a	0.22a	3.5a
C-S-WW+RC	1.79a	20.3 abc	724a	5.4ab	108a	12.1a	22.7ab	2.28ab	3.52a	0.20ab	3.4ab
WW-S-S	1.29ab	13.7cd	686a	12.3a	93ab	10.6a	20.9cd	2.13abc	2.96ab	0.16ab	2.8c
WW+RC-S-S	1.32ab	18.9 abc	702a	13.8a	109a	12.4a	22.2abc	2.29ab	3.21ab	0.19ab	3.0bc
P value											
Rotation	.01	<.001	.18	.01	.01	.22	<.001	.0002	.01	.01	<.0001

^aS, continuous soybean; C-S, corn-soybean; WW-S, winter wheat-soybean; WW+RC-S, winter wheat plus red clover-soybean; C-S-S, corn-soybean-soybean; C-S-WW, corn-soybean-winter wheat; C-S-WW+RC, corn-soybean-winter wheat plus red clover; WW-S-S, winter wheat-soybean-soybean; WW+RC-S-S, winter wheat plus red clover-soybean-soybean. The soil health indicators and soybean yield in C-S-S, WW-S-S and WW+RC-S-S rotations represent the average of the first and second year of soybean.

^bAverage yields of soybean grown in 2002 to 2018 (17 yr), whereas the soil health indicators were measured in soil samples in 2018.

^cMeans followed by the same letter are not significantly different at the .05 probability level according to the Tukey multiple comparison procedure.

least square latent variables were computed to explain most of the variations in both the response and the predictor variables. The number of PLS latent variables that minimized the predicted residual sum of squares was determined by using the split cross-validation method (CV = SPLIT). The variable importance of projection (VIP) statistic, as described by Wold (1995), measures the relative contribution of each predictor variable to the PLS latent variables. Only variables with VIP values greater than 0.8 were considered sufficiently influential to significantly explain the variation of the response variable (Wold, 1995). The predictive strength of the model was estimated by regressing the response variable measured values against the predicted values obtained by cross-validation using PROC REG in SAS (SAS Institute Inc., 2018).

3 | RESULT AND DISCUSSION

3.1 | Soil health indicators

Soil respiration varied significantly ($P = .01$) with cropping treatment (Table 2). Soil respiration rates were twice as high in C-S-WW+RC (1.79 mg C kg⁻¹ h⁻¹) and C-S-WW (1.77 mg C kg⁻¹ h⁻¹) compared with continuous soybean (0.79 mg C kg⁻¹ h⁻¹), but were not significantly different from the other rotations (1.29–1.63 mg C kg⁻¹ h⁻¹). It should be noted that these 3-yr rotations (C-S-WW+RC, C-S-WW) with higher respiration rates only had 1 yr of soybean in the rotations,

whereas all other 3-yr rotations had 2 yr of soybean (C-S-S, WW-S-S, and WW+RC-S-S). Increased crop diversity in rotations (i.e., C-S-WW, C-S-WW+RC) results in greater crop residue inputs and thereby more sequestration of atmospheric C and plant nutrient stored in soil organic matter (SOM) (Franzluebbers et al., 2018). Increased SOM typically means increased microbial biomass and hence increased respiration.

Lower respiration in the continuous soybean may also indicate increased C limitation (i.e., less labile C in the soil). There was a significant ($P < .001$) rotation effect on PMN (Table 2), which was 1.9–3.4 times greater for C-S (15.6 mg kg⁻¹ soil), WW-S (18.7 mg kg⁻¹ soil), WW+RC-S-S (18.9 mg kg⁻¹ soil), C-S-WW+RC (20.3 mg kg⁻¹ soil), C-S-WW (25.3 mg kg⁻¹ soil), and WW+RC-S (28.6 mg kg⁻¹ soil) than for continuous S (8.3 mg kg⁻¹ soil), but continuous S was not significantly different from the WW-S-S and C-S-S rotations (13.7–15.1 mg kg⁻¹ soil). Soil respiration rate was significantly and positively correlated with PMN ($r = .64$, $P < .001$, $n = 36$; Table 3), which may explain the lower PMN under continuous soybean because of the low availability of organic matter resources in the soil, which may further lower soil biological activities and soil health.

Permanganate oxidizable C was not affected by crop rotation (Table 2). Inorganic N was significantly greater ($P = .01$) for S (15.4 mg kg⁻¹ soil), C-S-S (14.3 mg kg⁻¹ soil), WW+RC-S-S (13.8 mg kg⁻¹ soil), WW+RC-S (13.8 mg kg⁻¹ soil), WW-S-S (12.3 mg kg⁻¹ soil), and WW-S

TABLE 3 Pearson correlation coefficients and significance levels for correlations for the soil variables in the data under soybean, including yield (Yield, t ha⁻¹), soil respiration (RESP, mg C kg⁻¹ h⁻¹), potentially mineralizable N (PMN, mg kg⁻¹ soil), permanganate oxidizable C (POXC, mg kg⁻¹ soil), inorganic N (IN, mg kg⁻¹ soil), total N (TN, g kg⁻¹ soil), total C (TC, g kg⁻¹ soil), water extractable organic C (WEOC, mg kg⁻¹ soil), water extractable organic N (WEON, mg kg⁻¹ soil), soil organic C (POMC, g kg⁻¹ soil), and soil organic N (POMN, g kg⁻¹ soil) of the particulate organic matter

	Yield	RESP	PMN	POXC	IN	TN	TC	WEOC	WEON	POMC	POMN
Yield	1										
RESP	0.38*	1									
PMN	0.49**	0.64***	1								
POXC	-0.06	0.33*	0.22	1							
IN	-0.47**	-0.37*	-0.36*	0.04	1						
TN	0.28	0.38*	0.63***	0.43**	-0.12	1					
TC	0.41*	0.68***	0.71***	0.42**	-0.29	0.83***	1				
WEOC	0.47**	0.55***	0.36*	0.18	-0.34*	0.19	0.50**	1			
WEON	0.28	0.49*	0.26	0.30	-0.21	0.33*	0.51**	0.82***	1		
POMC	0.43**	0.60***	0.67***	0.27	-0.29	0.29	0.56***	0.47**	0.29	1	
POMN	0.48**	0.47**	0.66***	0.21	-0.25	0.42*	0.54***	0.36*	0.24	0.91***	1

Note. Coefficients with values >0.31 and that are statistically significant are shown in bold font.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

***Significant at the .001 probability level.

(11.9 mg kg⁻¹ soil), than for C-S-WW (2.6 mg kg⁻¹ soil), but not different for the other rotations (5.4–8.8 mg kg⁻¹ soil; Table 2). Soil IN generally increased with increasing frequency of soybean in the crop rotation (i.e., S, C-S-S, WW+RC-S-S), suggesting that atmospheric N fixed by soybean-rhizobia directly contributed to soil N supply (Córdova et al., 2019; Salvaggiotti et al., 2008). Similarly, N fixation by red clover in WW+RC-S and WW+RC-S-S systems, combined with N mineralization from N-rich red clover residues, would contribute to greater IN pools in those systems. Soybean itself generates low amounts of residue with low C/N ratio relative to cereal crops, but rapid mineralization of this residue (Halvorson & Schlegel, 2012; Wright & Hons, 2004) may also have contributed to greater soil IN in S, C-S-S, WW+RC-S, WW+RC-S-S, and WW-S-S. Lower IN in soybean following corn phases may be due to immobilization of N as the high quantities of residual corn residues are decomposed by soil microbes (Vanhie et al., 2015).

The apparent immobilization of IN when high crop residues are present compared with crop removal were also observed in a stover removal field study at five residue removal rates (Drury et al., 2020). They found greater nitrate exposure when crop residues were removed, which resulted in greater N₂O emissions compared with the no-residue removal treatment.

Water extractable organic C was significantly ($P = .01$) affected by the cropping treatments (Table 2). Water extractable organic C was 1.4–1.5 times greater for C-S-WW+RC (108 mg kg⁻¹ soil), WW+RC-S-S (109 mg kg⁻¹ soil), WW+RC-S (109 mg kg⁻¹ soil), C-S (116 mg kg⁻¹

soil), and C-S-WW (119 mg kg⁻¹ soil), than continuous S (78 mg kg⁻¹ soil), but not different from S in the other rotations (93–106 mg kg⁻¹ soil). The greater WEOC under the 2-yr (C-S and WW+RC-S) and 3-yr (C-S-WW, C-S-WW+RC, and WW+RC-S-S) rotations than continuous S indicates that organic C (which serves as an energy source for soil microorganisms) was added to the soil by the cereal crops in the rotations (Haney et al., 2012). Water extractable organic N was not affected by crop rotation (Table 2).

Total soil C differed significantly ($P < .001$) between cropping treatments (Table 2). The WW+RC-S-S (22.2 g kg⁻¹ soil), WW+RC-S (22.4 g kg⁻¹ soil), C-S-WW+RC (22.7 g kg⁻¹ soil), and C-S-WW (22.8 g kg⁻¹ soil) rotations resulted in 13–16% greater TC compared with continuous S (19.7 g kg⁻¹ soil), but not significantly different from S in the other rotations (20.9–21.2 g kg⁻¹ soil). Cereal crops return high levels of C (via aboveground and belowground biomass and root exudates), which supports more active and abundant soil microbial life, as indicated by the strong positive correlation between TC and microbial respiration ($r = .68$, $P < .001$, $n = 36$; Table 3). Rotation had a significant effect ($P = .0002$; Table 2) on the total N, which was 12–13% greater in C-S-WW+RC (2.28 g kg⁻¹ soil), WW+RC-S-S (2.29 g kg⁻¹ soil), C-S-WW (2.31 mg kg⁻¹ soil), and WW+RC-S (2.31 g kg⁻¹ soil), compared with continuous S (2.04 g kg⁻¹ soil), but not different from S in the other rotations (2.11–2.13 g kg⁻¹ soil).

Particulate organic matter C was significantly ($P = .01$; Table 2) affected by rotation, with POMC being 73–91%

greater in C–S–WW+RC (3.52 g kg⁻¹ soil), C–S–WW (3.80 g kg⁻¹ soil), and WW+RC–S (3.88 g kg⁻¹ soil) than continuous S (2.03 g kg⁻¹ soil), but not different from S in the other rotations (2.96–3.27 g kg⁻¹ soil). Particulate organic matter N also differed significantly ($P = .01$) among rotations (Table 2), being 2.4–2.7 times greater for C–S–WW (0.22 g kg⁻¹ soil) and WW+RC–S (0.24 g kg⁻¹ soil) than for continuous S (0.09 g kg⁻¹ soil), but not different from S in the other rotations (0.16–0.20 g kg⁻¹ soil). The greater TC and POMC in the 2-yr (WW+RC–S) and 3-yr (C–S–WW and C–S–WW+RC) rotations when compared with continuous S may be due to greater C inputs to the soil because of greater root biomass and residue returns from corn and wheat and RC relative to continuous soybean. In our study, the quantity of residue produced after harvest is much greater for grain corn than for wheat or soybean (data not shown). In Ontario, Agomoh et al. (2020) found that POMC declined in crop rotations where wheat followed soybean compared with cereal crops, in part due to decreased residue being returned to the soil following soybean harvest. Lower concentrations of POMC and POMN in continuous soybean may indicate decreasing labile SOM available for nutrient cycling, maintenance of soil structure, and stabilization of soil macroaggregates (Cambardella & Elliott, 1992). Studies have found particulate organic matter (e.g., POMC and POMN) to be a readily available substrate for microbes, and the major pathway through which nutrients (especially N) are transferred from crop residues to the soil and made available for crop uptake (Gregorich et al., 2006; Haynes, 2005; Whalen et al., 2000). Unlike soybean, corn residues have been reported to have higher phenolic acid content, which is a crucial component of soil humic substances and aggregate stabilization (Martens, 2000b). It has also been reported that adding winter wheat in corn–soybean rotations increases N use efficiency (Gaudin, Janovicek et al., 2015) and enhances soil C and N pools (Franzluebbers et al., 1994a, 1994b). Hence, continuous S may result in lower accumulation and sequestration of C in stable soil aggregates because soybean residue has lower C/N ratios and phenolic acid content relative to corn and cereal residue (Martens, 2000a, 2000b). In this study, it is interesting to note that the increase in the labile organic matter pool (i.e., POMC) was considerably greater than the increase in total C. This is consistent with POMC being more sensitive than total C to agricultural practices that affect the amount of crop residue inputs (Gregorich et al., 2006; Haynes, 2005). Several studies have reported that among other factors, POMN may vary seasonally depending on the quantity and quality of organic residue inputs in the soil (Boone, 1994). We found that POMN had a larger pool (0.09–0.24 g kg⁻¹) of labile organic N than WEON (9.6–12.4 mg kg⁻¹) regardless of rotation. The POMN may represent a fraction of the soil organic N that is susceptible to decomposition early in the growing season, thereby boosting the supply of plant-available N to the subse-

quent crop when N requirement is high (Haynes, 2005). The greater TN in C–S–WW relative to continuous soybean agrees with Zuber et al. (2015) in Illinois, who found greater TN in the top 60 cm under C–S–WW than continuous soybean. The authors attributed this to greater residue returns from corn and wheat relative to soybean.

3.2 | Soybean yields

Soybean yields (average yield for the period from 2002 to 2018) varied significantly ($P < .0001$) with cropping treatment (Table 2). Soybean yield was 39–44% greater in the 2-yr (3.2–3.3 t ha⁻¹) and 22–52% greater in the 3-yr (2.8–3.5 t ha⁻¹) crop rotations compared with continuous S (2.3 t ha⁻¹). If the 3-yr rotations that had 2 yr of S in the rotation were excluded (i.e., C–S–S, WW–S–S, and WW+RC–S–S), then the 3-yr rotations with only 1 yr of soybean had yields of 3.4–3.5 t ha⁻¹ or a 48–52% increase in soybean yield with crop rotation compared with continuous soybean. Similarly, soybean yield was 25% greater for C–S–WW (3.5 t ha⁻¹) and 21% greater for C–S–WW+RC (3.4 t ha⁻¹) than WW–S–S (2.8 t ha⁻¹), and was 17% greater for C–S–WW than WW+RC–S–S (3.0 t ha⁻¹) rotation. This indicates that soybean yield is increased when the frequency of soybean in the rotation is reduced. Our results agree with other studies that have reported greater soybean yields under rotations (i.e., 2-yr C–S) when compared with continuous soybean (Al-Kaisi et al., 2016; Houx et al., 2014; Mazzilli & Ernst, 2019). In Missouri, Houx et al. (2014) reported that, averaged across the 2 yr of their study, soybean in a 2-yr C–S rotation yielded 0.43 Mg ha⁻¹ (12%) and 0.35 Mg ha⁻¹ (9.2%) more than continuous soybean under conventional tillage and no-tillage, respectively. Furthermore, other studies have reported that, in most situations, soybean yield was increased when the C–S rotation included winter wheat (Gagnon et al., 2019; Gaudin, Janovicek et al., 2015; Lund et al., 1993). In Québec, Gagnon et al. (2019) reported that, averaged across tillage systems (i.e., moldboard plow and reduced tillage), soybean yield was increased by 0.2 Mg ha⁻¹ (5.7%) when soybean was grown in rotation with corn and wheat (i.e., C–S–W) compared with continuous soybean. The authors attributed the reduced grain yields under continuous soybean to increasing pest predation and/or soil-borne plant pathogens.

The comparatively low aboveground and belowground biomass from soybean could reduce soil organic C levels, which may explain the lower soybean yields observed under continuous soybean. The higher yields of soybean under rotation compared with continuous soybean reflects the critical role that different crops (i.e., crop diversity) in the rotation play in improving soil properties, especially increased SOM (McDaniel et al., 2014; Smith et al., 2008). Inclusion of cereal crops (e.g., corn and winter wheat) and a cover crop

TABLE 4 Partial least square regression with the variable importance in the projection (VIP) and regression coefficients (*b*) for soil biochemical health indicators used to predict soybean yield averaged for 2006, 2012, and 2018

Variables ^a	VIP	<i>b</i>
RESP	0.95	0.08
PMN	1.24	0.11
POXC	0.15	-0.03
IN	1.19	-0.10
TN	0.70	0.06
TC	1.04	0.09
WEOC	1.18	0.10
WEON	0.72	0.06
POMC	1.08	0.09
POMN	1.21	0.11
PLS factors ^b	1	
Overall model PV ^c	34	
R ² (VIP >0.8) ^d	0.35	

Note. Soil health indicators with VIP values >0.8 are shown in bold font.

^aRESP, soil respiration; PMN, potentially mineralizable N; POXC, permanganate oxidizable C; IN, inorganic N; TN, total N; TC, total C; WEOC, water extractable organic C; WEON, water extractable N; POMC, soil organic C of the particulate organic matter; POMN, soil organic N of the particulate organic matter.

^bNumber of partial least square (PLS) factors derived from cross validation.

^cPercentage of variation (PV) that is explained by the PLS factor in the cross-validation step.

^dPredictive strength assessed by linear regression of measured values of response variables vs. predicted values obtained by cross-validation step using predictors with VIP >0.8.

(e.g., red clover) in the rotation increases the resource available for heterotrophic soil microbial communities, which in turn increases C and N cycling and thereby greater crop productivity compared with continuous soybean (Bullock, 1992; McDaniel et al., 2014). Lower yields coupled with lower root biomass from soybean likely resulted in decreased storage of soil C under continuous soybean relative to soybean in rotation with corn and winter wheat (i.e., WW+RC-S, C-S-WW, C-S-WW+RC, and WW+RC-S-S).

3.3 | Predicting the variability in soybean yield

A one-latent variable PLS model explained 34% of the total variability in soybean yield (Table 4). Seven soil biochemical health indicators (i.e., PMN, POMN, IN, WEOC, POMC, TC, and RESP) had a significant contribution to the model (VIP >0.8), which partially explained the variability in average soybean yields in 2006, 2012, and 2018. A PLS analysis indicated a generally positive correlation between soybean yield and the soil biochemical health indicators except for

soil IN in which yield was negatively correlated. Based upon VIP, the soil biochemical health indicators PMN, POMN, IN, WEOC, POMC, and TC had greater influence in explaining the variability in soybean yield under the conditions of this study. The greater soil biochemical indicators values (i.e., RESP, PMN, POMN, WEOC, TC, and POMC) in soybean under rotations (i.e., C-S-WW+RC, C-S-WW) when compared with continuous soybean reflects the greater quantity and diversity of resources (e.g., root exudates and crop residues) added to the soil under rotational cropping, which possibly promoted the growth and activity of different groups of soil microbial communities (e.g., heterotrophic soil bacteria) involved in nutrient cycling and the maintenance of soil organic matter, with an overall benefit of increasing the soil health (McDaniel et al., 2014; Paul, 2016; Tiemann et al., 2015).

4 | CONCLUSIONS

Our results indicate that growing soybean continuously can degrade soybean yields and soil health indicators appreciably. Soybean yield was reduced when it was grown as continuous, but greater when grown in rotation with cereal crops. Greater POMN, TC, and POMC in C-S-WW and C-S-WW+RC further indicates that corn, winter wheat, and red clover residues return more C and new C sources to the soil than soybean. This means improved soil C storage, microbial activity (via respiration activity), and increased crop yields when the frequency of soybean is reduced in rotations typical to Ontario, Canada.

Our results also suggest that negative impacts of soybean on soil health are mitigated by including cereal crops in rotation. Partial least squares regression analysis indicated that the soil biochemical health indicators, PMN, POMN, IN, WEOC, POMC, TC, and RESP explain 34% of the variation in soybean yield among rotations, with PMN, POMN, IN, WEOC, POMC, and TC being most influential. However, the predictive power of the model containing these soil biochemical health indicators was very low. Including additional indicators pertaining to soil microbial, chemical, and physical properties may boost the model's predictive power. Further research is needed to explore the influence of soybean rotations on indicators of soil microbial and physical health.

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AUTHOR CONTRIBUTIONS

Ikechukwu V. Agomoh, Conceptualization, Formal analysis, Investigation, Validation, Visualization, Writing-original draft, Writing-review and editing; Craig F. Drury, Conceptualization, Funding acquisition, Resources, Validation, Writing-review and editing; Xueming Yang, Conceptualization, Funding acquisition, Resources, Validation, Writing-review and editing; W. Daniel Reynolds, Conceptualization, Funding acquisition, Writing-review and editing.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

ORCID

Ikechukwu V. Agomoh  <https://orcid.org/0000-0002-5735-2272>

Craig F. Drury  <https://orcid.org/0000-0003-0986-0755>

Xueming Yang  <https://orcid.org/0000-0002-2665-5748>

Lori A. Phillips  <https://orcid.org/0000-0003-1005-5331>

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