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Impacts of winter wheat and cover crops on soil microbial diversity in a corn–soybean no-till cropping system in Quebec (Canada)

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Abstract

Intensive agriculture based on repeatedly plowing a monoculture is known to degrade the structural, chemical, and biological properties of soils. Conservation agriculture is gaining popularity worldwide with practices including reduced tillage, increased plant diversity, and implementation cover crops. The aim of this study was to assess the impact of crop rotation and cover crops on soil microbial communities. The hypothesis was that enhanced plant diversity would boost the diversity of the soil microbiome. Two rotation were tested by adding a cereal (corn [*Zea mays* L.]–soybean [*Glycine max* (L.) Merr.] and corn–soybean–wheat [*Triticum aestivum* L.]), as well as the implementation of cover crops. The enhanced plant diversity had no impact on total molecular biomass. The bacteria/fungi ratio varied across the plots but was not clearly linked to the enhanced plant diversity. Bacterial richness was not influenced by the treatments, whereas eukaryotic richness decreased in the presence of cover crops in one of the sites. Microbial composition was the most sensitive indicator to enhanced plant diversity. Differential relative abundance (log2 fold changes) identified proteobacterial amplicon sequence variants (ASVs) specifically related to each crop rotation system and to the presence of cover crops. There was more ASV of Actinobacteria associated with the three-crop rotation system and less ASV of Acidobacteria associated with the cover crops system compared to the two- and three-crop rotation systems, respectively. As for eukaryotes, the number of ASVs belonging to Ascomycota and Cercozoa phyla and associated with the three-crop rotation system is less important than for the two-crop rotation system. This study shows that conservation agricultural practices can influence soil microbial communities. The variations in some ASVs could have functional implications on organic matter decomposition or plant growth and in terms of soil ecosystem services and field crop sustainability.

1 | INTRODUCTION

According to the FAO (2020), soil health is defined as "the ability of soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems." Soil biology contributes to maintaining many soil functions. It plays a role in chemical processes by breaking down organic matter, which enables the acquisition and cycling of essential nutrients by cultivated plants. This degradation and the resulting products of microbial origin are also essential to the formation of clay-humus complexes that contributes to carbon storage and soil physical stability (Kallenbach et al., 2016; Kibblewhite et al., 2008; Lehmann & Kleber, 2015). Soil health is usually measured using physicochemical indicators or, to a lesser extent, biological indicators (Lehmann et al., 2020). The biological properties of a soil can be assessed using indicators such as organic matter content, microbial biomass, soil respiration, and enzymatic activity (Allen et al., 2011). The microbiota of agricultural soils is composed of complex microbial communities, and recent developments in metagenomic analyses have made it possible to study more precisely the behavior of these communities when exposed to various farming practices (Lehman et al., 2015; Wall et al., 2019). Assessing the richness and composition of bacterial and eukaryotic communities could enable the selection of appropriate biological indicators of agricultural soil health (Lehman et al., 2015; Sahu et al., 2018; Trivedi et al., 2016).

Deep plowing of agricultural soils is a weed management practice that mechanically alters the soil structure, causing surface erosion and deep compaction (Baumhardt et al., 2015). This practice has a direct impact on soil microbial communities and can decrease microbial biomass and activity (Lupwayi et al., 2012; Sapkota et al., 2012). Various plowing practices can also alter the composition of bacterial communities (Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013). Conservation agriculture combines practices such as minimal soil disturbance, enhanced plant diversity, and cover crops implementation (Kassam et al., 2018). Based on these three principles, the direct seeding on cover crops (DSCC) technique was first used in the southern hemisphere, mainly to control soil erosion caused by plowing (Carpentier et al., 2020; Séguy et al., 2012). When DSCC is used in field crops to minimize soil disturbance, weed management is often achieved using glyphosate-based herbicides in combination with glyphosate-resistant crops (Benbrook, 2016; Dill et al., 2008).

In conventional monocultures, the low genetic diversity of cover crops and soil microorganisms increases the risk of diseases as it enhances pathogen resistance (Mundt, 2002). The enhanced plant diversity associated with crop rotation can have positive effects such as boosting soil microbial biomass and richness as well as modifying the composition of microbial communities (C. Li et al., 2009; McDaniel et al., 2014; Tiemann et al., 2015; Venter et al., 2016; Xuan et al., 2011), even though modifications have not been observed for every indicator in the literature (C. Li et al., 2009; Peralta et al., 2018; Tiemann et al., 2015). Crop rotation can also have diverging effects on the balance between bacteria and fungi (Muhammad et al., 2021). Cover crops can alter the physicochemical properties of the soils. For example, they can help stabilize the soil structure with their root systems (Kaspar & Singer, 2011). Legumes used as cover crops can also boost the availability of organic carbon and nitrogen (Balota et al., 2014; Verzeaux et al., 2016). Cover crops can have positive effects on multiple soil health indicators, but multi-species cover crop mixes do not necessarily improve them better than one specie cover crop (Decker et al., 2022; Florence & McGuire, 2020; Reed & Morrissey, 2022). Moreover, cover crops can influence the soil microbiome. They can either boost microbial biomass (Balota et al., 2014; Hartwig & Ammon, 2002; Kim et al., 2020) or cannot (Romdhane et al., 2019; Sapkota et al., 2012). They can also impact microbial diversity positively (Manici et al., 2018), negatively (Peralta et al., 2018) or have neutral effects (Chamberlain et al., 2020; Romdhane et al., 2019).

In Quebec, cover crops are still rarely used in field crops, the harsh and long winter makes their establishment difficult. Their impacts on microbial communities have not yet been measured in this context. Moreover, studies using metagenomics tools are still scarce, and very few studies have assessed the impacts of cover crops and crop rotation on prokaryotes and eukaryotes, especially on fungi and protists.

The main goal of this study was to assess the impacts of an enhanced plant diversity on soil microbial communities in field crops in Quebec. The first specific objective was to assess the impacts of a more diversified crop rotation on the soil microbiome diversity by adding a cereal after soybean [*Glycine max* (L.) Merr.] culture in a corn (*Zea mays* L.)–soybean rotation design. The second specific objective was to assess the impacts of the presence or absence of cover crops on the soil microbiome diversity in cultivated crops. The hypothesis was that the treatments associated with an enhanced

Core Ideas

- Crop rotation and cover crops can both influence bacterial composition.
- Microbial composition is more sensitive to plant diversity than molecular biomass and microbial richness.
- Eucaryotic richness and composition are influenced negatively by plant diversity enhancement.

plant diversity would influence the quantity and diversity of microbial communities in the soils. The impacts of each treatment on the quantity of microorganisms as well as the richness and the composition of bacterial and eukaryotic communities were assessed.

2 | MATERIALS AND METHODS

2.1 | Cropping systems of the experimental plots

Soil sampling was carried out in the fields of two field crop producers in the south of Quebec, one located in Ste-Marthe (45°24,025′ N, −74°20,890′ O) and the other one in Montmagny (46°57,326′ N, 70°33,775′ O). Nine experimental plots measuring 12 m by 200 m were set up in 2016 at both study sites. In each location, the plots were lined up side by side in a single field, in clay soils in Ste-Marthe and in loamy-silty soils in Montmagny according to Info-Sols (MAPAQ, 2011).

The cropping systems tested were based on the rotation of corn, soybean, and common wheat (*Triticum aestivum* (L.)). Systems included direct seeding without cover crops associated with a two-crop rotation (corn-soybean) (DS2C) or a three-crop rotation (corn–soybean–wheat) (DS3C), as well as direct seeding with cover crops associated with a three-crop rotation (DSCC). Each plot kept the same cropping system throughout the years (DSCC, DS3C, or DSCC), but the type of culture varied every year (soybean, corn, or wheat) (Figure S1). Cover crops in the DSCC system were sown every year from 2016 with different species each year based on the annual crop of interest. Cover crops composition for the year preceding the sampling in spring 2019 is detailed below. In 2018 in Ste-Marthe, a rye cover (*Secale cereale* (L.)) was sown at the end of the season in plot number 3 (cultivated with corn), whereas an alfalfa cover (*Medicago sativa* (L.)) and a winter common wheat cover were, respectively, sown in the spring and at the end of the season in plot number 6 (cultivated with soybean). A multispecies cover was sown at the beginning of the summer in plot number 9 (cultivated with winter common wheat), which included alfalfa, sunflower (*Helianthus annuus* (L.)), phacelia (*Phacelia* Juss., 1789), pearl millet (*Pennisetum glaucum* (L.) R.Br., 1810), sorghum (*Sorghum bicolor* (L.) Moench, 1794), buckwheat (*Fagopyrum esculentum* Moench, 1794), oat (*Avena sativa* (L.)), radish (*Raphanus sativus* (L.)), crimson clover (*Trifolium incarnatum* (L.)), and garden pea (*Pisum sativum* (L.)). In Montmagny, no cover crops were sown at the beginning of the season in plots number 3 (cultivated with corn) and number 6 (cultivated with soybean), but there were germinated alfalfa seeds that had been sown during the previous year. Moreover, winter common wheat was sown in plot number 6 at the end of the season. In plot number 9 (cultivated with winter common wheat), a multispecies cover containing oat, hairy vetch (*Vicia villosa* Roth, 1793), broad bean (*Vicia faba* (L.)), 4010 pea, forage radish (*R. sativus* (L.) var. *oleifera*), sunflower, and common vetch (*Vicia sativa* (L.)) was sown in the fall.

2.2 | Soil sampling

Soil sampling was performed in the early spring of 2019 before the main crops were sown. Soil cores were collected in the 0- to 20-cm depth horizon of each plot using a 20-cm length and 3-cm diameter auger. Three different samples were collected in each plot. Each sample was a composite of three soil cores collected within 1 m of the GPS sampling point. Cores of each sample were then homogenized and kept at a temperature of −20°C until the analyses.

2.3 | Physicochemical analyzes of the soils

Soil texture was assessed by sedimentation (Gee & Bauder, 1986). Values for sand, loam, and clay contents are provided in Table S1. Soil contents in phosphorus, potassium, calcium, magnesium, aluminum, bore, copper, iron, manganese, zinc, sodium, nickel, cadmium, chrome, cobalt, and palladium were quantified with a Mehlich 3 extraction (Mehlich, 1984) followed by determination with an inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Optima 4300DV, Shelton, CT, USA). Content values are provided in Table S2.

2.4 | Analysis of soils microbial diversity

2.4.1 | DNA extraction and quality control

To analyze the soil microbiome, frozen soil samples were dried at room temperature for 72 h, and then ground, homogenized, and sieved through a 2-mm screen. Sub-samples of 400 mg of soils were then used to extract DNA following the protocol provided with the FastDNA SPIN Kit for Soil commercial kit (MP Biomedicals, Solon, OH, USA). DNA samples were eluted in 100 µl of elution solution (pyrogen and DNase-free distilled water). The quality of the DNA samples was controlled with a 1.6% (w/v) agarose gel electrophoresis and visualized with a fluorescent coloration using a GelDoc XR+ camera (Biorad, Hercules, CA, USA). DNA quantification was performed by spectrophotometry with a Biophotometer D30 (Eppendorf, Hamburg, Germany) using the 260 and 280 nm absorbance measurements and the A260/A280 ratio.

2.4.2 | Bacteria and fungi molecular counting

Molecular counting was performed with qPCR targeting the V6-V8 regions of bacterial 16S and fungal 18S rRNA. Amplification was performed using the eub338/eub518 primers for bacteria (Fierer et al., 2005) and FF390/FR1 for total fungi (Emerson et al., 2015) with the SYBR® green qPCR master mix (Qiagen, Toronto, ON, Canada). Detection was repeated twice on a CFX96 Touch System device (Biorad). Results are expressed as amplification units (A.U.) per gram of dry soil. It should be noted that the targeted genes can be detected multiple times in a single organism and in variable numbers between organisms during quantification; it is true for bacterial organisms (16S rRNA) as well as for fungal organisms (18S rRNA) (Smith & Osborn, 2009). Detection systems are based on a 4-log detection range with an efficiency rate of 89.1% ($R^2 = 0.99$) for total bacteria and 91.7% ($R^2 = 1$) for total fungi.

2.4.3 | Sequencing of rDNA amplicons

The metagenomic analyses evaluated the bacterial and eukaryotic diversity targeting V3–V4 region in bacterial 16S and V4 region in eukaryotes 18S. The amplification was performed with dual-indexed PCR approach using primers 515F (Parada et al., 2016) and 806RB (Apprill et al., 2015) for prokaryotes and E572F/E1009R (Comeau et al., 2011) for eukaryotes following the method described in Jeanne et al. (2019). Libraries were sequenced in a paired-end format with a reading of 300 base pairs on each side of the DNA strand using an Illumina MiSeq high throughput sequencer. These analyses were performed at the genomic analysis platform of the Institute of Integrative Biology and Systems (IBIS) at the Université Laval (Quebec, Canada).

2.5 | Bioinformatics

Bioinformatics analyses were performed using the bioinformatics platform of the Microbial Ecology Laboratory at the Research and Development Institute for the Agri-Environment. The DADA2 approach (Callahan et al., 2016) was used to validate sequences quality and identify amplicon sequence variants (ASVs) within the QIIME 2 platform (Bolyen et al., 2019). Taxonomic identification of ASV was performed using the following reference databases: Greengenes 13.5 for 16S (DeSantis et al., 2006), PR^2 (Guillou et al., 2013) for 18S, and SILVA (Quast et al., 2013) for 16S and 18S rRNA. Sequences were normalized at 10,000 per sample before statistical analyses.

2.6 | Statistical analyses

Analyses were performed with the R software (R Core Team, 2020) using the phyloseq package (McMurdie & Holmes, 2013), and figures were created using the ggplot2 package (Wickham, 2011). Statistical analyses of the microbial quantification data and log bacteria/fungi ratio were performed using the agricolae package (De Mendiburu & Simon, 2015). Data normality was assessed with a Shapiro–Wilk test. Normal data were compared using analysis of variance followed by Tukey tests for contrasts, whereas non-normal data were compared using a nonparametric Kruskal–Wallis test with a Benjamini-Hochberg correction. Microbial diversity was studied by measuring microbial richness on the one hand and microbial composition on the other. Shannon and Chao1 indexes of bacteria and eukaryotes were used to estimate microbial richness, using the statistical treatment previously described. Bacterial and eukaryotic composition were visualized using nonmetric multidimensional scaling of the Bray–Curtis dissimilarity matrix. Composition differences were assessed with permutational multivariate analysis of variance (PERMANOVA) statistical tests on QIIME 2 using the adonis function (Anderson, 2001; Oksanen et al., 2018). Relative abundance of the 10 main phyla were identified using the ampvis2 package in R (Andersen et al., 2018). A differential analysis of the bacterial and eukaryotic ASV abundance was performed using the DESeq2 package in R (Love et al., 2014). This analysis enables the visualization of contrasts between two treatments at the level of ASVs that have been grouped by phylum to facilitate visualization. A statistical significance threshold of 0.05 was used for all statistical analyses.

3 | RESULTS

3.1 | Quantification of bacteria and fungi using qPCR

Total number (A.U. g^{-1} dry soil) of bacteria and fungi detected in the soils at both study sites showed no significant difference ($p > 0.05$) between the three cropping systems when the plots cultivated with different crops of interest are combined (Figure S2). A detailed analysis of each plot showed that the plot number 6 in Montmagny (DSCC wheat; soybean as the previous crop) was the only one with a total number of fungi (A.U. g^{-1} dry soil) significantly higher than the others ($p = 0.001$) (Table S3).

3.2 | Bacteria/fungi ratio

At both study sites, no significant difference was found in the bacteria/fungi molecular quantification log-ratio between the

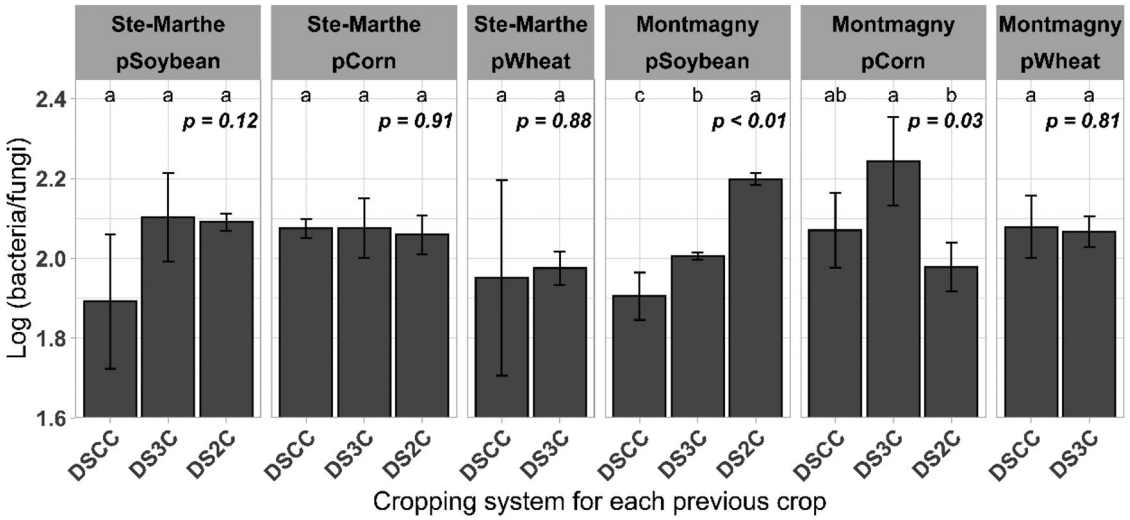

FIGURE 1 Ratio of molecular quantification for fungi/bacteria at both study sites between the three cropping systems based on the previous crop. DSCC, direct seeding on cover crops; DS2C, direct seeding without cover crops associated with a two-crop rotation (corn–soybean); DS3C, direct seeding without cover crops associated with a three-crop rotation (corn–soybean–wheat)

three cropping systems when the plots cultivated with different crops of interest are combined ($p = 0.27$). In Ste-Marthe, the bacteria/fungi molecular quantification log-ratio was not statistically different across all eight treatments ($p = 0.25$). As for the plots that were cultivated with soybean in 2018 in Montmagny, the ratio was lower in the DSCC plot than in both plots without cover crops (DS2C and DS3C) and also lower in the three-crop rotation (DS3C) than in the two-crop rotation (DS2C) (Figure 1).

It is worth noting that the same trend, although nonsignificant, was found in Ste-Marthe: the bacteria/fungi log-ratio was lower in the DSCC plot than in the two other plots where soybean was the previous crop. In Montmagny plots that were cultivated with corn in 2018, the ratio was lower in the DS2C plot than in the DS3C plot, whereas there was no difference between the DSCC plot and the other two plots. There was no difference between the plots that were cultivated with wheat in 2018 (Figure 1). When comparing the plots under the same cropping system, the bacteria/fungi ratio was significantly lower in the plots cultivated with soybean in 2018 than in the ones cultivated with corn for the DS3C design, whereas the opposite situation was observed for the DS2C design. No significant difference was observed between the plots associated with the DSCC design (Table S4).

3.3 | Bacterial and eukaryotic richness estimate

The Shannon and Chao1 indexes used to estimate bacterial species richness showed no statistically significant differences across the eight different treatments at both study sites ($p > 0.05$) (Table S5). Significant differences are observed between cropping systems when the plots cultivated with different crops of interest are combined. In Ste-Marthe, Shannon and Chao1 indexes for eukaryotic species were lower in the soils where cover crops were present (DSCC) than in the other soils (DS2C and DS3C) (Figure 2).

In plots cultivated with soybean in 2018, the Chao1 index for eukaryotes in the DSCC was lower than the DS2C system. No other difference in the eukaryotic richness indexes was observed between the different systems for the same previous crop. Moreover, previous crops showed no significant effect on richness indexes between plots of the same cropping system. In Montmagny, no significant difference was observed in the eukaryotic richness indexes across the eight treatments ($p > 0.05$) (Table S5). At both study sites, bacterial richness indexes were significantly higher than eukaryotic richness indexes ($p < 0.05$).

3.4 | Microbial composition

The PERMANOVA of the Bray–Curtis dissimilarity matrix showed no significant difference ($p > 0.05$) between the composition of every plot for bacteria as well as for eukaryotes. However, differences in composition were observed when the plots were combined based on cropping systems or previous crops (Table 1). Regarding bacteria in Ste-Marthe plots, the direct seeding without cover crops systems with a three-crop rotation (DS3C) and a two-crop rotation (DS2C) was significantly different ($F = 1.706$). However, the bacterial and eukaryotic compositions in the soils associated with the DSCC system did not differ from the DS2C and DS3C systems. In Montmagny plots, all three cropping systems had significantly a different composition, with the distance between centroids being larger between both three-crop systems (DSCC and DS3C) than with both systems without cover

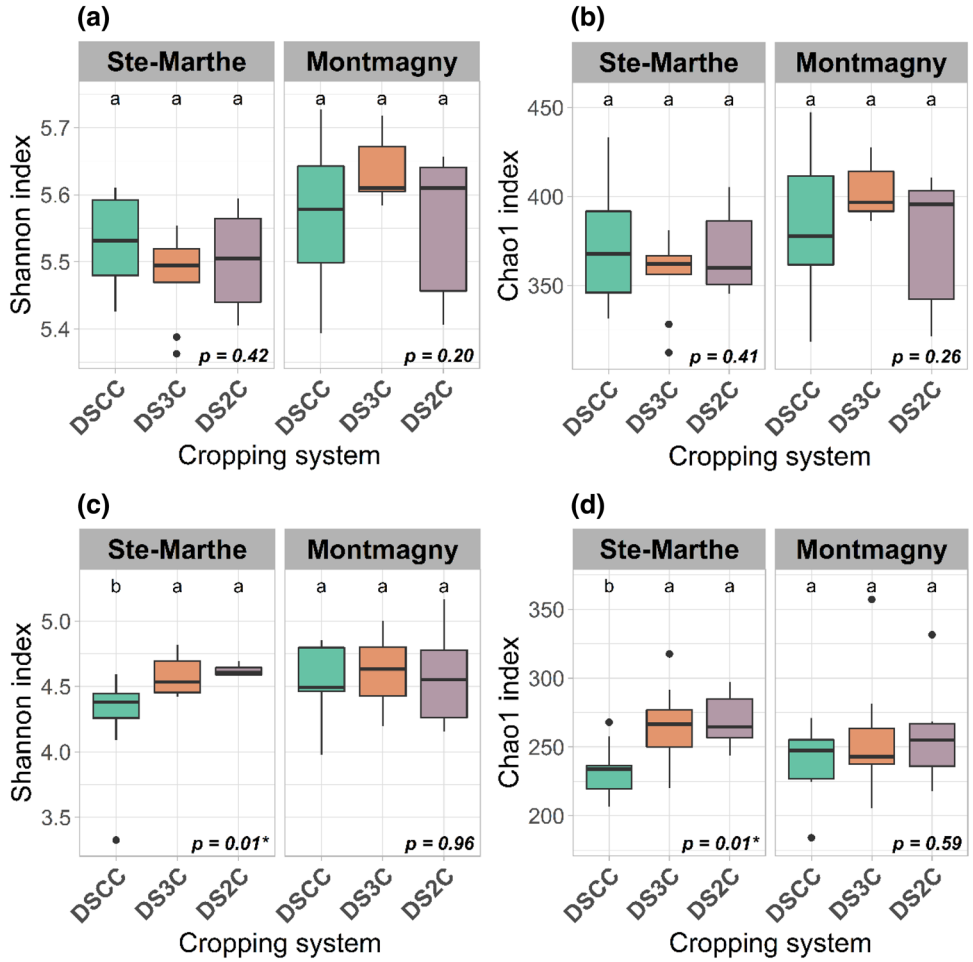

FIGURE 2 Richness estimate using Shannon and Chao1 indexes of bacteria (a and b) and eucaryotes (c and d) for each cropping system at both study sites. The color code is as follows: DSCC (green), DS3C (orange), and DS2C (purple). DSCC, direct seeding on cover crops; DS2C, direct seeding without cover crops associated with a two-crop rotation (corn–soybean); DS3C, direct seeding without cover crops associated with a three-crop rotation (corn–soybean–wheat)

TABLE 1 Permutational multivariate analysis of variance (PERMANOVA) of the microbial composition between cropping systems at both sites

	Cropping systems											
	Ste-Marthe						Montmagny					
	DSCC-DS3C		DS3C-DS2C		DSCC-DS2C		DSCC-DS3C		DS3C-DS2C		DSCC-DS2C	
PERMANOVA	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value
Bacteria	0.98	0.43	1.71	0.04*	1.18	0.19	1.92	<0.01*	1.41	0.02*	1.77	0.02*
Eukaryotes	1.18	0.14	1.42	0.03*	1.39	0.03*	1.45	0.11	1.47	0.01*	1.71	0.01*

Note: The asterisk shows a statistically significant difference (p value < 0.05). DSCC, direct seeding on cover crops; DS2C, direct seeding without cover crops associated with a two-crop rotation (corn–soybean); DS3C, direct seeding without cover crops associated with a three-crop rotation (corn-soybean-wheat)

crops (DS2C and DS3C) ($F = 1.920$ and $F = 1.407$). The analysis of the eukaryotic composition showed differences between the DS2C system and both three-crop rotation systems at both study sites, whereas no difference was found between both three-crop rotation systems with and without cover crops (Table 1).

Previous crops were not associated with an impact on bacterial composition. Plots cultivated with soybean in 2018 were the only ones that showed differences in terms of eukaryotic composition (Table 2). Details regarding the relative abundance of the 10 main bacterial and eukaryotic phyla can be found in Figure S3. Few differences were found

TABLE 2 Permutational multivariate analysis of variance (PERMANOVA) of the microbial composition between previous crops (2018) at both sites

	Previous crops											
	Ste-Marthe						Montmagny					
	Soybean-Corn		Soybean-Wheat		Corn-Wheat		Soybean-Corn		Soybean-Wheat		Corn-Wheat	
PERMANOVA	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value	*F*	*p* value
Bacteria	1.02	0.37	1.39	0.10	1.07	0.34	1.24	0.13	1.03	0.35	1.15	0.16
Eukaryotes	1.25	0.09	1.32	>0.05	1.29	0.06	1.79	0.01*	1.48	0.02*	1.11	0.29

Note: The asterisk shows a statistically significant difference (p value < 0.05).

between the eight plots at this taxonomic level. The most abundant bacterial phyla (>10%) in Ste-Marthe and Montmagny were, respectively, Actinobacteria (29.6%–33.7% and 19.7%–24.6%), Proteobacteria (18.0%–22.6% and 26.1%–29.1%), Acidobacteria (10.2%–11.5% and 12.6%–16.3%), and Chloroflexi (11.6%–13.4% and 10.9%–12.2%).

The most abundant eukaryotic phyla at both study sites belong to the fungi and protists kingdoms and are as follows: Ascomycota (38.7%–52.4% and 26.2%–49.2%), Cercozoa (8.5%–17.1% and 12.1%–25.0%), and Mucoromycota (12.2%–29.1% and 5.6%–16.6%). The DESeq2 analysis showed that treatments affected ASVs of those main phyla.

The bacterial DESeq2 analysis showed that more ASVs were more impacted by rotations (DS3C vs. DS2C) than by the presence of cover crops (DSCC vs. DS3C). The most impacted ASVs belong to the main phyla, but the impacts were different at both sites. Proteobacteria ASVs were impacted by both treatments; rotation had a positive impact in Ste-Marthe plots and a negative impact in Montmagny plots, whereas the cover crops had both a positive and negative impact in Ste-Marthe plots and a negative impact in Montmagny plots. Rotation had a positive impact on Actinobacteria ASVs in Ste-Marthe plots and both a positive and negative impact in Montmagny plots, whereas cover crops had a negative impact on Acidobacteria ASVs (Figure 3). The eukaryotic DESeq2 analysis showed that rotation impacted more ASVs than cover crops, but both aspects had minor impacts on the most abundant phyla including fungi. In general, rotation had a negative impact on Ascomycota ASVs in Ste-Marthe plots and Cercozoa ASVs in Montmagny plots (Figure 4).

4 | DISCUSSION

4.1 | Impacts of cropping systems on microbial biomass

4.1.1 | No visible impact of cropping systems

Findings from this study suggest that neither crop rotation nor cover crops had an impact on molecular microbial biomass. Quantification with qPCR has only recently started being used to estimate molecular microbial biomass (Albright et al., 2020; Kuske et al., 2019). Data regarding the use of molecular biomass to evaluate the effects of cover crops on microbial communities are still scarce. Microbial biomass is often estimated using methods such as microbial biomass carbon (MBC) analysis, microbial biomass nitrogen analysis, or phospholipid fatty acids (PLFA) analysis. Meta-analyses have shown that microbial biomass tends to rise in the presence of cover crops (Kim et al., 2020) and with crop rotation (McDaniel et al., 2014); however, it is not always influenced by an enhanced vegetal diversity according to some studies (Sapkota et al., 2012; Tiemann et al., 2015). The diversity of plant families could be more important than the sole number of species, potentially because of the various root exudates associated with functionally different species (Steinauer et al., 2016). Using a similar method, Romdhane et al. (2019) also concluded that bacterial abundance was not impacted by cover crops nor by different levels of plant diversity ranging from two to eight species from various families (legumes, Poaceae, Cruciferae, and other). The various methods used to estimate biomass can lead to divergent results, especially when comparing the MBC method, commonly used in the past to estimate microbial biomass, with the PLFA and qPCR methods. In fact, the MBC method seems to be less reproducible (Zhang et al., 2017).

The sampling campaign in this study took place in the spring before the sowing of cover crops and main crops. Results therefore reflect the absence of long-term visible effects after the winter, despite a 3-year cycle of crop rotation and a DSCC system. A meta-analysis from Kim et al. (2020) showed that the impacts of cover crops on microbial biomass are more or less visible based on the timing of the sampling and the presence or absence of a plant cover and the main commercial crop. However, the moment when the effects of the cover crops were the most visible differed according to the method used to estimate biomass (MBC or PLFA). Climate can also have an influence on the observed results. Cover crops were initially developed to control soil erosion in southern countries, which could partially explain the differences between these results and other studies. Indeed, it seems that the impacts of cover crops on biomass tend to be smaller in continental climate than in tropical or temperate climate

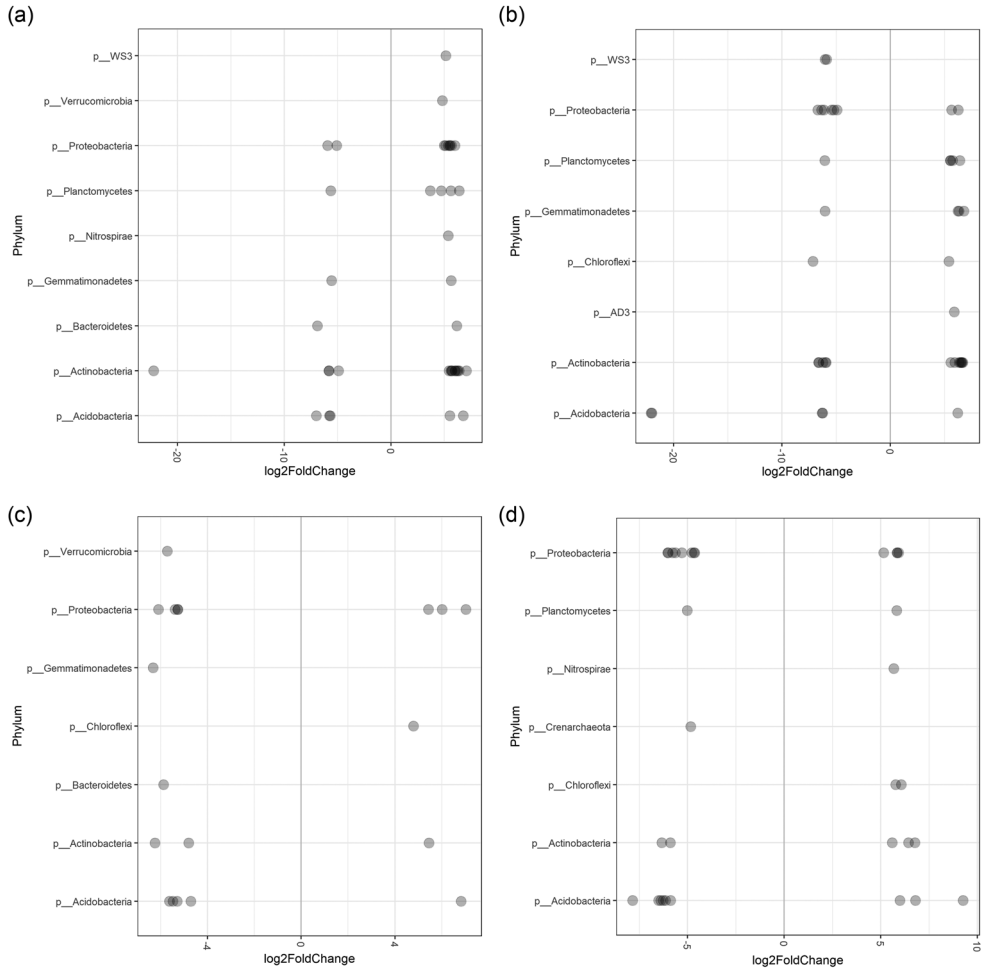

FIGURE 3 DESeq2 analysis comparing bacterial amplicon sequence variants (ASVs) impacted by a three-crop rotation (DS3C) compared to a two-crop rotation (DS2C) in Ste-Marthe (a) and Montmagny (b), as well as bacterial ASVs impacted by the presence of cover crops (DSCC) compared with the same rotation without cover crops (DS3C) in Ste-Marthe (c) and Montmagny (d). DSCC, direct seeding on cover crops; DS2C, direct seeding without cover crops associated with a two-crop rotation (corn–soybean); DS3C, direct seeding without cover crops associated with a three-crop rotation (corn–soybean–wheat)

(Kim et al., 2020). Annual precipitations and soil structure also have an influence on the observed effects of cover crops on microbial biomass (Muhammad et al., 2021).

4.1.2 | Impacts of cropping systems on the bacteria/fungi ratio

In this study, we found significant differences of bacteria/fungi ratio between plots without cover crops, between plots with different cropping systems (SD2C and DS3C), and between plots with different previous crops (soybean or corn) at Montmagny site. Bacteria/fungi ratio was also lower in one DSCC plot at the same study site. No differences between plots were found at Ste-Marthe site.

In the DSCC cropping system at Montmagny, we observed a lower bacteria/fungi ratio in the soil of plot with soybean as a previous crop rather than in soil of the plot with corn. The C/N ratio and hemicellulose content of crop affect the decomposition rate of plant tissues (Beyaert & Paul Voroney, 2011; Broder & Wagner, 1988), which can impact microbial biomass (Hsiao et al., 2019). Soybean can be degraded more rapidly and favor bacteria in the short term, but corn favors fungi in the long term (Broder & Wagner, 1988), while wheat residues composition may also favor fungi (D. Li et al., 2019). In these plots, soybean was preceded by corn and corn by wheat, which may explain the higher presence of fungi in the soybean previous crop. However, in the DS2C system, fungi were favored in the plot with corn grown the previous year compared to the plot with soybean grown the previous year. In these plots, only corn and soybean were rotated. The previous corn crop was grown twice in the last 3 years, including during the first year of the project. The degradation of the older corn residues in this plot may have favored fungi, while bacteria were favored in the previous soybean plot by the new soybean residues.

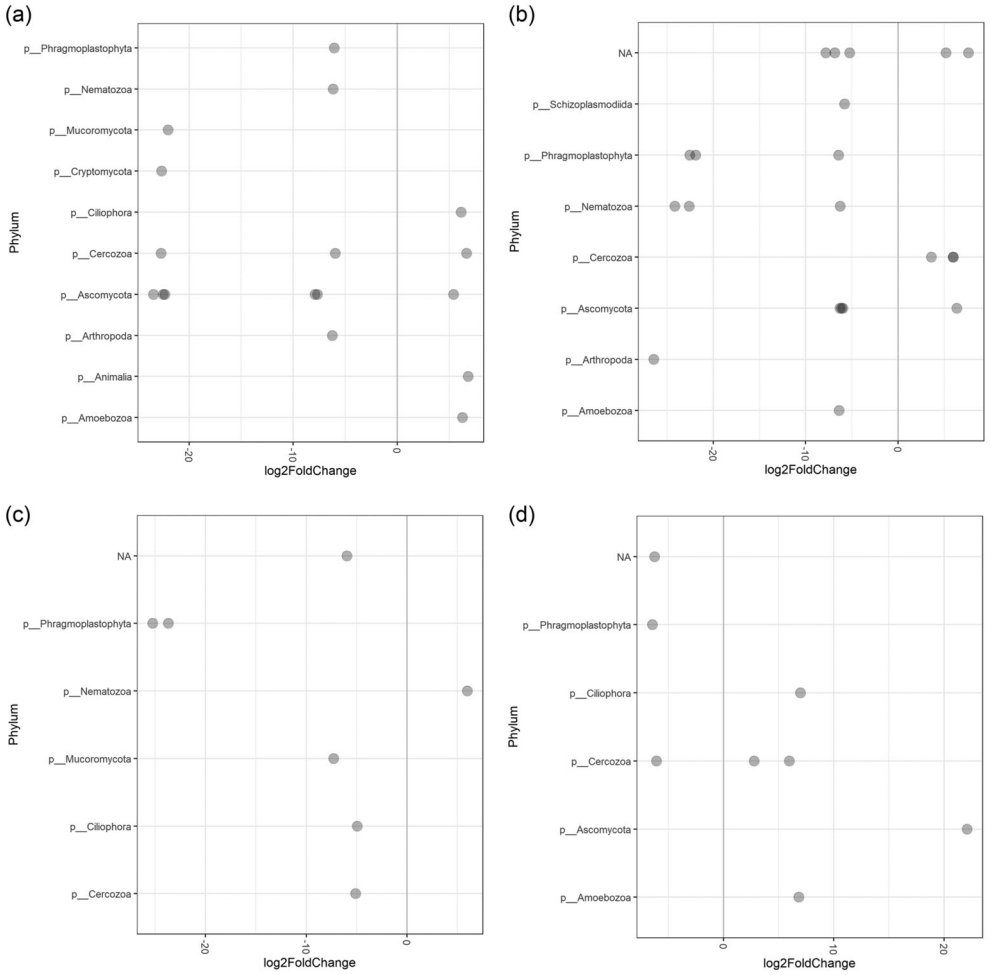

FIGURE 4 DESeq2 analysis comparing eukaryotic amplicon sequence variants (ASVs) impacted by a three-crop rotation (DS3C) compared with a two-crop rotation (DS2C) in Ste-Marthe (a) and Montmagny (b) as well as eukaryotic ASVs impacted by the presence of cover crops (DSCC) compared with the same rotation without cover crops (DS3C) in Ste-Marthe (c) and Montmagny (d). DSCC, direct seeding on cover crops; DS2C, direct seeding without cover crops associated with a two-crop rotation (corn–soybean); DS3C, direct seeding without cover crops associated with a three-crop rotation (corn–soybean–wheat)

The effects of crop rotation on the bacteria/fungi ratio differed in Montmagny based on the previous crops, whereas they were not visible in Ste-Marthe. In the plots without cover crops, the lowest ratio was associated with the three-crop rotation design DS3C in the plots with soybean grown the previous year, whereas it was associated with the two-crop rotation design DS2C in the plots with corn grown the previous year. However, it was expected that crop rotation would have favored fungi over bacteria. Six et al. (2006) showed that more diverse rotations can increase the presence of fungi in the soils. However, in our study, the plots with the lowest plant diversity were minimally associated with a two-crop rotation design and the addition of wheat in the three-crop rotation design only happened once since the plots were set up in 2016. After 10 years of cultivation ranging from a monoculture to a 5-crops rotation, Tiemann et al. (2015) observed no difference in the bacteria/fungi ratio based on plant diversity in aggregates bigger than 0.25 mm, whereas a smaller ratio was observed in the microaggregates associated with the most diversified rotation. Indeed, bacteria are more abundant and have a higher diversity in microaggregates (Bach et al., 2018).

In this study, fungi significantly benefited from the presence of cover crops in the plots with soybean grown the previous year in Montmagny plots. The same trend was observed in Ste-Marthe. Fungi can indeed be favored over bacteria when the plant functional diversity is high (Finney et al., 2017; Lange et al., 2014). This increase could be linked to a higher root biomass and variations in root exudates (Eisenhauer et al., 2017). However, in the plots with previous crops other than soybean, no difference in the ratio was observed, which is surprising, especially in the plots cultivated with wheat, where an impact could have been expected due to the diversity of cover crops sown the year before (10 species from 6 families in Ste-Marthe and 7 species from 4 families in Montmagny). Even though some variations in the bacteria/fungi microbial balance were observed, it is unlikely

that the impact on soil functions such as the accumulation of organic matter by fungi could be significant in the absence of microbial biomass variations (Kallenbach et al., 2016; Six et al., 2006).

4.2 | Impact of cropping systems on bacterial diversity

4.2.1 | Impacts of crop rotation

No impact of crop rotation was found on bacterial richness estimated with Shannon and Chao1 indexes. However, variations in composition were observed between the three-crop rotation and the two-crop rotation at both study sites. The integration of wheat–corn–soybean rotation impacted the microbial composition after a single 3-year rotation cycle. Variations were detected through an analysis of specific ASVs within each phylum, even though the relative abundance of phyla was similar between plots. However, no difference was observed based on previous crops.

Contrary to our findings, Venter et al. (2016) conducted a meta-analysis where an enhanced plant diversity had positive impacts on Shannon indexes for bacteria, fungi, and archaea. However, findings depend on the method used. Studies based on sequencing showed a decrease in the Shannon index, but data obtained using this method are still scarce. In other studies, no variation in bacterial richness was observed when transitioning from a monoculture to a corn–soybean rotation (Chamberlain et al., 2020) nor when adding wheat to a corn–soybean rotation (Peralta et al., 2018).

Chamberlain et al. (2020) also found differences in bacterial composition between rotation systems in the fall and in the spring at lower taxonomic levels, whereas no difference in composition was found between the main crops when associated with the same rotation system. This shows that rotation has an impact on composition, but it differs only slightly from one crop to another. On the other hand, Peralta et al. (2018) observed no variation in microbial composition between a corn–soybean rotation and a corn–soybean–wheat rotation after 12 years of cultivation.

4.2.2 | Impacts of cover crops

The implementation of cover crops had no impact on the bacterial richness observed at the beginning of the season, but bacterial composition differed significantly in the presence and absence of cover crops at Montmagny site. This was not observed at Ste-Marthe site, but it should be noted that cover crops had more difficulty setting in. Even though cover crops were in place during the previous years, our sampling campaign took place before the sowing of the new cover crops, which means that the area covered with plants in each plot was similar at the moment of the sampling (data not shown). Romdhane et al. (2019) had similar findings, both at the beginning and at the end of the growing season. In another study where the sampling took place at the beginning of the season, after the sowing but before the germination of the cover crops, the highest diversity indexes were associated with rotation designs without cover crops. This could be due to the fact that there were more weeds in the plots without cover crops, which could have impacted microbial communities (Peralta et al., 2018). Other studies showed that bacterial richness can be positively affected by cover crops, but these effects are more visible when plowing practices are used in comparison with conservation agriculture practices, as these can protect microbial communities from the negative effects of plowing (Kim et al., 2020). The fact that there was no soil preparation in our plots could explain the absence of differences in richness when compared with other studies.

Chamberlain et al. (2020) showed that cover crops had no influence on bacterial composition during the first implementation year. However, variations in soil communities were linked to variations in pH and organic matter. Among the different seed mixes of cover crops tested in Romdhane et al. (2019), the only mixes that affected the microbial composition in comparison with the bare soil were the ones composed of two species without legumes. However, the study showed that differences in cover crops elimination strategies (frost, rolling, or glyphosate) had a larger impact on bacterial communities by altering the soil content in organic carbon and total nitrogen. Peralta et al. (2018) showed that composition modifications associated with an enhanced plant diversity can also have a positive impact on the potential for disease reduction. However, in their study, composition was mainly explained by the physicochemical characteristics of the soil (texture and moisture).

4.2.3 | Possible functional implications

Both aspects of the cropping systems (rotation and cover crops) had positive and negative impacts on Proteobacteria ASVs, the most abundant phylum in the soils. Even though this phylum includes numerous cultivable species, a lot of sequences have not been associated with a genus yet, and knowledge on this bacterial group is still scarce (Janssen, 2006; Spain et al., 2009). Proteobacteria have, for instance, been positively associated with carbon mineralization (Fierer et al., 2007). Some genera such as *Pseudomonas* can be used as rhizobacteria to foster plant growth and boost resistance to soil-borne diseases (Lugtenberg & Kamilova, 2009). Rotations mainly had a positive effect on some Actinobacteria ASVs. Actinobacteria can be found in various ecosystems and account for 13% of bacteria on average (Janssen, 2006).

They are more abundant in soils with a high pH (Lauber et al., 2009) or soils that are rich in organic matter (Barka et al., 2016). They play an important role in the decomposition of organic matter, namely because of their capacity to break down plant cellulose, which makes them essential to the carbon cycle (Lewin et al., 2016). They are known to have beneficial interactions with plants but can also be pathogens (Barka et al., 2016). Cover crops had a negative effect on Acidobacteria ASVs, which account for 20% of bacterial communities on average. Despite their high abundance, they have been overlooked for a long time as they are difficult to grow in the lab (Janssen, 2006). The various groups included in this phylum are pH sensitive, among other things (Jones et al., 2009). Genomic studies of these subgroups show important differences in their metabolism of carbon and nitrogen, but the various environmental studies performed at the phylum level do not give precise information on ecological implications (Kielak et al., 2016). As an example, Acidobacteria were associated with low carbon mineralization rates (Fierer et al., 2007) as well as an enhanced organic carbon availability (Jones et al., 2009). Other differences in composition between both rotation designs and based on the presence or absence of cover crops were also detected in the ASVs of rarer phyla. Moreover, in our study, ASVs of the different phyla could scarcely be assigned to particular species or genus. Even though modern sequencing methods help to know which taxa are present, it is still necessary to elucidate the functional implications, including by evaluating absolute abundances instead of relative abundances (Fierer, 2017).

4.3 | Impacts of cropping systems on eukaryotic diversity

The sowing of cover crops influenced the eukaryotic richness, as lower Shannon and Chao1 indexes were observed in soils with cover crops compared with soils without one. The Chao1 index is impacted by the rarest groups, which could explain why the PERMANOVA showed no modification in composition. However, crop rotation had some impacts on the ASVs belonging to the most abundant phyla of fungi (Ascomycota) and microfauna (Cercozoa). The PERMANOVA showed a significant difference in the eukaryotic composition, which means that the rarest phyla have likely been affected. In H. Liu, Pan, et al. (2019), the input of plant diversity through crop rotation reduced diversity indexes for fungi, affecting the composition of both pathogenic and beneficial groups, potentially having a positive impact on cultural crops. By contrast, J. Liu, Yao, et al. (2019) also observed an enhanced diversity with crop rotation as well as impacts on pathogenic fungi. In Ai et al. (2018), fungi composition was compared between a soybean-wheat rotation and a corn–soybean rotation. Crop rotation had no impact on the relative abundance of the most abundant phylum of fungi (Ascomycota) but affected multiple families, similarly to our study. Ascomycota are known to be greatly involved in cellulose degradation (De Boer et al., 2005). Schmidt et al. (2019) showed that cover crops had a negative impact on the relative abundance of Ascomycota, with a rise in phylogenetic diversity. In their study, the most abundant taxa were less affected by cover crops than by plowing, which suggests that other farming practices can have large impacts on fungi.

5 | CONCLUSION

The aim of this study was to assess for the first time the impact of enhanced plant diversity using cover crops on microbial communities in the context of field crops in Quebec, where knowledge is currently scarce. The addition of wheat to a corn–soybean rotation and the implementation of cover crops had no impact on the total microbial biomass and bacterial richness. However, the bacteria/fungi ratio and the eukaryotic richness were slightly affected by these treatments. Moreover, the enhanced plant diversity influenced the bacterial and eukaryotic composition after a single rotation cycle. This confirms our hypothesis that an enhanced plant diversity can influence the diversity of soil microbial communities. However, this finding did not confirm that an increase in plant diversity influences microbial biomass. Thus, microbial composition, both bacterial and eukaryotic, could be a more sensitive indicator of soil health than other indicators commonly used such as microbial biomass. Our study shows that conservation agriculture practices that increase plant diversity through more diversified rotations and the establishment of cover crops have an impact on the microbial communities of agricultural soils in field crops in Quebec. By better understanding the functional implications associated with the current findings on various microbial groups, it could be possible to assess more precisely the impacts of conservation agriculture practices on the production capacity of the soils as well as the maintenance of the ecosystem services they offer.

AUTHOR CONTRIBUTIONS

Blandine Giusti: Conceptualization, data curation, formal analysis, investigation, methodology, validation, and writing—original draft. **Richard Hogue**: Conceptualization, data curation, formal analysis, funding acquisition, methodology, resources, supervision, validation, and writing—review and editing. **Thomas Jeanne**: Data curation, formal analysis, methodology, supervision, validation, and writing—review and editing. **Marc Lucotte**: Conceptualization, funding acquisition, investigation, project administration, resources, supervision, and writing—review and editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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