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# Cross-Effect Between Cover Crops and Glyphosate-Based Herbicide Application on Microbiote Communities in Field Crops Soils

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**Abstract:** This study investigates how cover crops (CC) and different application rates of glyphosate-based herbicide (GBH) may affect soil microbial communities. Our hypothesis was that the use of CC would promote the presence of certain microbial communities in soils and mitigate the potential impact of GBH on these communities. CC can promote biodiversity by increasing plant diversity in fields, while GBH may have non-target effects on species that utilize the shikimate pathway. Crop managements in an experimental field in Southern Québec (Canada) consisted in Glyphosate-based Herbicide (GBH) applications rates at 0.84, 1.67 and 3.33 L ha$^{-1}$ in corn, soybean and wheat fields cultivated with Direct Seeding along with CC (DSCC) and at 3.33 L ha$^{-1}$ in similar crops cultivated with direct seeding but without CC (DS). DSCC did not significantly impact microbial richness compared to DS, but did alter specific abundance among prokaryotes and eukaryotes. A permutational multivariate analysis revealed that the type of crop (soybean, wheat, maize) significantly influenced the composition of eukaryotic communities in 2018 and 2019, but not prokaryotic communities. Importantly, the study identifies a cross-effect between CC and GBH application rates suggesting that herbicide use in soybean plots can influence Anaeromyxobacter populations. Also, higher abundance of Enoplea and Maxilopoda were observed in plots with the lower application rate of GBH. Both eukaryotes group are known to be sensitive to crop management. These findings emphasize the need for a holistic approach to agricultural practices, considering the combined effects of both CC and GBH application rates on soil microbial health. Ultimately, the study calls for sustainable agricultural practices that preserve microbial diversity, which is essential for maintaining ecosystem services and soil health.

**Keywords:** cover crops; richness index; abundance index; prokaryote; eukaryote; soil microorganisms content

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## 1. Introduction

Soil degradation remains a major issue worldwide whereas approximately 33% of agricultural lands are currently in a precarious situation [1,2]. Soil degradation can be linked to compaction, loss of fertility, limitation of bioavailable nutrients needed by crop plants, poor water infiltration and increased salinity [1–3]. The FAO emphasizes that the loss of soil resources and functions can be avoided with sustainable practices [1].

Conventional agriculture with mechanical tillage such as ploughing is still a widely used soil management practice and is largely responsible for soil degradation [4–6]. To challenge this issue, conservation agriculture (CA) has been put forward [7–9]. CA is designed to assure biodiversity and natural biological processes in order to assure soil sustainability and to increase agricultural productivity [7]. Limiting tillage and maintaining a permanent vegetal cover are two out of the three pillars of this approach [9,10]. Maintaining crop residues on the soil is a largely used approach and has amply demonstrated its potential for limiting erosion in direct seeding systems (DS) [8,9]. By considerably reducing tillage, DS remain vulnerable to surface soil compaction, limiting field operations and influencing soil structure [11,12]. The problems of soil compaction observed in DS could also influence soil microbiota [13–15]. Healthy, fertile soil provides greater resilience to biotic and abiotic stresses, while sustaining high productivity over the long term [16]. Soil productivity is closely linked to the biodiversity of its biota [14,17]. Prokaryotes and Eukaryotes organisms are known to be the cornerstone for maintaining soil functions and availability of essential nutriments for crops [15,18]. Soil organisms community are sensitive to soil management, making them early indicators for interpreting the level of disturbance or benefit according to a given agricultural management [16].

The main objective of this research is to observe whether the use of cover crops (CC) combined with different application rates of glyphosate-based herbicides (GBH) can influence soil microbiota compared to DS without CC. Our hypothesis is that the use of CC may have a positive influence on the richness, abundance, and composition of certain microbial communities in soils of glyphosate tolerant (GT) soybean and corn fields. In addition, CC could also mitigate the impact of GBH on soil microorganisms, depending on the application rates compared to DS without CC.

CC has the property of increasing the root diversity present in soils [19–21], which produces a wider range of products from plant exudation and senescence. This promotes the heterogeneity of food resources and diversify microhabitats, which leads to greater diversity in soil biota [17]. The presence of CC also promotes higher soil aggregation, which can further contribute to increased microbiota diversity [14,17,20]. On the other hand, non-selective herbicides such as glyphosate-based ones are frequently used in no-till farming [6,22]. At certain application rates, their use may have a negative impact on soil microorganisms [23–25], which can have an antagonistic effect on the benefits expected from the use of cover crops. Fungi and a limited number of microorganisms (bacteria and protozoa) possess the shikimate pathway and amino acid synthesis targeted by GBH [26], meaning they may be affected by exposure to GBH. Some studies suggest that the use of CC has the potential to capture some of the applied GBH, thus reducing the presence of glyphosate in soils and mitigating their impact on prokaryotic populations [25]. The originality of this study lies in the fact that few studies have compared species richness and relative abundance in soil between DS and DSCC and even fewer have investigated the combined effect of CC use with different GBH application rates.

## 2. Materials and Method

### *2.1. Experimental Design*

The project was carried out over two years (2018 and 2019) in an open field at the Grain Research Center (CEROM) at St-Mathieu-de-Beloeil, Quebec, Canada (45.5828 N, −73.2374 W). Soil sampling was carried out with an auger prior to plot establishment to determine the soil mineral content on the 0–20 cm horizon with a Mehlich 3 extraction [27] (Figure 1). The soil type at the site is a humic Gleysol with a heavy clay texture (Figure 1). The experimental design included three crops in rotation (soybean-corn-wheat). Four different weed management with application of GBH (Roundup Ready

Wheatermax®) (CEROM, St-Mathieu-de-Beloeil, QC, Canada) were applied [DS 3.3: direct seeding without CC + 3.3 L $ha^{-1}$ in 2 applications (1804 g a.i), DSCC 3.3: direct seeding with CC + 3.3 L $ha^{-1}$ in 2 applications (1804 g a.i), DSCC 1.67: direct seeding with CC + 1.67 L $ha^{-1}$ in 2 applications (902 g a.i) and DSCC 0.84: direct seeding with CC + 0.84 L $ha^{-1}$ in two applications (451 g a.i)]. Overall, this experimental setting represents twelve different cropping practices replicated four times for a total of 48 plots arranged on four randomized complete blocks (Figure 1). The tweed managements were T1: Corn DS 3.33, T2: Corn DSCC 3.33, T3: Corn DSCC 1.67, T4: Corn DSCC 0.84, T5: Soybean DS 3.33, T6: Soybean DSCC 3.33, T7: Soybean DSCC 1.67, T8: Soybean DSCC 0.84, T9: Wheat DS 3.33, T10: Wheat DSCC 3.33, T11: Wheat DSCC 1.67 and T12: Wheat DSCC 0.84. Each plot measured 9 m × 20 m with a distance of 2.50 m between each plot and 12 m between each block. The cultivars used and sowing dates are shown in Figure 1 and have been defined according to the recommendations of the Quebec Ministry of Agriculture, Fisheries and Food (MAPAQ) agronomists.

GT corn and soybean cultivars were used in this study. Two sequential GBH applications were realized (12 May and 3 June 2018, and 12 May and 13 June in 2019 in corn plots; 3 June and 27 June in 2018 and 18 May and 24 June in 2019 in soybean plots). Embutox at 2.25 L $ha^{-1}$ was applied 6 June 2018, and 2019 in wheat plots.

Corn plots were fertilized with 95 kg $ha^{-1}$ of N added 29 June 2018, and 90 kg $ha^{-1}$ of N and 60 kg $ha^{-1}$ of P added 28 June 2019. In wheat plots, 50 kg $ha^{-1}$ of N and 65 kg $ha^{-1}$ of P were added 9 May with an additional 60 kg $ha^{-1}$ of N 20 June 2018. In 2019, 90 kg $ha^{-1}$ of N was added June 6th in wheat plots. Soybean plots were not fertilized in 2018 and 2019. The cover crops sown in the DSCC plots are presented in Figure 1. The cover crops were sown in August in wheat plots sown after the harvest (wheat harvest: 10 August 2018 and 15 August 2019). The autumn cover crops were sown a few weeks before the harvest of soybean (soybean harvest: 5 October 2018 and 15 October 2019) and corn (corn harvest: 12 November 2018 and 27 October). No cover crop was sown in DS plots.2.2. Prokaryotes and eukaryotes content in soil.

#### 2.1.1. Soil Sampling

Three soil samples were collected in 2018 and 2019 with an auger in the 0–20 cm horizon in each plot. The sampling periods used for the metagenomic analysis took place 4 November 2018, and 24 October 2019 in order to obtain a picture of the microorganism communities at the end of the production season and close to the crop harvesting period. The soil cores were then homogenized and kept at a temperature of −20 °C until analyses.

#### 2.1.2. Soil Physicochemical Analyses

The elemental contents were determined using the Mehlich-3 extraction method for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sodium (Na), nickel (Ni), cadmium (Cd), chromium (Cr), cobalt (Co), and lead (Pb) [27]. All elemental contents were quantified using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Optima 4300DV, Perkin Elmer Inc., Waltham, MA, USA).

#### 2.1.3. DNA Extraction

All soil samples were then ground and sieved through a 2 mm screen after having been dried at room temperature for 72 h. From each soil sample, 400 mg of soil were used as sub-samples for DNA extraction. The extraction was executed based on the instruction provided by FastDNA SPIN Kit for Soil commercial kit (MP Biomedicals, Solon, OH, USA). Elution solution of 100 µL (pyrogen and DNase-free distilled water) including eluted DNA

samples were prepared. Quality control of the DNA samples and the DNA quantification were carried out according to the procedure recommended in a previous study [28].

#### 2.1.4. Metagenomic Analyses

Molecular counting was carried out using qPCR targeting the V6–V8 regions of bacterial 16S and fungal 18S rRNA. Amplification was performed with the eub338/eub518 primers for bacteria and FF390/FR1 primers for total fungi, using the SYBR® Green qPCR master mix (Qiagen, Toronto, ON, Canada). Detection was repeated twice on a CFX96 Touch System device (Biorad, Nicosia, Cyprus). 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 at varying levels between organisms during quantification, both for bacterial (16S rRNA) and fungal (18S rRNA) organisms. Detection systems operate within a 4-log detection range with an efficiency rate of 89.1% (R2 = 0.99) for total bacteria and 91.7% (R2 = 1) for total fungi.

Metagenomic analyses evaluated bacterial and eukaryotic diversity by targeting the V3–V4 region of bacterial 16S and the V4 region of eukaryotic 18S rRNA. Amplification was conducted using a dual-indexed PCR approach with primers 515F and 806RB for prokaryotes, and E572F/E1009R for eukaryotes [29]. Libraries were sequenced in a paired-end format, with 300 base pair reads 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 Université Laval (Quebec, QC, Canada).

#### 2.1.5. Bioinformatics Analyses

Bioinformatics analyses were conducted 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 employed to assess sequence quality and identify amplicon sequence variants (ASVs) within the QIIME 2 platform [30,31]. Taxonomic identification of ASVs was performed using the following reference databases: Greengenes 13.5 for 16S, PR2, 18S, SILVA and for 16S and 18S rRNA [32–34]. Sequences were rarefied to 10,000 per sample prior to statistical analyses [28].

#### 2.1.6. Statistical Analyses

All analyses on metagenomic data were performed with the R software version 4.1.1 (R Core team). A Shapiro-Wilk test was performed to test the normal distribution of residuals. An analysis of variance (ANOVA) was performed when distributions of residuals were normal, and a Wilcoxon analysis was performed for non-parametric distribution. The phyloseq package was used for Shannon index, Chao 1, Observed index evenness and composition analyses [35]. The microorganisms composition was defined through an ordination using nonmetric multidimensional scaling of the Bray-Curtis (Figure 2). Then, the eukaryotic and prokaryotic composition were assessed with permutational multivariate analysis of variance (PERMANOVA) and the adonis function [36]. The vegan package was used to performing the adonis function [36]. The abundance of the main genus between managements were identified with the ampvis2 package [37] and the Operational Taxonomic Units [38]. In this study, only the 25 more abundant taxa were used for the prokaryotic taxonomic groups. Genus was the most precise identified taxonomic unit used here whenever possible for eukaryotes and prokaryotes.

**Experimental design**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bloc #4 | T12 DSCC 3.3 | T10 DSCC 0.84 | T6 DSCC 0.84 | T7 DSCC 1.67 | T1 DS 3.3 | T8 DSCC 3.3 | T11 DSCC 1.67 | T5 DS 3.3 | T2 DSCC 0.84 | T9 DS 3.3 | T4 DSCC 3.3 | T3 DSCC 1.67 |
| Bloc #3 | T9 DS 3.3 | T3 DSCC 1.67 | T4 DSCC 3.3 | T5 DS 3.3 | T11 DSCC 1.67 | T2 DSCC 0.84 | T7 DSCC 1.67 | T8 DSCC 3.3 | T1 DS 3.3 | T6 DSCC 0.84 | T10 DSCC 0.84 | T12 DSCC 3.3 |
| Bloc #2 | T10 DSCC 0.84 | T8 DSCC 3.3 | T12 DSCC 3.3 | T3 DSCC 1.67 | T6 DSCC 0.84 | T5 DS 3.3 | T4 DSCC 3.3 | T2 DSCC 0.84 | T9 DS 3.3 | T7 DSCC 1.67 | T1 DS 3.3 | T11 DSCC 1.67 |
| Bloc #1 | T3 DSCC 1.67 | T1 DS 3.3 | T7 DSCC 1.67 | T9 DS 3.3 | T8 DSCC 3.3 | T10 DSCC 0.84 | T12 DSCC 3.3 | T11 DSCC 1.67 | T2 DSCC 0.84 | T5 DS 3.3 | T4 DSCC 3.3 | T6 DSCC 0.84 |

| 2018 | 2019 | **GBH application (Roundup Wheater Max)** |
|---|---|---|
| Corn | Soybean | 3.3 = 3.3 L ha$^{-1}$ in 2 applications |
| Wheat | Corn | 1.67 = 1.67 L ha$^{-1}$ in 2 applications |
| Soybean | Wheat | 0.84 = 0.84 L ha$^{-1}$ in 2 applications |

| Soil Texture | Rate (%) | Soil elements | Content |
|---|---|---|---|
| Clay | 72.6 ± 0.9 | P | 12.87 ± 2.51 mg kg$^{-1}$ |
| Loam | 27.4 ± 0.9 | K | 313.50 ± 20.84 mg kg$^{-1}$ |
| Sand | 0 ± 0.0 | C | 2.94 ± 0.22 g kg$^{-1}$ |
| | | Mg | 803.17 ± 48.27 mg kg$^{-1}$ |
| | | Al | 1056.71 ± 19.32 mg kg$^{-1}$ |
| | | Cu | 11.00 ± 0.47 mg kg$^{-1}$ |
| | | Fe | 217.54 ± 13.92 mg kg$^{-1}$ |
| | | Mn | 24.92 ± 5.72 mg kg$^{-1}$ |
| | | Zn | 2.33 ± 0.18 mg kg$^{-1}$ |
| | | Na | 47.60 ± 3.06 mg kg$^{-1}$ |

**Types of crops and cover crops used in the experiment.**

| Years | Crops | Cultivars | Date of seeding | Cover crops mix sowned | |
|---|---|---|---|---|---|
| | | | | 1rst seeding date | 2e seeding date |
| 2018 | Corn GT: | Elite E65G82R | May 11th 2018 | **October 26th:** autumn rye (200 kg ha$^{-1}$). Sown with a Great Plains® seed drill. | **August 13th:** buckwheat (5 kg ha$^{-1}$), sunflower (5 kg ha$^{-1}$), faba bean (15 kg ha$^{-1}$), forage radish (3kg ha$^{-1}$), phacelia (1 kg ha$^{-1}$), 4010 forage pea (25 kg ha$^{-1}$) and oats (20 kg ha$^{-1}$). Sown with a Great Plains® seed drill. |
| | Soybean GT: | Altitude R2 | May 11th 2018 | **October 6th:** autumn wheat (200 kg ha$^{-1}$). Sown with a Great Plains® seed drill. | n/a |
| | Wheat: | Moka | May 10th | **May 10th:** berseem (10 kg ha$^{-1}$) and crimson clover (10 kg ha$^{-1}$). Sown manually. | n/a |
| 2019 | Corn GT: | P9188AM | May 8th | **June 19th:** crimson clover (5 kg ha$^{-1}$), tillage radish (3 kg ha$^{-1}$ and tillage turnips (2 kg ha$^{-1}$). Sown manually. | **September 8th:** autumn rye (50 kg ha$^{-1}$), tillage radish (3kg ha$^{-1}$), tillage turnip (2kg ha$^{-1}$) and common vetch (10kg ha$^{-1}$). Sown with a Great Plains® seed drill. |
| | Soybean GT: | Altitude R2 | May 18th | **September 6th:** autumn wheat (225 kg ha$^{-1}$). Sown with a Great Plains® seed drill. | n/a |
| | Wheat: | Hoffman HRF | May 8th | **May 12th:** berseem (5 kg ha$^{-1}$) and crimson clover (5 kg ha$^{-1}$). Sown manually. | **August 27th:** buckwheat (5 kg ha$^{-1}$), sunflower (5 kg ha$^{-1}$), faba bean (15 kg ha$^{-1}$), forage radish (3kg ha$^{-1}$), phacelia (1 kg ha$^{-1}$), 4010 forage pea (25 kg ha$^{-1}$) and oats (20 kg ha$^{-1}$). Sown manually. |

**Figure 1.** Soil texture, soil elementary content, the cultivars and the cover crops used in the experimental design. Elementary contents were obtained for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminium (Al), bore (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sodium (Na), nickel (Ni), cadmium (Cd), chrome (Cr), cobalt (Co) and lead (Pb) are presented as means ± standard error on the mean.

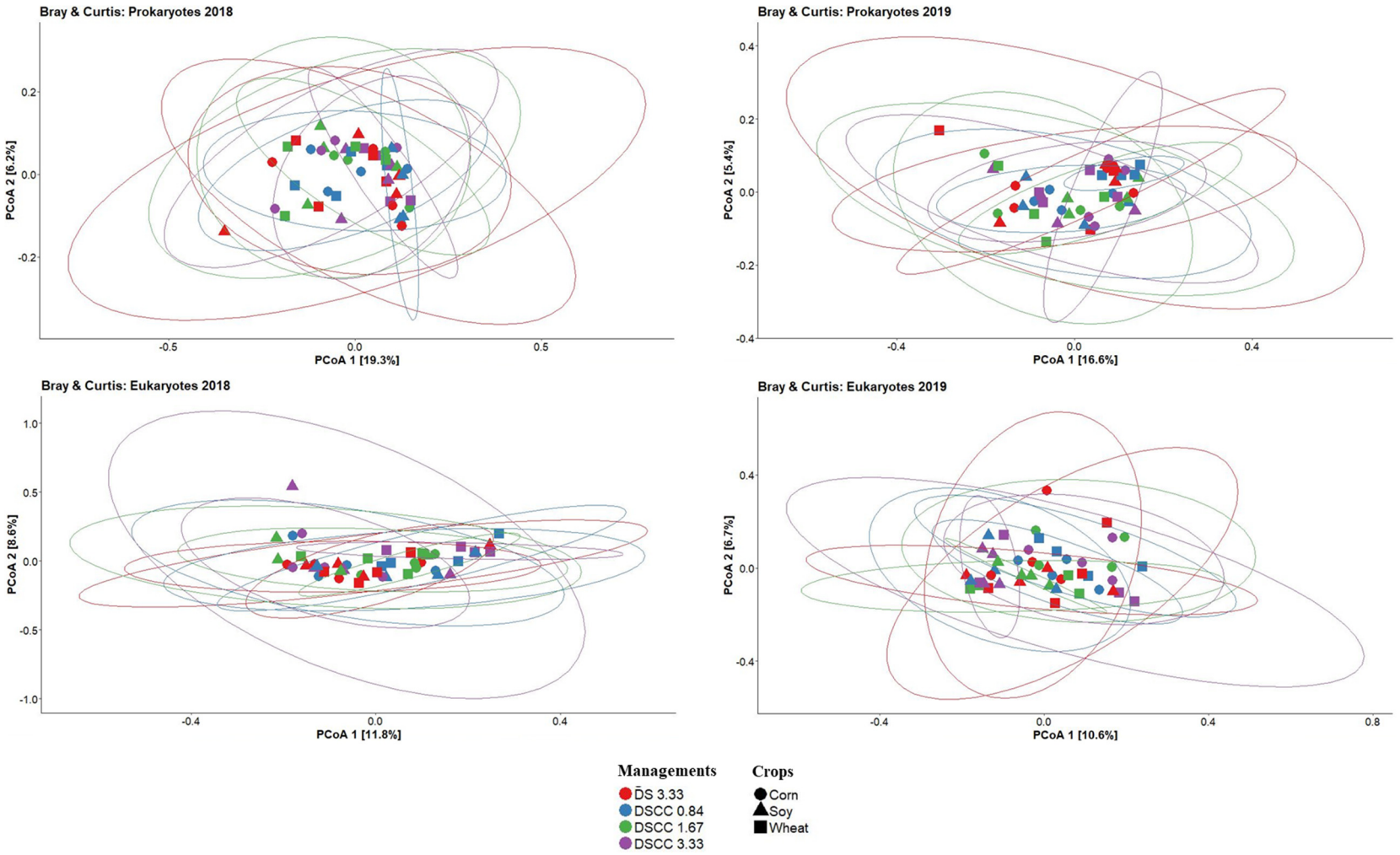


**Figure 2.** Principal coordinates analysis (PCoA) using Bray-Curtis dissimilarity test on procaryotes and eucaryotes composition in soil between the different crop managements in corn, soy and wheat. An analysis with contrast was performed to assess significant difference between DS and DSCC plots. Also, a univariate analysis was performed to assess significant difference between all crops managements. A threshold of 0.05 was used to assessed statistical significance for all statistical analyses. A post-hoc letters test was performed when statistical significances were observed. Then, relative abundance of all taxonomic group was represented by phyloseq bar plots for each crop for 2018 and 2019.

## 3. Results

### *3.1. Soil Organisms Content Index Values*

In 2018, no significant difference was observed for the eukaryotic richness and diversity between crop managements according to total eukaryotes Observed index, the Shannon index and the Chao 1 index (Figure 3A). No significant differences were assessed based on the total eukaryotes Observed index, the Shannon index and the Chao 1 index in 2019 (Figure 3B).

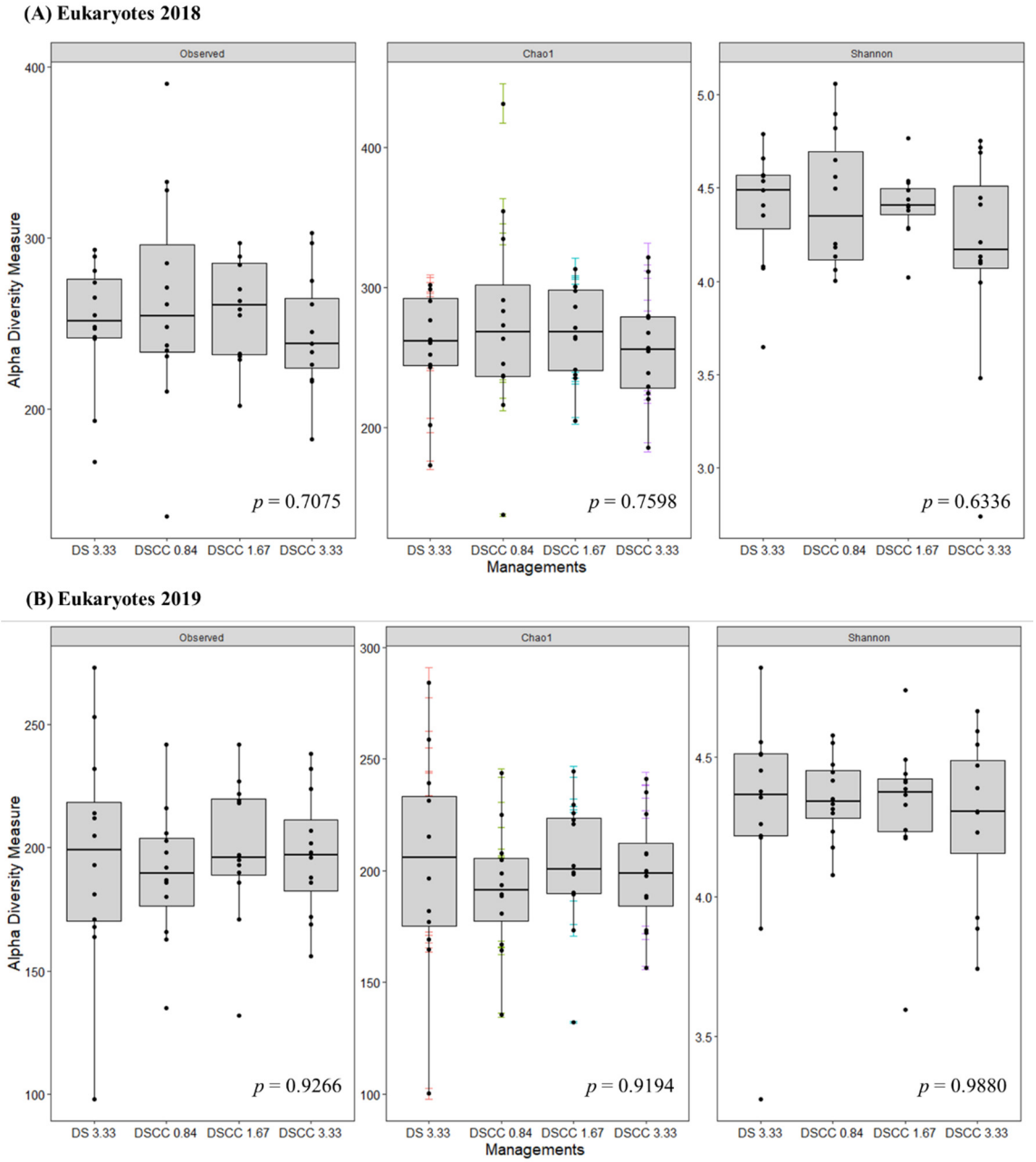


**Figure 3.** *Cont.*

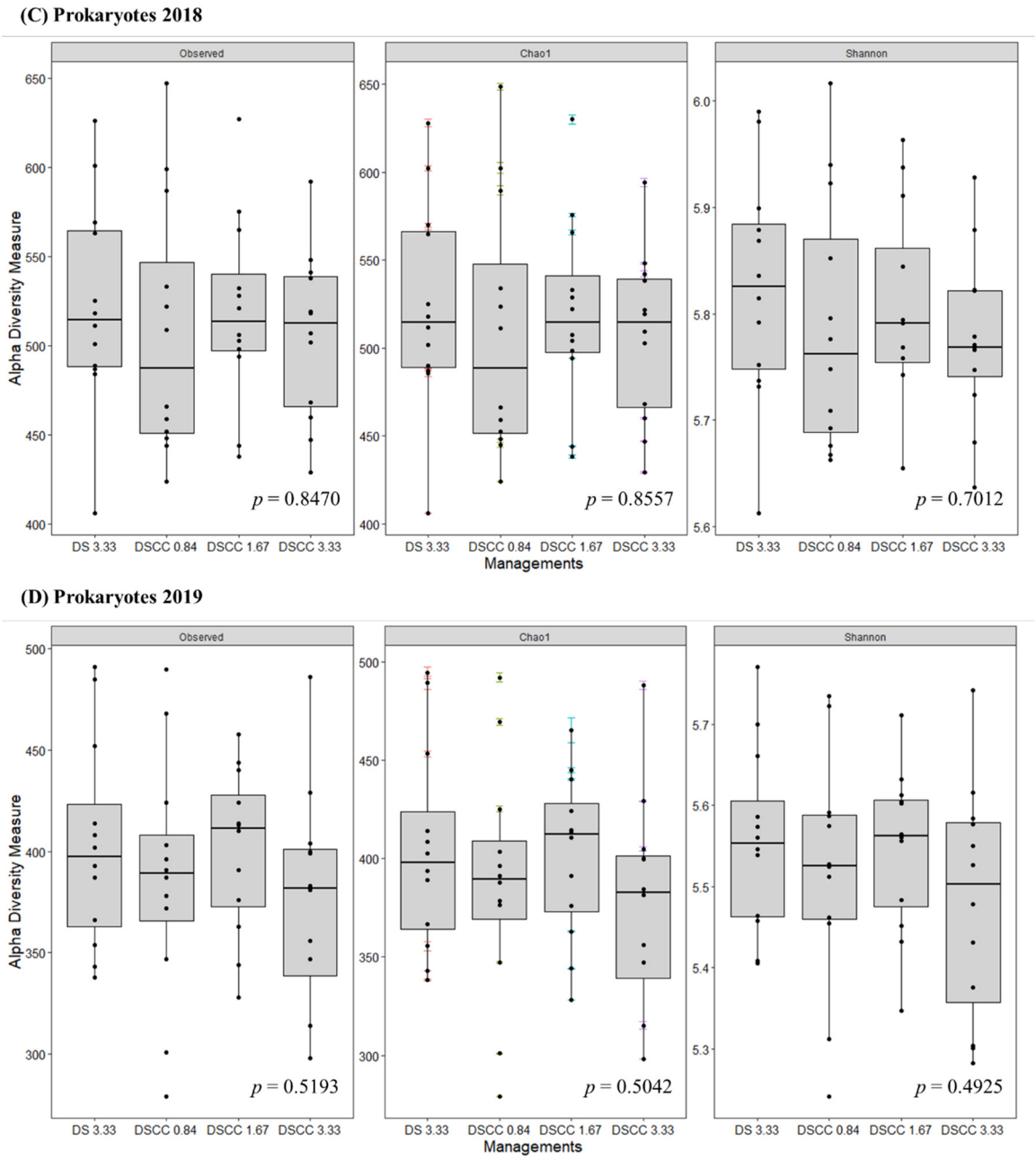


**Figure 3.** Values of eukaryotic (**A**,**B**) and prokaryotic (**C**,**D**) richness index in soil (n = 96) between crop managements (DS 3.33, DSCC 0.84, DSCC 1.67 and DSCC 3.33).

Similarly, it was not possible to observe any significant difference on the prokaryotic richness over two years based the Observed index, Shannon index (5.82 ± 0.11 in DS and Chao 1 index in 2018 (Figure 3C). Also in 2019, no significant difference was observed based on the prokaryotic Observed index, Shannon index and Chao 1 index (Figure 3D).

In 2018 and 2019, no significant difference was observed between crop managements based on Evenness index for eukaryotes and prokaryotes (Figure 4).

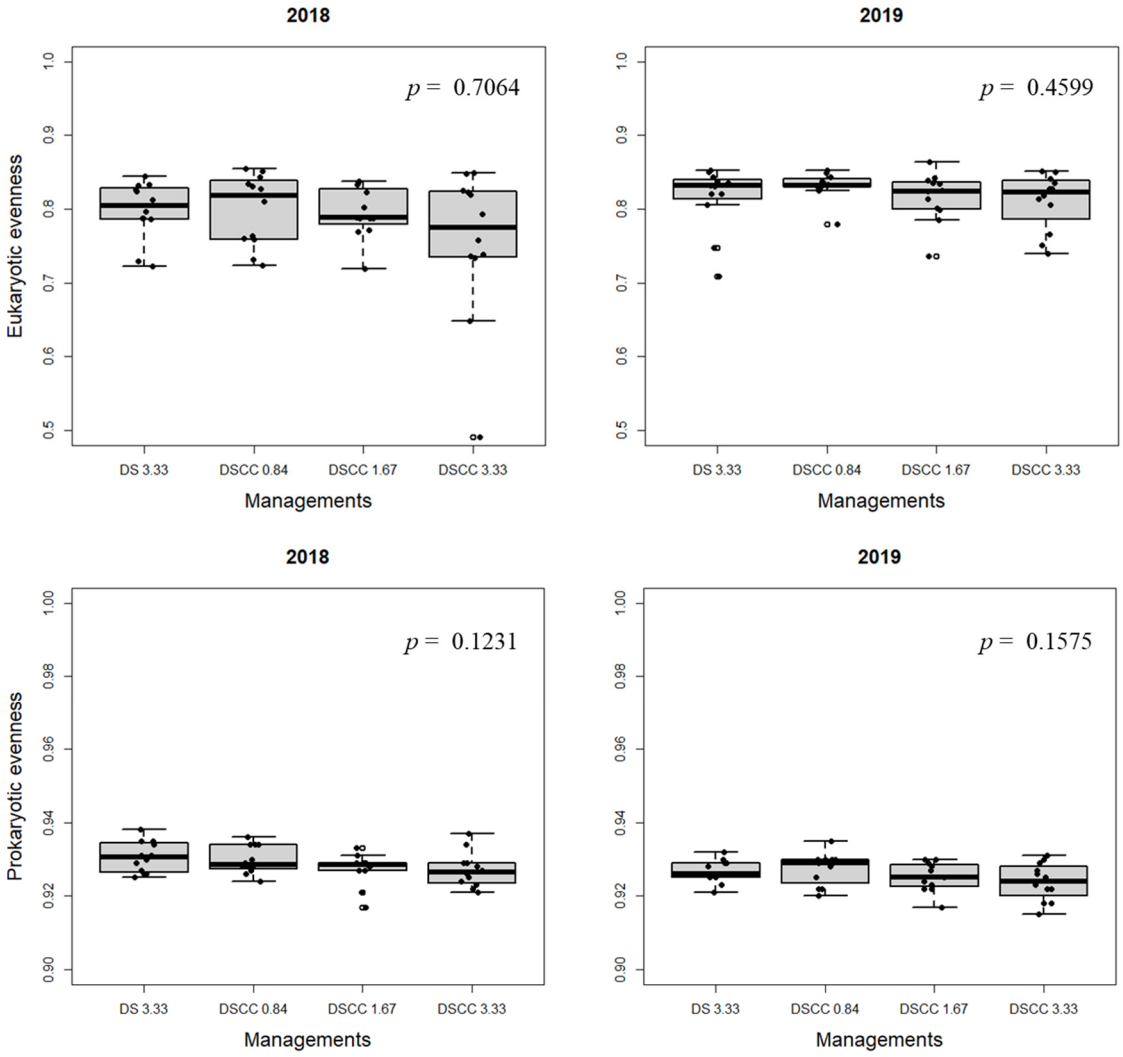


**Figure 4.** Eukaryotic and prokaryotic evenness between crop managements in 2018 and 2019.

### *3.2. Microbiota Composition*

According to the results of the PERMANOVA analysis; only the type of cultivated crop seemed to have a significant effect on the eukaryotic composition in 2018 ($p$ = 0.016) and 2019 ($p$ = 0.001) (Table 1). The different managements and the mixed effect of the type of crops*managements seemed to have no significant effect on the eukaryotic composition for both years (Table 1). In 2018 and 2019; the type of crops; the different managements and the mixed effect of crops*managements seemed to have no significant effect on the prokaryotic composition (Table 1).

### *3.3. Abundance of Taxonomic Group*

Based on the contrast analysis, the only significant differences in eukaryotes were observed for the class Maxillopoda in soybean plots ($p$ = 0.0176) between DS plots and DSCC plots in 2018 (Table 2). In 2019, Significant differences between DS plots and DSCC plots were observed for the Other Eukaryota ($p$ = 0.0364) with more striking difference in wheat plots ($p$ = 0.001) (Table 2). Differences were also observed for the class Cephalopoda ($p$ = 0.0193), for Insecta in the corn plots ($p$ = 0.0049) and for the class Maxillopoda ($p$ = 0.0482) between DS plots and DSCC plots in 2019 (Table 2).

**Table 1.** Permutational multivariate analysis of variance (PERMANOVA) of the eukaryotic and prokaryotic composition between crops management in 2018 and 2019.

| Permanova | Eukaryotes 2018 | | | | Eukaryotes 2019 | | | |
|---|---|---|---|---|---|---|---|---|
| | Df | F.Model | $r^2$ | *p* Value | Df | F.Model | $r^2$ | *p* Value |
| Crops | 2 | 1.3682 | 0.0577 | 0.016 * | 2 | 1.7259 | 0.0716 | 0.001 * |
| Managements | 3 | 0.9209 | 0.0583 | 0.758 | 3 | 0.8662 | 0.0539 | 0.921 |
| Crops*Managements | 6 | 0.9830 | 0.1244 | 0.539 | 6 | 1.0261 | 0.1277 | 0.333 |
| Permanova | Prokaryotes 2018 | | | | Prokaryotes 2019 | | | |
| | Df | F.Model | $r^2$ | *p* value | Df | F.Model | $r^2$ | *p* value |
| Crops | 2 | 0.8696 | 0.0376 | 0.736 | 2 | 0.8830 | 0.0383 | 0.744 |
| Managements | 3 | 0.8946 | 0.0580 | 0.756 | 3 | 0.8617 | 0.0560 | 0.875 |
| Crops*Managements | 6 | 0.9784 | 0.1268 | 0.537 | 6 | 0.9671 | 0.1257 | 0.583 |

Note: The * indicate that the main effect of crops, crop managements or the mixed effect of crops* crop managements are significant based on the *p* value threshold ($p < 0.05$).

**Table 2.** Contrast analysis of eukaryotic content between plots with (DSCC) or without cover crops (DS) for 2018 and 2019.

| Taxon | | | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC |
| Eukaryota | Cephalopoda | F value | na | na | na | na | 6.00 | 4.5 | 4.5 | 0.00 |
| | | *p* value | na | na | na | na | 0.0193 * | 0.0408 * | 0.048 * | 1.00 |
| | Eimeriidae | F value | 0.1412 | 0.8305 | 0.0169 | 0.1525 | 0.0013 | 0.3802 | 0.0951 | 0.9734 |
| | | *p* value | 0.7093 | 0.3682 | 0.8971 | 0.6984 | 0.9718 | 0.5414 | 0.7596 | 0.3304 |
| | Enoplea | F value | 0.0212 | 0.1509 | 0.177 | 0.081 | 3.2424 | 1.0483 | 0.552 | 1.828 |
| | | *p* value | 0.885 | 0.6999 | 0.6765 | 0.7776 | 0.0801 | 0.3127 | 0.4623 | 0.1848 |
| | Insecta | F value | na | na | na | na | 3.00 | 0.00 | 0.00 | 9.00 |
| | | *p* value | na | na | na | na | 0.0918 | 1.00 | 1.00 | 0.0049 * |
| | Malacostraca | F value | 0.3333 | 1.00 | 0.00 | 0.00 | na | na | na | na |
| | | *p* value | 0.5673 | 0.324 | 1.00 | 1.00 | na | na | na | na |
| | Maxillopoda | F value | 2.6844 | 6.1875 | 0.0015 | 0.0968 | 4.1841 | 0.028 | 1.3172 | 6.5665 |
| | | *p* value | 0.11 | 0.0176 * | 0.9689 | 0.7575 | 0.0482 * | 0.8681 | 0.2587 | 0.0147 |
| | Pirsonia Clade | F value | 1.3631 | 0.1272 | 0.2687 | 1.3158 | 1.1757 | 1.2491 | 0.4289 | 2.0031 |
| | | *p* value | 0.2507 | 0.7234 | 0.6074 | 0.2589 | 0.2854 | 0.2711 | 0.5167 | 0.1656 |
| | Reticulomyxidae | F value | 0.3333 | 0.00 | 1.00 | 0.00 | 0.3996 | 1.1989 | 0.00 | 0.00 |
| | | *p* value | 0.5673 | 1.00 | 0.324 | 1.00 | 0.5313 | 0.2808 | 1.00 | 1.00 |
| | Other Eukaryota | F value | 0.3933 | 0.2379 | 0.299 | 0.0027 | 4.7244 | 0.016 | 12.8407 | 0.0948 |
| | | *p* value | 0.5345 | 0.6287 | 0.5879 | 0.959 | 0.0364 * | 0.9 | 0.001 * | 0.7599 |
| Fungi | Agaricomycetes | F value | 0.7723 | 0.5021 | 0.7052 | 0.0007 | 0.8307 | 0.0016 | 0.0016 | 2.4922 |
| | | *p* value | 0.3853 | 0.4832 | 0.4066 | 0.9792 | 0.3681 | 0.9687 | 0.9687 | 0.1232 |
| | Saccharomucetes | F value | 0.3982 | 0.2602 | 0.1327 | 0.8973 | 0.3323 | 1.0044 | 0.00 | 0.00 |
| | | *p* value | 0.532 | 0.6131 | 0.7177 | 0.3498 | 0.5679 | 0.3229 | 0.9947 | 0.9976 |
| | Sordariomycetes | F value | 0.0282 | 0.7358 | 0.1935 | 0.0161 | 0.0033 | 0.0183 | 0.8453 | 0.4687 |
| | | *p* value | 0.8675 | 0.3967 | 0.6626 | 0.8998 | 0.9545 | 0.8932 | 0.364 | 0.498 |
| | Other Fungi | F value | 0.279 | 0.5934 | 0.1072 | 0.2228 | 0.0097 | 0.6375 | 0.0292 | 0.6364 |
| | | *p* value | 0.6006 | 0.4461 | 0.7452 | 0.6398 | 0.9223 | 0.4298 | 0.8653 | 0.4303 |

Note: The * indicate significant difference between direct seeding plots with cover crops (DSCC) or without cover crops (DS) based on the *p* value threshold ($p < 0.05$). The "na" means not applicable.

The results of contrast analyses for the prokaryotes showed a difference for the genus Anaeromyxobacter between DS plots and DSCC plots ($p$ = 0.015), particularly in the soybean plots ($p$ = 0.0062) in 2018 (Table 3). For the prokaryotes in 2019, the result of the analysis with contrast showed significant differences for the genus the genus Nistropira ($p$ value = 0.0482), and the genus Rhodoplanes ($p$ = 0.0205) between DS plots and DSCC plots. The difference for the Nistropira is more striking in the corn plots ($p$ = 0.0357) in 2019.

**Table 3.** Contrast analysis of prokaryotic content between plots with (DSCC) or without cover crops (DS) for 2018 and 2019.

| Taxon | | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC |
| Aeromicrobium | F value | 0.2345 | 0.093 | 0.3358 | 0.0021 | 0.4448 | 0.0338 | 0.1551 | 0.8932 |
| | *p* value | 0.6311 | 0.7621 | 0.5658 | 0.9638 | 0.5091 | 0.8552 | 0.6961 | 0.3509 |
| Agromyces | F value | 0.2786 | 1.1231 | 0.1559 | 0.2919 | 0.3765 | 1.1043 | 0.4964 | 1.9856 |
| | *p* value | 0.6008 | 0.2963 | 0.6953 | 0.5923 | 0.5433 | 0.3003 | 0.4856 | 0.1674 |
| Anaeromyxobacter | F value | 6.5229 | 8.4698 | 0.7623 | 0.4099 | 0.3907 | 0.0342 | 0.7139 | 0.1785 |
| | *p* value | 0.0150 * | 0.0062 * | 0.3884 | 0.5261 | 0.5359 | 0.8544 | 0.4037 | 0.6752 |
| Arthrobacter | F value | 1.0408 | 2.0036 | 2.8977 | 2.1911 | 0.6268 | 0.9421 | 0.6153 | 0.1473 |
| | *p* value | 0.3144 | 0.1655 | 0.0973 | 0.1475 | 0.4337 | 0.3382 | 0.4379 | 0.7034 |
| Balneimonas | F value | 0.0039 | 0.5802 | 0.5395 | 0.0067 | 0.806 | 0.5842 | 0.4341 | 0.0174 |
| | *p* value | 0.9503 | 0.4512 | 0.4674 | 0.9354 | 0.3753 | 0.4497 | 0.5142 | 0.8959 |
| Candidatus Nitrososphaera | F value | 0.625 | 3.5294 | 1.7767 | 3.3941 | 0.5934 | 1.2691 | 0.2494 | 0.085 |
| | *p* value | 0.4344 | 0.0684 | 0.1909 | 0.0737 | 0.4461 | 0.2674 | 0.6206 | 0.7722 |
| DA101 | F value | 0.2995 | 0.8562 | 0.0998 | 0.0861 | 0.0912 | 0.0021 | 0.6403 | 0.0533 |
| | *p* value | 0.5876 | 0.361 | 0.7539 | 0.7709 | 0.7643 | 0.9634 | 0.4289 | 0.8188 |
| Gemmata | F value | na | na | na | na | 5.4564 | 2.85 | 1.4945 | 1.2886 |
| | *p* value | na | na | na | na | 0.0252 | 0.1 | 0.2295 | 0.2638 |
| Hyphomicrobium | F value | 0.1253 | 0.898 | 0.2804 | 1.0634 | 0.8193 | 2.9984 | 0.2897 | 0.4928 |
| | *p* value | 0.7254 | 0.3496 | 0.5997 | 0.3093 | 0.3714 | 0.0919 | 0.5938 | 0.4872 |
| Iamia | F value | 1.0891 | 1.5793 | 0.7708 | 0.107 | 0.1047 | 0.0365 | 0.0015 | 0.5087 |
| | *p* value | 0.3036 | 0.217 | 0.3858 | 0.7455 | 0.7482 | 0.8496 | 0.9697 | 0.4803 |
| Kaistobacter | F value | 0.3799 | 0.0067 | 2.1368 | 0.2269 | 2.5859 | 0.0076 | 4.2604 | 0.4021 |
| | *p* value | 0.5415 | 0.935 | 0.1525 | 0.6367 | 0.1166 | 0.9311 | 0.0463 * | 0.53 |
| Kribbella | F value | 0.2361 | 2.7448 | 3.787 | 0.305 | 2.0322 | 1.0765 | 1.9017 | 0.0028 |
| | *p* value | 0.63 | 0.1063 | 0.0595 | 0.5842 | 0.1626 | 0.3064 | 0.1764 | 0.9584 |
| Marmoricola | F value | 0.3086 | 0.0689 | 1.5718 | 0.0009 | 2.131 | 2.5889 | 0.1189 | 0.3302 |
| | *p* value | 0.582 | 0.7945 | 0.218 | 0.9769 | 0.153 | 0.1164 | 0.7323 | 0.5691 |
| Methylibium | F value | 1.4876 | 0.1639 | 4.0987 | 0.243 | 0.677 | 0.0181 | 0.0463 | 1.1569 |
| | *p* value | 0.2305 | 0.6879 | 0.0504 | 0.6251 | 0.416 | 0.8938 | 0.8309 | 0.2893 |
| Mycobacterium | F value | 0.0019 | 1.4361 | 0.4214 | 0.225 | 0.0215 | 0.0286 | 0.0247 | 0.0584 |
| | *p* value | 0.9657 | 0.2386 | 0.5204 | 0.6381 | 0.8843 | 0.8666 | 0.876 | 0.8103 |
| Nitrospira | F value | 0.809 | 0.1569 | 0.2015 | 2.2652 | 4.1841 | 0.4003 | 0.5294 | 4.7642 |
| | *p* value | 0.3744 | 0.6944 | 0.6562 | 0.141 | 0.0482 * | 0.5309 | 0.4716 | 0.0357 * |
| Nocardioides | F value | 0.01 | 1.6356 | 0.871 | 0.2688 | 0.5821 | 0.1636 | 0.792 | 0.0007 |
| | *p* value | 0.9211 | 0.2091 | 0.3569 | 0.6073 | 0.4505 | 0.6882 | 0.3794 | 0.9786 |
| Pedomicrobium | F value | 0.0077 | 0.0026 | 0.0848 | 0.2438 | 0.6524 | 1.7834 | 0.5823 | 0.4893 |
| | *p* value | 0.9306 | 0.9599 | 0.7726 | 0.6245 | 0.4245 | 0.1901 | 0.4504 | 0.4887 |
| Pirellula | F value | 0.0129 | 0.0011 | 0.3481 | 0.1816 | 0.01 | 0.088 | 0.2206 | 0.352 |
| | *p* value | 0.9102 | 0.974 | 0.5589 | 0.6725 | 0.921 | 0.7684 | 0.6414 | 0.5567 |
| Pseudonocardia | F value | 0.0523 | 1.3196 | 0.0251 | 0.83 | 0.2804 | 4.3372 | 1.0254 | 0.0234 |
| | *p* value | 0.824 | 0.2582 | 0.875 | 0.3683 | 0.5997 | 0.0445 * | 0.318 | 0.8794 |
| Rhodoplanes | F value | 0.345 | 0.1975 | 0.0197 | 0.1872 | 5.8762 | 3.0914 | 1.6575 | 1.3293 |
| | *p* value | 0.5606 | 0.6594 | 0.8892 | 0.6678 | 0.0205 * | 0.0872 | 0.2062 | 0.2565 |

**Table 3.** *Cont.*

| Taxon | | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC | DS vs. DSCC | Soybean DS vs. DSCC | Wheat DS vs. DSCC | Corn DS vs. DSCC |
| Skermanella | F value | 0.0046 | 0.3633 | 0.5194 | 0.0556 | 0.8042 | 0.3513 | 1.9887 | 0.2022 |
| | *p* value | 0.9461 | 0.5505 | 0.4758 | 0.8149 | 0.3758 | 0.5571 | 0.1671 | 0.6557 |
| Solirubrobacter | F value | 1.4444 | 2.1522 | 0.5978 | 0.0252 | 0.1223 | 0.0963 | 0.4421 | 0.0631 |
| | *p* value | 0.2373 | 0.151 | 0.4445 | 0.8749 | 0.7286 | 0.7581 | 0.5103 | 0.8031 |
| Steroidobacter | F value | 0.6637 | 0.1424 | 0.0778 | 0.5696 | 0.0761 | 2.5559 | 0.0976 | 3.1121 |
| | *p* value | 0.4206 | 0.7081 | 0.7819 | 0.4553 | 0.7842 | 0.1186 | 0.7565 | 0.0862 |
| Streptomyces | F value | na | na | na | na | 0.6263 | 0.0301 | 0.3006 | 0.4209 |
| | *p* value | na | na | na | na | 0.4339 | 0.8631 | 0.5869 | 0.5206 |

Note: The * indicate significant difference between direct seeding plots with cover crops (DSCC) or without cover crops (DS) based on the *p* value threshold ($p < 0.05$).

In 2018, no significant difference was observed on the abundance of eukaryotic taxonomy groups between crop managements (Figure 5). For the prokaryotes, one difference was observed for the abundance of the Anaeromyxobacter genus between crop managements ($p = 0.0331$) (Figure 6). Higher content was measured in DSCC 1.67 plots and DSCC 3.33 plots compared to DS 3.33 plots ($p = 0.0189$ and $p = 0.0317$ respectively) (Figure 6).

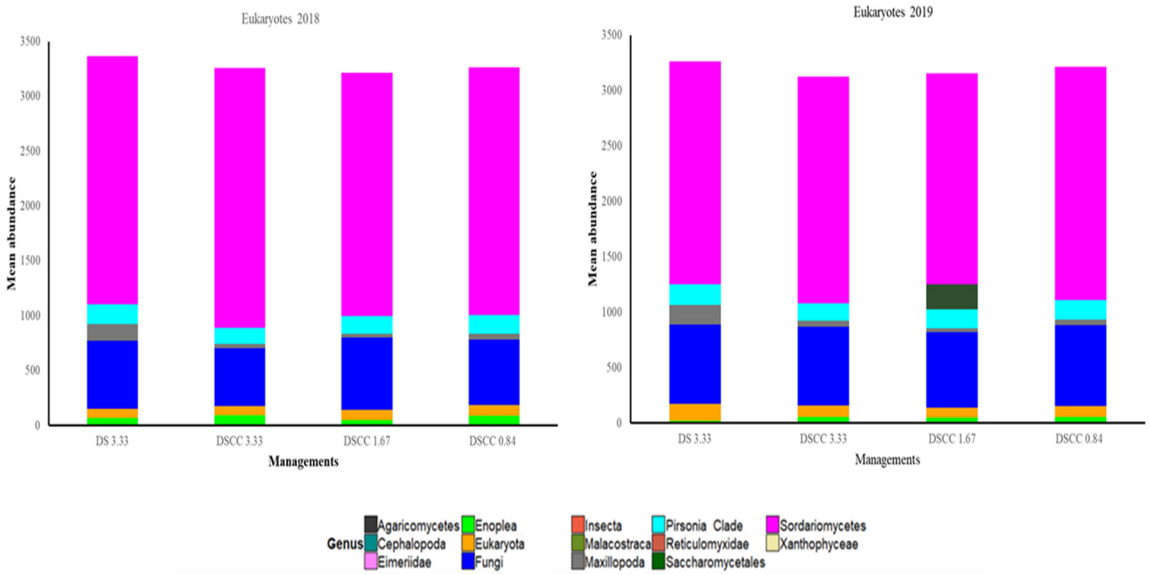


**Figure 5.** Abundance of eukaryotes between crop managements in 2018 and 2019.

In 2019, significant differences were observed for the Enoplea family ($p = 0.0494$) and the Agaricomycetes class ($p = 0.0341$) (Figure 5). A more abundant content of Enoplea was observed in DSCC 0.84 plots compared to DSCC 3.33 plots ($p = 0.0065$). The DSCC 3.33 plots had higher abundance of Agaricomycetes compared to DSCC 1.67 plots ($p = 0.0050$) (Figure 5). Also, one difference was observed for the prokaryotes (Figure 6). Indeed, the Marmoricola genus was more abundant in DSCC 0.84 plots compared to DS 3.33 plots ($p = 0.0162$) and to DSCC 1.67 plots ($p = 0.0099$).

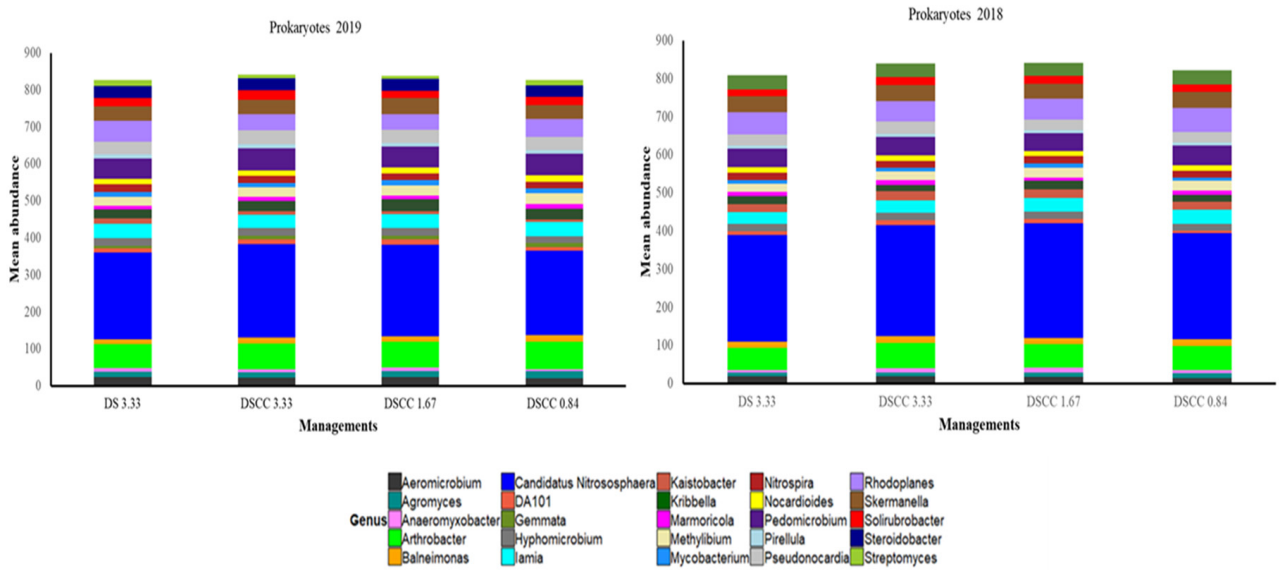


**Figure 6.** Abundance of prokaryotes between crop managements in 2018 and 2019.

Over 2018 and 2019 years, the relative abundance of each taxon was calculated for the different crops (Figure 7A). In the case of eukaryotes, the highest proportion is attributed to the fungus group where the genus Sordariomycetes is the more abundant (67.3 ± 0.1%) followed by other unidentified fungi (20.3 ± 0.5%) and Agaricomycetes (0.22 ± 0.02%). The other eukaryotes represented less than 6% of the total eukaryotic composition in soil and where the more abundant taxon is Pirsonia Clade (5.2 ± 0.2%) followed by other unidentified Eukaryota (3.0 ± 0.1%). The relative abundance of the other taxonomic groups represent less than 1%. Although it was not possible to observe any different relative abundance between crop mangements in corn plots, few differences have been observed in wheat and soybean plots. In soybean plots, a difference was observed for Agaricomycetes between the different crop managements ($p$ = 0.0244) and the highest relative abundance values were observed in DSCC 0.84 plots (0.41 ± 0.02%) and the lowest values in DSCC 3.33 plots (1.43 ± 0.35%) and DS 3.33 plots (1.04 ± 0.19%) (Figure 7A). In wheat plots, one difference was observed between crop managements for the Sordariomycetes genus ($p$ = 0.0241) (Figure 7A). In wheat plots, the highest relative abundance values were measured in the DSCC 3.33 plots (70.6 ± 4.5%) and the lowest in the DS 3.33 plots (63.7 ± 1.9%).

Relative abundance was also calculated for the 25 most abundant prokaryotes taxa where no differences were observed between crop managements in corn and soybean plots (Figure 7B). Among them, Candidatus Nitrososphaera have the higher relative abundance value (30.9 ± 0.4%), followed by Arthobacter (7.5 ± 0.2%), Rhodoplanes (7.2 ± 0.1%) and Pedomicrobium (6.7 ± 0.1%). All other groups of prokaryotes have a specific contribution of 5% or less of total abundance. Differences were only observed in the wheat plots between the different crop managements. In wheat plot, a difference was observed for the relative abundance of Arthrobacter ($p$ = 0.046). The highest value was measured in the DSCC 3.33 plots (7.6 ± 0.4%) and the lowest values in the DS 3.33 plots (5.6 ± 1.7%$^2$) (Figure 7B).

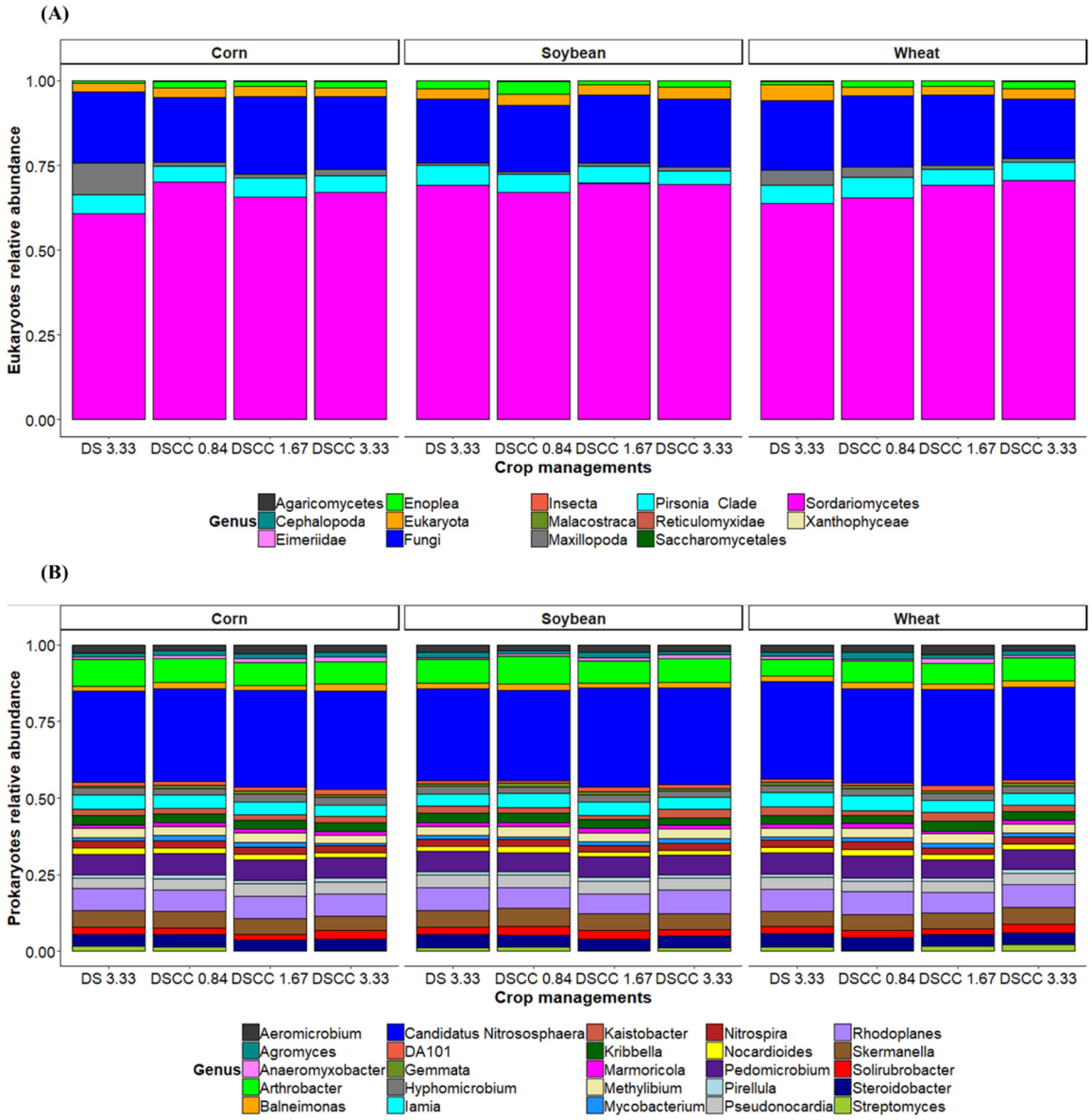


**Figure 7.** Relative abundance of eukaryotes (**A**) and prokaryotes (**B**) taxa and comparison between crops managements.

### *3.4. Elementary Content in Soil*

The data obtained from the elemental contents in the soils after two years show differences between crop managements and types of crops. In corn, lower Mn content was observed in DSCC 0.84 plots (Table 4), and higher Co content in DSCC 1.67 plots (Table 4). In wheat, Mg content was lower in DSCC 1.67 plots (Table 4). Mn content was also lower in DSCC 1.67 plots, but also in DSCC 0.84 plots (Table 4). The only lower content observed in DS 3.3 plots was that of Ni (Table 4). The greatest number of differences, however, was observed in soybean crops, where the contents of K, Mg, B, Fe, Mn, Zn, Ni, and Cd were different between crop managements (Table 4).

**Table 4.** Elementary contents between crop managements with different glyphosate-based herbicides application rates.

| Element (mg * kg$^{-1}$) | Corn | | | | | Soybean | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DS 3.3 | DSCC 3.3 | DSCC 1.67 | DSCC 0.84 | *p* Value | DS 3.3 | DSCC 3.3 | DSCC 1.67 | DSCC 0.84 | *p* Value |
| P | 27.72 ± 14.94 | 10.64 ± 1.32 | 12.96 ± 1.81 | 22.22 ± 7.66 | 0.4636 | 12.30 ± 0.59 | 11.32 ± 1.29 | 13.02 ± 1.77 | 8.58 ± 0.67 | 0.0723 |
| K | 313.17 ± 10.33 | 304.33 ± 12.39 | 327.33 ± 13.45 | 314.83 ± 10.22 | 0.5875 | 333.00 ± 4.45 a | 334.33 ± 3.37 a | 321.33 ± 8.80 ab | 311.67 ± 5.55 b | 0.0424 |
| Ca | 2989.67 ± 65.64 | 2908.33 ± 91.01 | 2896.33 ± 85.88 | 2874.50 ± 35.15 | 0.7017 | 2850.17 ± 151.18 | 3019.83 ± 100.57 | 2673.50 ± 75.01 | 2934.50 ± 49.47 | 0.0789 |
| Mg | 797.33 ± 30.72 | 850.83 ± 18.17 | 814.17 ± 8.89 | 826.50 ± 9.44 | 0.2702 | 717.67 ± 7.47 c | 739.33 ± 33.56 bc | 777.33 ± 11.97 ab | 828.33 ± 7.22 a | 0.0022 |
| AL | 1055.00 ± 11.19 | 1056.83 ± 5.72 | 1050.17 ± 5.87 | 1038.00 ± 8.35 | 0.3735 | 1050.33 ± 7.28 | 1045.83 ± 5.59 | 1066.33 ± 9.23 | 1043.83 ± 12.82 | 0.3192 |
| B | 0.753 ± 0.036 | 0.696 ± 0.010 | 0.765 ± 0.012 | 0.723 ± 0.027 | 0.2008 | 0.685 ± 0.048 b | 0.781 ± 0.029 a | 0.651 ± 0.013 b | 0.716 ± 0.015 ab | 0.0384 |
| Cu | 11.42 ± 0.37 | 11.60 ± 0.47 | 11.28 ± 0.20 | 10.93 ± 0.17 | 0.5445 | 10.75 ± 0.18 | 11.35 ± 0.29 | 11.30 ± 0.20 | 10.75 ± 0.18 | 0.1020 |
| Fe | 216.33 ± 5.35 | 217.83 ± 2.65 | 216.17 ± 4.64 | 233.17 ± 12.14 | 0.2493 | 226.33 ± 7.09 a | 215.67 ± 2.25 ab | 229.33 ± 5.90 a | 204.50 ± 2.58 b | 0.0077 |
| Mn | 24.67 ± 2.02 a | 19.25 ± 1.57 b | 25.93 ± 1.44 a | 19.17 ± 1.40 b | 0.0115 | 17.44 ± 3.85 b | 24.43 ± 2.11 a | 17.27 ± 0.92 b | 26.10 ± 1.40 b | 0.0249 |
| Zn | 2.79 ± 0.30 | 2.66 ± 0.17 | 2.56 ± 0.11 | 2.49 ± 0.06 | 0.6816 | 2.34 ± 0.05 b | 2.30 ± 0.02 b | 2.50 ± 0.05 a | 2.09 ± 0.06 c | 0.0001 |
| Na | 46.02 ± 2.04 | 44.73 ± 1.82 | 43.63 ± 1.56 | 44.58 ± 1.05 | 0.7897 | 41.85 ± 1.17 | 46.70 ± 1.46 | 47.20 ± 1.98 | 47.10 ± 1.59 | 0.0739 |
| Ni | 1.38 ± 0.08 | 1.40 ± 0.05 | 1.40 ± 0.05 | 1.44 ± 0.02 | 0.9138 | 1.57 ± 0.13 a | 1.33 ± 0.04 bc | 1.47 ± 0.03 ab | 1.12 ± 0.05 c | 0.0031 |
| Cd | 0.089 ± 0.004 | 0.091 ± 0.003 | 0.091 ± 0.003 | 0.092 ± 0.002 | 0.8851 | 0.093 ± 0.001 a | 0.090 ± 0.003 a | 0.087 ± 0.002 ab | 0.081 ± 0.002 c | 0.0226 |
| Cr | 0.294 ± 0.005 | 0.293 ± 0.007 | 0.292 ± 0.005 | 0.284 ± 0.010 | 0.7896 | 0.286 ± 0.004 | 0.291 ± 0.006 | 0.281 ± 0.006 | 0.285 ± 0.005 | 0.5929 |
| Co | 0.489 ± 0.042 ab | 0.414 ± 0.027 b | 0.523 ± 0.027 a | 0.399 ± 0.034 b | 0.0441 | 0.406 ± 0.056 | 0.498 ± 0.035 | 0.394 ± 0.023 | 0.488 ± 0.030 | 0.1455 |
| Pb | 3.69 ± 0.24 | 3.69 ± 0.22 | 3.76 ± 0.11 | 3.65 ± 0.17 | 0.9820 | 3.64 ± 0.18 | 3.83 ± 0.21 | 3.70 ± 0.15 | 3.30 ± 0.16 | 0.2201 |

| Element (mg * kg$^{-1}$) | Wheat | | | | |
|---|---|---|---|---|---|
| | DS 3.3 | DSCC 3.3 | DSCC 1.67 | DSCC 0.84 | *p* value |
| P | 12.84 ± 3.17 | 11.15 ± 1.15 | 12.36 ± 1.04 | 11.77 ± 1.02 | 0.9626 |
| K | 311.88 ± 9.17 | 322.38 ± 6.08 | 340.88 ± 12.86 | 332.00 ± 8.25 | 0.1779 |
| Ca | 2972.25 ± 44.77 | 2931.00 ± 102.28 | 2818.25 ± 71.30 | 2887.75 ± 45.92 | 0.4627 |
| Mg | 814.625 ± 6.47 a | 794.5 ± 8.23 ab | 775.38 ± 8.71 b | 809.88 ± 11.11 a | 0.0163 |
| AL | 1044.13 ± 8.51 | 1049.25 ± 9.42 | 1045.63 ± 9.58 | 1046.13 ± 9.13 | 0.9826 |
| B | 0.72 ± 0.02 | 0.74 ± 0.02 | 0.69 ± 0.03 | 0.67 ± 0.02 | 0.1314 |
| Cu | 11.16 ± 0.30 | 11.24 ± 0.24 | 11.06 ± 0.16 | 11.09 ± 0.23 | 0.9534 |
| Fe | 211.375 ± 2.76 | 222.88 ± 8.23 | 220.13 ± 3.93 | 219.25 ± 5.51 | 0.5023 |
| Mn | 25.79 ± 1.12 a | 25.08 ± 3.60 a | 19.39 ± 1.33 b | 20.6 ± 1.33 b | 0.0020 |
| Zn | 2.48 ± 0.16 | 2.57 ± 0.11 | 2.54 ± 0.09 | 2.47 ± 0.14 | 0.9270 |
| Na | 45.38 ± 1.76 | 44.24 ± 0.94 | 42.03 ± 0.99 | 41.88 ± 1.06 | 0.1478 |
| Ni | 1.25 ± 0.04 b | 1.35 ± 0.06 ab | 1.47 ± 0.06 a | 1.32 ± 0.06 ab | 0.0463 |
| Cd | 0.086 ± 0.008 | 0.088 ± 0.002 | 0.089 ± 0.002 | 0.085 ± 0.004 | 0.6212 |
| Cr | 0.302 ± 0.010 | 0.288 ± 0.008 | 0.281 ± 0.006 | 0.295 ± 0.004 | 0.2485 |
| Co | 0.517 ± 0.026 | 0.486 ± 0.028 | 0.440 ± 0.023 | 0.449 ± 0.022 | 0.1368 |
| Pb | 3.59 ± 0.16 | 4.00 ± 0.22 | 3.73 ± 0.13 | 3.51 ± 0.14 | 0.2050 |

Note: The * and different small letters indicate that mean values are significantly different between crop managements based on the *p* value threshold ($p < 0.05$).

However, the results from the ANOVA analyses with contrasts show that significant differences were only observed in wheat plots between DSCC plots and DS plots, regardless of GBH application rates (Table 5). These differences were observed for only three elements: P, K, and Zn (Table 5). K and Zn content were higher in the DSCC plots compared to the DS plots. In contrast, higher P content was observed in DS plots (Table 5).

**Table 5.** Contrast analysis of elementary contents between crop managements for 2018 and 2019.

| Element (mg × g$^{-1}$) | DS vs. DCC | | Corn DS vs. DSCC | | Soybean DS vs. DSCC | | Wheat DS vs. DSCC | |
|---|---|---|---|---|---|---|---|---|
| | F Value | *p* Value | F Value | *p* Value | F Value | *p* Value | F Value | *p* Value |
| P | 0.0261 | 0.8729 | 1.1465 | 0.2934 | 0.7935 | 0.3806 | 4.9135 | 0.0349 * |
| K | 0.0476 | 0.8288 | 1.6846 | 0.2049 | 0.3530 | 0.5572 | 5.0182 | 0.0332 * |
| Ca | 1.8144 | 0.1888 | 0.5848 | 0.4508 | 0.3518 | 0.5579 | 0.6945 | 0.4117 |
| Mg | 0.1278 | 0.7234 | 0.1517 | 0.6998 | 2.3576 | 0.1359 | 1.6951 | 0.2035 |
| Al | 0.4915 | 0.4891 | 3.3792 | 0.0767 | 0.7900 | 0.3817 | 0.0871 | 0.7701 |
| B | 0.7897 | 0.3817 | 0.6611 | 0.4230 | 0.3544 | 0.5564 | 0.0188 | 0.8918 |
| Cu | 0.0719 | 0.7906 | 1.6083 | 0.2152 | 0.1522 | 0.6994 | 0.1522 | 0.6994 |
| Fe | 0.0288 | 0.8665 | 1.4428 | 0.2397 | 0.1156 | 0.7364 | 1.2717 | 0.2690 |
| Mn | 0.8698 | 0.3590 | 0.4034 | 0.5305 | 0.1443 | 0.7069 | 1.8555 | 0.1840 |
| Zn | 0.0312 | 0.8610 | 0.7200 | 0.4033 | 0.7200 | 0.4033 | 5.4641 | 0.0268 * |
| Na | 0.1060 | 0.7471 | 0.5416 | 0.4679 | 1.7681 | 0.1944 | 1.3674 | 0.2521 |
| Ni | 0.0107 | 0.9183 | 0.5648 | 0.4586 | 0.2285 | 0.6364 | 1.0726 | 0.3092 |
| Cd | 0.2332 | 0.6329 | 0.2467 | 0.6233 | 0.2565 | 0.6165 | 0.0263 | 0.8724 |
| Cr | 1.8885 | 0.1803 | 2.1776 | 0.1512 | 0.2788 | 0.6017 | 2.0939 | 0.1590 |
| Co | 1.0200 | 0.3212 | 0.6982 | 0.4105 | 0.0512 | 0.8226 | 1.3122 | 0.2617 |
| Pb | 0.2854 | 0.5974 | 0.0093 | 0.9240 | 0.3794 | 0.5429 | 2.6538 | 0.1145 |

Note: The * indicate significant difference between DSCC crops or DS crops based on the *p* value threshold ($p < 0.05$).

## 4. Discussion

### *4.1. No Significant Difference in Richness and Evenness Along with Crop Managements*

The results show that there is no significant difference in eukaryotic and prokaryotic richness, nor in the diversity and evenness indices (Observed, Shannon, Chao1) between DS and DSCC crop managements in both 2018 and 2019 (Figure 3A–D and Figure 4). These results are in line with those observed in a similar geographical and soil context who also observed that richness was similar between DS and DSCC crop managements at similar GBH application rates [28]. This could indicate that other environmental or agricultural factors such as ploughing, climate or crop type, play a more dominant role in the short term [39,40]. It was observed in a previous study that even after a long-term implantation of winter crops, the influence of CC could not be distinguished from that of DS, and that the main benefits in terms of microbiota richness seemed to arise from stopping ploughing than from using CC [40,41].

### *4.2. Influence of Crop Type on Prokaryotic and Eukaryotic Composition*

The use of PERMANOVA analysis allows for the capture of multivariate effects to better understand interactions between crop types, crop managements, and microbial communities. The analysis indicates that crop type has a significant effect on eukaryotic composition in both 2018 and 2019. This may be explained by the fact that the crop itself (soybean, wheat, corn) influences the structure of eukaryotic soil communities, likely due to differences in the rhizosphere associated with each crop [42–44]. The type of crop also dictates the cover crop (CC) mixture used, which in turn directly affects the content of certain soil elements. This was particularly observed for P, K, and Zn content in wheat plots (Table 5). The highest levels were found in DSCC plots compared to DS plots when we excluded the potential influence of GBH application rates. Crops may affect soil microbiota through root exudates, plant debris and symbiotic associations, or direct alteration of the supply of carbon to the soil, nutrient availability and soil structure [45]. It has already been observed that the use of different maize genotypes can influence the composition of eukaryotes in the soil, significantly increasing the presence of phytophagous nematodes and mycorrhizal fungi, compared to a site where maize has not been cultivated [44]. Interestingly, although the different crop managements and crop types appear to influence the composition of eukaryotic communities, they do not seem to have influenced the fungal group. It has been observed in the past that fungi, especially arbuscular mycorrhizal fungi, are much more sensitive to mechanical soil disturbance [46]. In no-till crop managements, the fungal composition can be maintained through the use of mulch from previous crop residues, which can serve as both a support and a resource, as is the case in the DS plots of this study.

Notable effects on specific taxonomic groups

#### 4.2.1. Procaryotes

Significant differences are observed in certain prokaryotic genera, such as Anaeromyxobacter in 2018 or Nistropira and Rhodoplanes in 2019 (Table 3), highlighting that specific bacterial groups may be sensitive to differences between DS and DSCC crop maagements. The difference for Anaeromyxobacter is greater in soybean plots. This could be explained by the fact that soybean may influence the nitrogen-fixing bacterial community and other nitrogen-transforming microbial communities such as Anaeromyxobacter [47,48]. Anaeromyxobacter is a genus of bacteria that plays a role in the biogeochemical cycling of organic matter, often involved in the reduction of oxygen and other electron acceptors in soils [49,50]. Soil oxygenation and nutrient availability are key factors in determining the microbial communities involved in organic matter decomposition, and fluctuations in

these parameters can influence the distribution and activity of specific microorganisms like Anaeromyxobacter [51,52].

Similarly, significant differences for Nistropira and Rhodoplanes in 2019 may also be linked to changes in soil pH, nitrogen availability, or organic matter dynamics under different management practices [51,53]. The presence of clover in corn plots could have influenced the presence of Nitrospira which is also supported by the fact that cover crop mixture containing more legume support bacteria associated with nutrient cycling and nitrification [54].

#### 4.2.2. Eukaryotes

Significant differences have been observed in 2019 for three taxonomic groups: the class Cephalopoda, Maxillopoda, and the group of other Eukaryota, between DSCC plots and DS plots (Table 2). Cephalopoda and Maxillopoda being considered as aquatic organisms, it is unlikely these species be quite involved in agricultural soil functions. The relative abundance of these species remains very low (<1%) and negligible compared to other taxonomic groups observed in the study. Another explanation may originate from the marine deposits the soil under study is derived from. It is possible that the use of CC has facilitated the mobility of certain trace compounds and their detection through metagenomic analysis. However, the link with the use of CC and their abundance is not obvious in the context of this study. As for the other Eukaryota, the presence of a vegetation cover and structural and functional root diversity may have increased the resources needed by certain eukaryotes, thus stimulating the growth of their population [41].

### *4.3. The Cross-Effect Between CC and GBH Application Rates on Soil Microbiota Content*

Our results indicate that GBH application rates have no significant effects on the richness, uniformity, or composition of eukaryotic and prokaryotic communities in the soils during the two years studied (2018 and 2019). Some studies reported a reduction in the biomass, activity or richness of soil microorganisms following the use significantly higher GBH application rates than those used in this study [55–57]. However, it is important to highlight that GBH application rates in this study are resembling those generally used by farmers in Québec. The GBH application rates seem to have more influence on the abundance of taxonomic group in short term. The relationship between GBH application rates and microbial diversity is often rate-dependent [58,59]. At lower application rates, the herbicide might have a subtle effect on microbial communities, possibly reducing the abundance of sensitive species without causing significant shifts in overall diversity. However, at higher application rates, more pronounced changes might occur, such as a decrease in microbial diversity or a shift toward glyphosate-tolerant species. Higher application rates might lead to the selection of glyphosate-tolerant microorganisms, altering community dynamics such as a decrease in microbial diversity or a shift toward tolerant species [58,60]. Also, GBH impact on soil microbial communities might not be immediately visible but could accumulate over time. Multiple application cycles could lead to long-term shifts in microbial community structure and function that become apparent after reaching the threshold at which GBH levels begin to significantly impact soil health and microbial communities [23].

The cross-effect between CC and GBH appears to be influenced by the type of crops. In this study, this effect seems more pronounced in soybean crops compared to corn and wheat crops (Table 4). Soybean plots exhibit greater contrasts in elementary contents with different crop managements. This is particularly the case for elements essential to crop development, such as B, Fe, K, Mg, Mn, and Zn (Table 4), as well as certain soil microorganisms [61]. Although it has been shown that the use of CC alone does not explain the differences in

elementary contents in soybean plots (Table 5), the variations in these contents do not either follow a GBH application rate-dependent relationship (Table 4). This is particularly true for B, Mn, Ni, and Zn. In some cases, the content of these elements was even lower in plots with the lowest GBH application rate (0.84 L $ha^{-1}$). On the other hand, for other elements like Mg, the influence of higher GBH application rate seems more obvious. It is well known that glyphosate has chelation properties [62], which could explain the lower Mg content in plots with a 3.3 L $ha^{-1}$ GBH application rate. However, it is still unclear whether it can influence metal bioavailability in soils, potentially contributing to either increased toxicity or nutrient limitations for soil organisms and plants [62]. Here, this potential causal link between GBH application rates and elemental content is not straight forward, which partly explains why these differences were not observed in wheat or corn crops. This highlights the importance of the interaction between the effects of GBH application rates and the legacy left by the type of cover crops used in previous crops. Subsequently, this cross-effect can significantly influence the abundance of certain microorganisms, as observed in this study.

The cross-effect between CC and GBH application rates on the Prokaryotes content

Prokaryotes are assumed to be the organisms potentially impacted by different GBH applications [23,24,60]. While most eukaryotes do not function with the shikimate pathway, certain bacteria and fungi do, as an essential step of the synthesis of aromatic amino acids [23,25]. In 2018, the abundance of Anaeromyxobacter was higher in DSCC 3.33 and DSCC 1.67 plots (Figure 6). As mentioned earlier, Anaetromyxobacter are widely involved in soil functions and health. The interaction between CC and GBH application rates seems to have favored certain weeds species, itself being corroborated by a lower weed cover rate in these plots [63].

Interestingly, higher abundance of Marmoricola was observed in DSCC 0.84 plots (Figure 6). Marmoricola is a Gram-positive and chemoorganotropic prokaryote genus that has already been considered in other studies as an interesting indicator for soil microbiota activity such as soil dehydrogenase, acid phosphatase, pH, TK, and C/N cycling all promoting high crop yields [64–66]. Like other actinobacteria, the presence of Marmoricola seems to be sensitive to certain environmental conditions and agricultural practices, which can be an asset in determining the level of soil health, even in the short term [15,67]. It can be seen that with GBH application rates of 1.67 L $ha^{-1}$ and above the abundance of Marmoricola is lower. If different GBH application rates caused specific shifts in bacterial community composition (for example, a decline in nitrogen-fixing bacteria at higher rates), this could highlight the potential rate-specific impacts of GBH applications on critical soil functions. GBH can indirectly affect soil health through its impact on microbial populations involved in key processes like nutrient cycling and organic matter decomposition [68,69]. Higher GBH application rates or more frequent exposition to them might impair these processes by suppressing microbial taxa essential for breaking down organic matter, releasing nutrients, and maintaining soil structure.

The cross-effect between CC and GBH application rates on Eukaryotes content

A difference is observed between crop managements for Enoplea, a class of nematodes (Figure 5) the only representative of this genus in this study being Longidorus genus. This genus counts 176 species and generally includes phytopathogenic species, an external parasite of plant roots in the rhizosphere [70,71]. Longidorus abundance is higher in plots with CC and significantly more present in DSCC 0.84 plots (Figure 5). The lowest abundance of this type of nematode was observed in the DS 3.33 plots (Figure 5). That may be explained by the fact that the effect of glyphosate could potentially be more pronounced, particularly among sensitive organisms such as fungi, nematodes, and certain protozoa [72]. It has been observed that even at low GBH application rates, glyphosate can induce oxidative stress in

nematodes [73], and that the persistence of glyphosate in the environment can influence the structure, abundance, and recovery of various nematode communities in the long term [74]. Here, the effects are more pronounced and harmful to the nematodes beyond 0.84 L $ha^{-1}$ in two applications. On the other hand, these results may also represent the more pronounced presence of vegetation in these plots. The presence of CC increases root diversity and root exudates, which probably favours the presence of nematodes [75–79]. However, the direct influence of the use of CC on Longidorus abundance is not demonstrable in this study, which suggests that another factor could explain their higher abundance in DSCC 0.84 plots. In these plots, a higher weed cover rate was measured, compared with plots with other weed managements and higher GBH application rates [63].

Agaricomycetes are the other eukaryote group where significant differences were observed between crops managements (Figure 5). This class of fungi comprises almost 36,000 species, some closely associated with wood rot [80,81]. Schizophyllum abundance was highest in DSCC 3.33 plots compared to DSCC 1.67 plots (Figure 5). Fungi, particularly arbuscular mycorrhizal fungi, can be sensitive to glyphosate and their response to different application rates could vary [82,83]. However, their abundance was very low here and it is known that the influence of crop managements on Schizophyllium varies greatly according to the species known in this genus [81,84].

It is important to note that the effects of GBH may become more pronounced with longer exposure, resulting from higher application rates or more frequent applications [72,85,86]. In crop managements where GBH are applied at higher rates, the impact on microbial communities might be more pronounced in DS compared to DSCC. These interactions could modulate microbial diversity and soil health in complex ways. If GBH are used along with DS as part of a weed control strategy in conventional farming, their potential effects on soil microorganisms could interact with the broader impacts of the management practices themselves (e.g., ploughing, inorganic fertilizers, other pesticides) [17,87]. For example, DSCC, which may involve more sustainable practices (e.g., cover cropping or reduced tillage), could mitigate some of the negative impacts of glyphosate on soil health by enhancing soil structure or organic matter content, which in turn might act as a buffer against some harmful effects of GBH on the microbial communities [25]. However, the number of studies comparing DS and DSCC with GBH applications on soil microbiota richness remains limited, particularly in temperate regions, despite the increasing interest in the use of CC [41].

Also, another important factor to consider is the potential development of resistance to glyphosate among soil microorganisms. While this is more commonly associated to weeds, there is growing evidence that certain soil bacteria and fungi can develop tolerance to glyphosate over time [88,89]. It was reported that *P. lilacinum* has the ability to degrade glyphosate to a considerable extent and to utilise the chemical as a P source, without showing rate-dependent negative effects on its growth [89]. The use of higher GBH application rates for effectively controlling weeds along with climate change might also lead to collateral damage to non-target organisms, including beneficial microbes. It is crucial to assess whether the benefits in terms of weed control outweighs the potential negative effects on microbial diversity and ecosystem functioning at higher rates.

## 5. Conclusions

In this study, no significant difference in microbial richness, evenness, or diversity between DS and DSCC crop managements in both 2018 and 2019 are observed, suggesting that other factors such as climate, or crop type may play a more dominant role in shaping soil microbial communities in the short term. However, specific changes observed in prokaryotic and eukaryotic groups, highlight the complex interactions between crop

management practices, GBH application rates, and soil microbial communities. While GBH application rates does not significantly affect microbial richness, certain taxonomic groups, particularly Anaeromyxobacter, Marmoricola and Enoplea, show varying responses to different GBH application rates and crop management. The presence of CC seems to facilitate the growth of certain microbial populations, possibly by increasing resource availability through root diversity and exudates. While GBH application rates show a subtle impact on microbial communities, their effects may become more pronounced over time with higher rates or repeated applications. Additionally, the combined effects of GBH and CC on microbial abundance are still complex and require further exploration. This relationship appears even more complex in soybean plots, where many differences in the content of certain soil elements were also observed, potentially influencing the abundance of certain taxonomic group. Long-term studies are needed to fully understand the cumulative impact of glyphosate on soil health and microbial dynamics. Moreover, the potential for microbial resistance to glyphosate must be considered, especially considering increasing herbicide use in field crop agriculture with climate change. These results underline the importance of considering both the direct effects of GBH application and the broader management practices in maintaining soil biodiversity and ecosystem functions.

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