

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

**AVANTAGES D'IMPLANTER DES PLANTES DE COUVERTURES EN GRANDES
CULTURES DE SOJA GT(*GLYCINE MAX* (L.) MERR.) ET MAÏS (*ZEA MAYS* (L.)
TOLÉRANTES AUX HERBICIDES À BASE DE GLYPHOSATE**

THÈSE
PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN SCIENCES DE L'ENVIRONNEMENT

PAR
JÉRÔME BERNIER BRILLON

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DÉDICACE

« Pour tirer le meilleur parti des connaissances acquises, pour en extraire toute la richesse, il importe de ne pas s'y habituer trop vite, de se laisser le temps de la surprise et de l'étonnement » (Hubert Reeves - L'espace prend la forme de mon regard)

AVANT-PROPOS

Cette thèse a été réalisée dans le cadre du projet MYGROG (*Maintaining high yields in Quebec field crops while reconsidering the option of using glyphosate*) et dirigée par le professeur Marc Lucotte, détenteur de la chaire de recherche en durabilité des grandes cultures. Ce projet a été financé par le programme de partenariat stratégique Conseil de Recherches en Sciences Naturelles et en Génie du Canada (CRSNG). La thèse est présentée sous la forme de trois articles scientifiques rédigés en anglais (chapitres I, II et III), précédés d'une introduction générale, rédigée en français. En date du dépôt de la thèse, deux chapitres ont été acceptés pour publication, le premier dans *Physiologia* et le second dans *Agriculture* et le troisième chapitre a été soumis dans *Agriculture*. Le Centre de recherche sur les grains (CEROM) a contribué à la réalisation des travaux décrits dans les trois chapitres, par la mise en place du protocole expérimental et par le travail des techniciens agronomiques sur son site à St-Mathieu-de-Beloeil. Un agronome du Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), Gilles Tremblay, a aussi contribué à la planification du design expérimental et a agit comme conseiller agronomique. L'auteur de cette thèse est l'auteur principal des trois articles scientifiques et la contribution de tous.tes les co-auteurs.trices est détaillée ci-dessous.

Chapitre I : Influence of no-till system with or without cover crops on stomata sensitivity of glyphosate-tolerant soybeans to vapor pressure deficit.

Co-auteurs : Jérôme Bernier Brillon, Matthieu Moingt et Marc Lucotte

Cet article est publié dans *Physiologia* (*Physiologia* 2023, 3, 531–541. <https://doi.org/10.3390/physiologia3040039>). L'auteur principal a participé à la planification du

design expérimental et de l'approche méthodologique, à l'échantillonnage effectué sur le terrain, à l'analyse et la validation des résultats, à la rédaction, à la revision et à la publication de l'article. Matthieu Moingt et Marc Lucotte ont participé à la planification du design expérimental, à la supervision des tâches, à la validation des résultats, à l'acquisition de ressources et à la revision de l'article. L'administration générale du projet et le financement associé ont été principalement orchestrés par Marc Lucotte.

Chapitre II : Using cover crops as means for controlling weeds and reducing the applied quantity of glyphosate-based herbicide in no-till glyphosate tolerant soybean and corn.

Co-auteurs : Jérôme Bernier Brillon, Marc Lucotte, Ariane Bernier, Myriam Fontaine et Matthieu Moingt

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Chapitre III : Cross-effect between cover crops and glyphosate-based herbicide application on microbiote communities in field crops soils.

Co-auteurs : Jérôme Bernier Brillon, Marc Lucotte, Gilles Tremblay, Blandine Giusti et Matthieu Moingt

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

2,4-D	Acide (2,4-dichlorophénoxy) acétique
%	Pourcentage
°C	Degré Celsius
AC	Agriculture de conservation
ACCase	Acétyl-coenzyme A carboxylase
Acétyl-CoA	Acétyl-coenzyme A
AICc	Akaike information criterion with correction
Al	Aluminium
ALS	Acétolactate synthase
AMPA	Acide aminométhylphosphonique
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
A.U.	Amplification units
B	Bore
C	Carbone
C3	Plante avec fixation du carbone en C3 (3-phosphoglycérate)
Ca	Calcium
CA	Conservation agriculture
CC	Cultures de couverture
CCs	Cover crops

Cd	Cadmium
CEROM	Centre de recherche sur les grains
CH ₄	Méthane
CI	Confidence intervals
cm	Centimètre
C/N	Carbon-to-Nitrogen ratio
CO	Monoxyde de carbone
Co	Cobalt
CO ₂	Dioxyde de carbone
CP4	Gène de souche <i>Agrobacterium tumefaciens</i>
Cr	Chrome
CRSNG	Conseil de Recherches en Sciences Naturelles et en Génie du Canada
Cu	Cuivre
Df	Degrees of freedom
DistVein	Distance entre les nervures foliaires secondaire
DS	Direct seeding
DSCC	Direct seeding on cover crops
DW	Dry weight
EPSP	5-Énolpyruvylshikimate-3-phosphate
FAO	Organisation des Nations unies pour l'alimentation et l'agriculture
Fe	Fer
F value	Value of Fisher test
FW	Fresh weight
g	Gramme
GBH	Glyphosate-based herbicide

GES	Gaz à effet de serre
GLM	General linear model
GOX	Gène Glycolate oxidase
Gs	Conductance stomatique
Gsmax	Conductance stomatique maximum
GT	Glyphosate tolerant
h	Heure
H ₂ O	Eau
ha	Hectare
HBG	Herbicide à base de glyphosate
hPa	Hectopascal
IBIS	Institute of Integrative Biology and Systems
ICC	Intraclass correlation coefficient
IWM	Integrated weed management
K	Potassium
km	Kilomètre
kg	Kilogramme
L	Litre
LL	Lower limits
m	Mètre
mg	Milligramme
MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
Mg	Magnésium
mm	Millimètre
mmol	Millimole

Mn	Manganèse
MYFROG	Maintaining high yields in Quebec field crops while considering the option of using glyphosate
N	Azote
Na	Sodium
Ni	Nickel
N ₂ O	Protoxyde d'azote
OGM	Organisme génétiquement modifié
OTU	Operational Taxonomic Units
P	Phosphore
<i>P</i>	Valeur de probabilité qui permet de tester l'hypothèse nulle
Pb	Plomb
PERMANOVA	Permutational analysis of variance
qPCR	Quantitative polymerase chain reaction
r^2	Coefficient de détermination
R2	Stade reproductive #2 des plants
R3	Stade reproductive #3 des plants
s	Seconde
SE	Standard error on mean
SEM	Scanner electron microscope
SD	Semis direct
SOC	Soil organic carbon
StoDen	Stomatal density
StoIndex	Stomatal index
StoLength	Stomatal length
StoSize	Stomatal size

StoWidth	Stomatal width
t	Tonne
TDR	Time-domain reflectometry
TG	Tolérant au glyphosate
TK	Total potassium content
UL	Upper limits
µm	Micromètre
V2	Stade végétatif #2 des plants
Vpd	Déficit de pression de vapeur
Vpd.c	Valeur centrée du déficit de vapeur
v/v	Pourcentage volume/volume
VWC	Voumetric water content
Zn	Zinc

RÉSUMÉ

Le recours combiné aux cultivars tolérants au glyphosate (TG) et à l'utilisation d'herbicides à base de glyphosate (HBG) a facilité l'adoption de pratiques agricoles à travail réduit du sol, telles que le semis direct (SD), dans les grandes cultures à l'échelle mondiale. L'intégration de cultures de couverture (CC) pourrait accroître les avantages associés aux pratiques de semis direct, contribuant ainsi à la productivité et à la durabilité des cultures TG. L'objectif de cette thèse, menée sur deux ans, était d'évaluer si l'association des CC avec des cultures TG en semis direct pouvait constituer une stratégie efficace pour améliorer la productivité, la résilience et la durabilité des grandes cultures tout en réduisant l'usage de HBG. L'étude visait en particulier à observer les effets de l'utilisation des CC sur les stress hydriques vécus par les cultures, le contrôle des adventices et la diversité des microorganismes du sol dans les systèmes TG traités avec des HBG. Grâce à un design expérimental reproduisant les pratiques agricoles courantes en grandes cultures TG, les résultats obtenus permettront de mieux guider les producteurs dans l'adoption ou non des CC pour une transition écologique réussie.

Dans le chapitre 1 de cette thèse, il est question de déterminer si l'utilisation de CC peut influencer le potentiel d'échange gazeux du soja TG lorsque le déficit de pression de vapeur (Vpd) augmente. L'étude a été menée dans des parcelles cultivées en semis direct avec CC (DSCC) ou sans CC (DS) exposées à une quantité de HBG appliqué à $3,3 \text{ L ha}^{-1}$. La conductance stomatique (Gs) a été mesurée cinq fois sur les mêmes feuilles identifiées après l'application d'un herbicide à base de glyphosate. Ces feuilles ont ensuite été prélevées afin d'observer les stomates et les traits foliaires à l'aide d'un microscope à balayage électronique. Le Vpd a été calculé en même temps que les mesures de Gs à la surface des feuilles. Les résultats obtenus suggèrent que l'utilisation des CC favorise la plasticité du soja à travers des changements phénotypiques des feuilles (une nervation plus élaborée et une densité stomatique abaxiale plus élevée), ce qui peut à son tour améliorer leur tolérance aux conditions plus sèches. En 2019, les valeurs de Gs étaient jusqu'à 29% plus élevées dans les parcelles avec CC par rapport à celles sans CC avec des valeurs Vpd similaires. Cette étude montre que les avantages de l'utilisation des CC peuvent être observés à travers les stratégies de développement morphologique des plantes cultivées et leur plus grande tolérance à la sécheresse.

Dans le cas du chapitre 2 de cette thèse, le but est d'évaluer si l'utilisation de CC dans les productions de soja et de maïs TG peut aider à lutter contre les mauvaises herbes et réduire la quantité de HBG nécessaire pour lutter contre celles-ci. L'échantillonnage a été réalisé sur 32

parcelles expérimentales (4 traitements culturaux avec 4 répétitions sur les deux cultures). La gestion des cultures a consisté en des applications de HBG à des taux de 0,84, 1,67 et 3,3 L ha⁻¹ dans les parcelles DSCC et à des taux de 3,3 L ha⁻¹ dans les parcelles DS. Les principales variables considérées étaient le taux de couverture des adventices, les paramètres des plantes (poids frais et sec et hauteur), les rendements en grains. Les teneurs en eau et en cations dans le sol ont également été considérées comme des indicateurs de la compétition interspécifique potentielle des cultures avec les CC ou les adventices. Les résultats obtenus au cours des deux années montrent qu'il est possible de réduire l'utilisation de HBG de 50 % dans les parcelles avec CC par rapport aux parcelles sans CC en utilisant un taux d'application de HBG de 3,33 L ha⁻¹ (DS 3,33). Cependant, les adventices ont eu un impact important sur la teneur en eau du sol, ce qui s'est traduit par des plantes plus petites et des rendements plus faibles dans les parcelles où seulement 0,84 L ha⁻¹ de HBG a été appliqué. Dans le contexte de l'étude, l'utilisation des CC semble faciliter le développement d'une agriculture plus durable, tout en réduisant les quantités de HBG généralement utilisées.

Le chapitre trois de la thèse a pour objectif d'observer si l'utilisation de CC peut favoriser la richesse et l'abondance des communautés eucaryotes et procaryotes par rapport à une gestion DS sans CC. L'étude a été effectuée grâce à un design expérimental en blocs aléatoires qui intégraient un total de 48 parcelles (12 traitements culturaux en soja, maïs et blé répliqués quatre fois). Des taux d'application de HBG de 0,84, 1,67 et 3,33 L ha⁻¹ ont été appliqués dans les parcelles DSCC et de 3,33 L ha⁻¹ dans les parcelles DS. Le sol échantillonné a permis de mesurer la richesse, l'abondance et la composition des communautés de microorganismes. Les résultats obtenus sur deux ans n'ont montré aucune différence pour l'indice de richesse entre les cultures. Peu de différences ont été observées pour la diversité bêta entre les cultures en fonction du nombre d'unités taxonomiques opérationnelles. Pour les eucaryotes, les valeurs d'abondance les plus élevées ont été observées dans les parcelles DSCC 0,84 ($54,8 \pm 9,7$ pour *Enoplea*) et DSCC 3,33 ($5,25 \pm 1,51$ pour *Agaricomycetes*) en 2019. Pour les procaryotes, l'abondance d'*Anaeromyxobacter* était plus élevée dans les parcelles DSCC 3,33 ($11,92 \pm 1,90$) et DSCC 1,67 ($12,00 \pm 1,76$) en 2018 et celle de *Marmoricola* dans les parcelles DSCC 0,84 en 2019. Les résultats obtenus ici démontrent que l'utilisation de CC peut influencer l'abondance totale et l'abondance relative de certains groupes taxonomiques de procaryotes et d'eucaryotes. Ces résultats montrent que l'usage de CC peut augmenter les bénéfices obtenus dans les régies en SD, et démontre l'intérêt d'implanter à plus long terme des CC comme une solution durable pour promouvoir l'abondance métagénomique des sols dans les grandes cultures TG. Cette thèse a également démontré que l'usage de CC est un outils indispensable pour le producteurs et pourrait permettre d'atteindre tous les objectifs généraux du plan d'agriculture durable du Québec. Les différents bénéfices observé avec l'usage des CC dans cette étude sont complémentaires et essentiels pour améliorer l'intégrité, la santé et la résilience des sols agricoles et des grandes cultures.

Mots clés : Culture de couverture, herbicide à base de glyphosate, gestion des adventices, grandes cultures tolérantes au glyphosate, paramètres agronomiques, déficit de pression de vapeur, trait foliaire, communauté des microorganismes

INTRODUCTION

I. ÉTAT DES GRANDES CULTURES DANS LE MONDE

I.1 Portrait de la problématique

Selon la FAO, environ 11% des superficies terrestres sont cultivées, soit 36% des terres arables ou jugées acceptables pour être cultivées (FAO, 2021). Une grande part des superficies agricoles et de production agricole mondiale sont attribuées aux grandes cultures. Celles-ci sont majoritairement liées à la production de grains tels que des céréales (ex. maïs, blé et riz), des oléagineux (ex. tournesol, canola, arachide, olive), des protéagineux (ex. soja, pois et d'autres légumineuses). Les quatre productions les importantes en grandes cultures, le maïs, le soja, le blé et le riz, représentent 74% de la production de grain, 35% des superficies cultivées et 30% du volume de la production agricole mondiale (FAO, 2021). Au cours des dernières années dans le monde, près de 38% des grains produits en grandes cultures ont été consommés par des humains, 30% par le bétail et 32% en produits transformés, dont 6% en biocarburant (MAPAQ, 2020).

Les grandes cultures sont souvent associées à l'agriculture conventionnelle et à un système de production fortement mécanisé qui nécessite des quantités importantes d'intrants de synthèse (fertilisants et pesticides) (Abston, 2018; Sumberg et Giller, 2022). Ce type de système de production découle de la révolution verte et de la motivation à développer des systèmes agricoles plus intensifs et spécialisés (Abston, 2018). Un des principaux objectifs de la révolution verte était d'obtenir des rendements élevés tout en limitant les superficies agricoles cultivées (Abston, 2018). Malgré que cette intensification et cette spécialisation des productions ont permis d'augmenter la

production agricole de 146% entre 1961 et 2000, cette approche n'est toutefois pas soutenable sur le long terme (FAO et ITPS, 2015). Lors des dernières décennies, l'agriculture conventionnelle a entraîné la dégradation et la perte des sols arables, la perte de biodiversité et l'augmentation de la résistance aux traitements chimiques chez certains ravageurs et adventices (FAO et ITPS, 2015; FAO *et al.*, 2020; Perotti *et al.*, 2020). Avec les changements climatiques actuels et à venir, ces enjeux risquent d'être exacerbés (Li *et al.*, 2009; Li et Fang, 2016; Rhodes, 2014; Ziska, 2016) ce qui représentera un défi colossal pour d'une part, les producteurs, mais également pour l'atteinte de l'objectif de produire plus pour nourrir une population mondiale de plus de 9 milliards en 2050 (van Dijk *et al.*, 2021).

I.2 Dégradation des sols

L'intensification des pratiques agricoles a d'une part, permis d'augmenter de façon considérable la production mondiale de denrées alimentaires, mais également d'impacter négativement la qualité des superficies cultivées. Actuellement, environ 33% des superficies cultivées sont dégradées (FAO et ITPS, 2015; Ferreira *et al.*, 2022). Selon la FAO (2024), la dégradation du sol représente les modifications chimiques, physiques ou biologiques pouvant influencer l'état de santé du sol et entraîner une diminution de la capacité de l'écosystème à fournir des biens et services pour ses bénéficiaires, donc maintenir les rendements de la production agricole dans la présente étude. Une des principales raisons de cette dégradation est liée à l'utilisation de la machinerie lourde et au travail mécanique des sols (de Oliveira Silva *et al.*, 2019; Lee *et al.*, 2021; Strudley *et al.*, 2008; Yu, Z. *et al.*, 2020). Plusieurs types de labours existent et les impacts sur les sols sont différents selon ceux-ci (Catania *et al.*, 2018; de Oliveira Silva *et al.*, 2019). Toutefois, un travail intensif et répété du sol effectué par le labour a des répercussions importantes sur la fertilité, le contenu en eau via et la biodiversité du biote des sols agricoles (Catania *et al.*, 2018; de Oliveira Silva *et al.*, 2019; Morugan-Coronado *et al.*, 2022; Vanwalleghem *et al.*, 2017). Il est commun d'observer un minimum de 5-6 passages par saison de croissance de machinerie pour obtenir un sol meuble pour produire un lit de semences en agriculture conventionnelle (Liu *et al.*, 2016). Le poids de la

machinerie combiné à la fréquence de passage brise les agrégats du sol, crée une couche compacte du sol située à la base du labour et favorise le ruissellement des eaux en surface (Liu *et al.*, 2016; Vanwalleghem *et al.*, 2017). Dans le contexte où les sols sont généralement laissés à nu et dépourvu de végétation, les petites particules produites lors du labour deviennent facilement lessivables ce qui contribue à l'érosion, à une diminution du stock de carbone (Liu *et al.*, 2016; Vanwalleghem *et al.*, 2017; Yu, Z. *et al.*, 2020) et à la perte d'autres nutriments dans les sols nécessaires aux cultures (Pimentel *et al.*, 1995; Robertson *et al.*, 2014; Vanwalleghem *et al.*, 2017).

Afin de maintenir les sols fertiles, le volume d'eau et la quantité d'intrants synthétiques azotés et phosphorés utilisés ont été multipliés de 2, 7, 3 fois respectivement sur des superficies cultivées lors des dernières décennies (FAO et ITPS, 2015; Liu *et al.*, 2015; Motesharezadeh *et al.*, 2017). Actuellement, l'utilisation moyenne d'engrais est de 1 M t an⁻¹ dans plus de 18 pays (Liu *et al.*, 2015) ce qui représente une charge économique et une augmentation à long terme du coût de production à l'hectare. De plus, une part élevée des engrais appliqués ne seront pas absorbés par les cultures (Zhang *et al.*, 2021) ce qui favorise leur lixiviation vers les réseaux hydrologiques avoisinants (Blesh et Drinkwater, 2013). Le travail mécanique du sol est aussi une source importante de gaz à effet de serre (GES). Le labour est responsable de la perte de matière organique sous forme gazeuse en CO, CO₂, N₂O et CH₄ (Pimentel *et al.*, 1995; Robertson *et al.*, 2014). Il a été estimé que l'apport de GES provenant du secteur agricole est très élevé et représente 26 à 36% des émissions globales dont 10 à 14% de celles-ci sont directement liées aux pratiques agricoles en champ (Robertson *et al.*, 2014).

I.3 Usage d'herbicides et résistance des adventices

Bien que l'usage de pesticides remonte à l'antiquité, leur usage à des fins herbicides est beaucoup plus récent dans l'histoire de l'agriculture (Timmons, 2005). L'utilisation du jus de lime contre la prêle en Allemagne et l'utilisation du sulfate de cuivre contre la moutarde des champs en France sont des exemples de précurseurs des herbicides apparus au 19^e siècle (Timmons, 2005). L'action

des herbicides telle qu'on la connaît aujourd'hui remonte à 1940 avec la mise en marché de l'herbicide à base d'acide (2,4-dichlorophénoxy) acétique aussi connu sous l'appellation 2,4-D. Lors des décennies qui vont suivre, la production de nouveaux herbicides sera fulgurante. Entre 1950 et 1969, on passe de 25 à 120 herbicides différents disponibles sur le marché (Timmons, 2005).

Avec l'avènement de la révolution verte, les herbicides sont devenus un complément important au travail mécanique en agriculture conventionnelle (Clay, 2021; Timmons, 2005). L'utilisation des herbicides a augmenté de façon spectaculaire en raison du faible coût des produits, de leur facilité d'utilisation et de leur capacité à réduire la quantité de travail nécessaire pour lutter contre les adventices (Clay, 2021; Timmons, 2005). Les adventices sont des plantes présentes de façon non intentionnelle et pouvant entrer en compétition avec les plantes de cultures pour différentes ressources telles que l'espace pour croître, la luminosité, les nutriments, la disponibilité en eau (Harlan et deWet, 1965; Osipitan *et al.*, 2019; Timmons, 2005). La diminution de la diversité des herbicides sur le marché et la stagnation dans le développement de nouveaux mécanismes d'action depuis les années 1990 a grandement contribué à diminuer les alternatives de lutte chimique contre les adventices (Heap et Duke, 2018; Timmons, 2005). Actuellement, 60% des herbicides présents sur le marché sont attribués à seulement quatre mécanismes (EPSP synthase, auxine, acétolactate synthase, acétyl-CoA carboxylase) (Fernando *et al.*, 2016; Heap et Duke, 2018). L'utilisation répétée d'herbicides avec des mécanismes d'actions similaires a exercé une forte pression sélective sur les adventices ce qui a favorisé le développement de cas de résistance (Duke *et al.*, 2018; Heap et Duke, 2018). Plusieurs facteurs intrinsèques à une espèce peuvent la prédisposer à développer de la résistance (Clay, 2021; Heap et Duke, 2018). Parmi ceux-ci on considère la durée du cycle de vie qui est défini par la longévité d'une plante ce qui inclut son stade sous forme de semences, sa germination et son développement végétatif et reproductif. La production, la distribution et la longévité des semences influencent le potentiel de résistance et la capacité d'un individu ou d'une population à maintenir et transmettre son bagage génétique ce qui peut avoir également des incidences sur le développement de résistance (Kumar et Jha, 2016; Renton *et al.*, 2014). La

plasticité génétique qui se définit par la capacité d'un individu, d'une population ou d'une espèce à s'adapter à différentes pressions ce qui peut également influencer le capacité de devenir résistant à certains herbicides (Beckert *et al.*, 2011). En 1968, le Sèneçon vulgaire ou commun (*Senecio vulgaris*) est reconnu pour être le premier cas recensé d'adventice résistante à un herbicide, l'atrazine, un herbicide inhibiteur de photosystème II (Heap, 2014). Actuellement, on recense de par le monde près de 530 cas de résistance aux herbicides, avec 272 espèces au total qui comptent 155 dicotylédons et 177 monocotylédons (Heap, 2024). Les différents mécanismes de résistance peuvent être spécifiques ou non spécifiques au site d'action d'un herbicide (Bo Bo *et al.*, 2017; Délye *et al.*, 2013) (Figure I.1). Une résistance spécifique à un site d'action peut s'exprimer principalement par une augmentation ou la mutation d'un site d'action ce qui diminue l'inhibition de certaines voies de synthèse et une diminution de l'affinité de l'ingrédient actif sur le site d'action respectivement (Bo Bo *et al.*, 2017; Délye *et al.*, 2013) (Figure I.1). La résistance non spécifique à un site d'action peut se définir par une limitation du déplacement de l'herbicide dans la plante via la réduction de son absorption, l'altération de sa translocation dans les différents compartiments de la plante et son stockage dans les cellules (Bo Bo *et al.*, 2017; Délye *et al.*, 2013; Ghanizadeh et Harrington, 2017) (Figure I.1). L'augmentation de l'activité physiologique pour augmenter la métabolisation de l'herbicide en différents métabolites secondaires ou pour augmenter la production d'enzymes antioxydantes dans le but de compenser ou protéger l'impact des herbicides sur la plante est également considérée comme des mécanismes de résistance non sélectifs (Délye *et al.*, 2013; Ghanizadeh et Harrington, 2017) (Figure I.1). La résistance croisée, qui représente un mécanisme unique qui permet la résistance à plus d'un herbicide, est également possible (Bo Bo *et al.*, 2017; Délye *et al.*, 2013). Les adventices qui ont la capacité de développer des mécanismes de résistances contre l'action de divers herbicides complexifie grandement leur contrôle et diminue les alternatives chimiques pour y arriver (Beckie *et al.*, 2020a; Clay, 2021; Cousens et Fournier-Level, 2018; Moss *et al.*, 2019). Tel est le cas de l'ivraie rigide (*Lolium rigidum*) et le pâturin annuel (*Poa annua*) qui sont résistants à 12 mécanismes d'actions différents ainsi que le panic pied-de-coq (*Echinochloa crus-gali*) et l'amaranthe de Palmer (*Amaranthus palmeri*), résistants à 10 et 9 mécanismes d'actions respectivement (Bo Bo *et al.*, 2017; Heap, 2024).

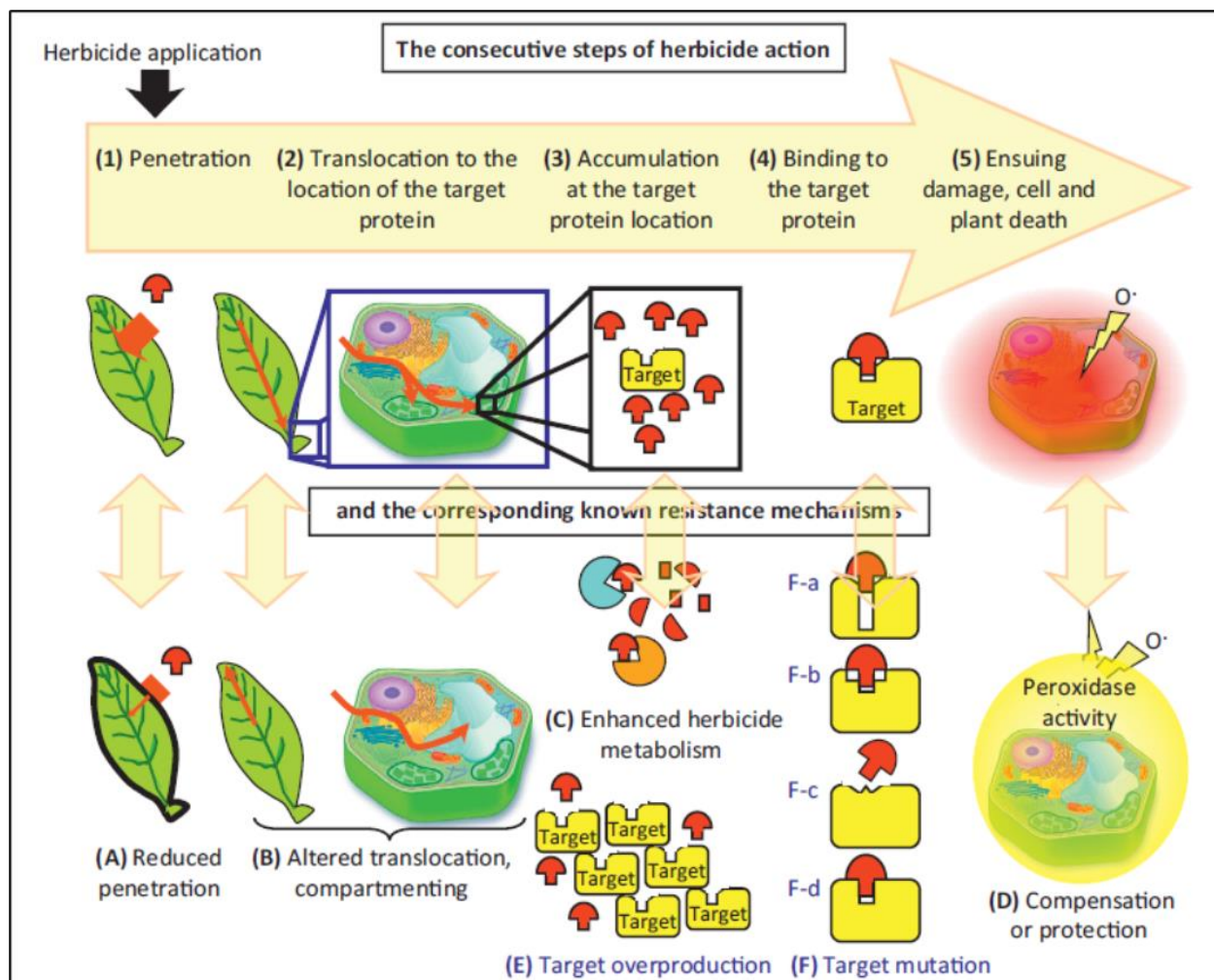


Figure I.1 Chronologie de l'action des herbicides (en haut) à la suite de leur application et les mécanismes de résistances (en bas) des adventices pour chacune des étapes. Tirée de Délye *et al.*, 2013

Nommé « l'herbicide du siècle », le glyphosate ($C_3H_8NO_5P$; N-(phosphonomethyl)glycine) a été synthétisé en 1950 et breveté pour ses propriétés comme chélateur de métaux en 1964 (Duke *et al.*, 2018). En 1970, le glyphosate est devenu l'ingrédient actif des herbicides à bas de glyphosate

(HBG) développé par le chimiste de Monsanto®, John E. Franz (Duke *et al.*, 2018). Les HBG ont été mis en marché par Monsanto® comme herbicide systémique en pré-émergence ou en jachère (brûlage) (Duke *et al.*, 2018). Le glyphosate inhibe l'action catalytique de l'enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase qui limite la métabolisation des acides aminés aromatiques phénylalanine, tyrosine et tryptophane, essentiels au développement des plantes (mauvaises herbes et cultures incluses) (Gomes *et al.*, 2014). Une propriété intéressante des HBG est qu'ils peuvent agir efficacement contre un large spectre d'adventices (Benbrook, 2016; Duke, 2017; Duke *et al.*, 2018). Avec la commercialisation des premières semences OGM de cultivars tolérants au glyphosate (TG) en 1996, les HBG peuvent dorénavant être utilisés comme herbicide sélectif en post-émergence (Duke *et al.*, 2018). Les cultivars TG ont la propriété d'être résistants aux HBG ce qui a permis aux producteurs d'appliquer ces herbicides lorsque la culture d'intérêt est présente, ce qui n'était pas le cas auparavant. Avant l'usage de semences TG, les producteurs étaient obligés d'appliquer les herbicides en prélevée ou en post récolte ce qui limitait la fenêtre d'opportunités pour lutter contre les adventices. Entre les années 1970 et le milieu des années 1990, aucun cas de résistance aux HBG n'avait été observé ce qui a permis à ceux-ci de pallier aux problèmes des adventices résistances aux herbicides couramment utilisés antérieurement en grandes cultures (ex. Inhibiteurs de PSII (années 1970 à 1980), ALS (1980) et ACCase (1990)) (Duke, 2017; Duke *et al.*, 2018; Timmons, 2005). Un autre avantage des HBG est que son utilisation est jugée comme facile et peu coûteuse. À cause de son indice de toxicité aiguë faible et à sa demi-vie relativement courte dans les sols, les HBG ont contribué à mettre de côté l'utilisation d'herbicides fortement utilisés dans le passé et avec un niveau de toxicité plus élevé pour les producteurs et l'environnement tels que l'atrazine, les métolachlores, le diquat et le paraquat (Duke, 2020; van Bruggen *et al.*, 2018). Ultimement, l'utilisation des HBG offre des rendements agricoles intéressants ce qui a permis de mettre en pause la nécessité d'augmenter les superficies agricoles (Brookes *et al.*, 2017). Les HBG rivalisent actuellement avec le 2,4-D pour le statut d'herbicide le plus étudié dans l'histoire avec plus de 20 000 publications scientifiques et brevets qui portent sur le glyphosate (Duke, 2017; Duke *et al.*, 2018; Timmons, 2005). L'efficacité et l'intérêt grandissant pour le glyphosate ont comme répercussion une diminution du financement dans la recherche sur

d'autres herbicides ce qui cause la stagnation en recherche et développement de nouveaux mécanismes d'action herbicides (Duke, 2017; Duke *et al.*, 2018; Timmons, 2005).

Les HBG sont victimes de leur succès alors que les premiers cas de résistance aux HBG ont été observés aux alentours des années 2000. Il a été observé que des cas de résistance pouvaient se produire sur des individus ou populations d'adventices exposés à une haute dose d'HBG ce qui se traduit également par une haute pression sélective (Bo Bo *et al.*, 2017; Délye *et al.*, 2013; Heap, 2014). Une faible pression sélective peut également se produire ce qui est représenté par cas de résistance à des petites doses fréquentes d'HBG (Heap, 2014). Actuellement avec 59 espèces résistantes réparties à travers le monde, le glyphosate arrive au second rang derrière l'atrazine pour le nombre d'adventices résistantes à un ingrédient actif (Heap, 2024) (Figure I.2). De plus, une grande majorité d'adventices résistantes au glyphosate sont aussi résistantes à d'autres herbicides tels que plusieurs espèces de Poacées et d'Amaranthacées (Beckie *et al.*, 2020b; Beckie *et al.*, 2020a; Brookes *et al.*, 2017; Moss *et al.*, 2019). Cette problématique oblige les producteurs à augmenter la dose et la fréquence d'application d'HBG ou de les jumeler avec d'autres mécanismes d'action (ex. paraquat, diquat, dicamba et 2,4-D) et/ou pratique de gestion des adventices (ex. le labour) (Heap et Duke, 2018; Yu, Z. *et al.*, 2020). L'efficacité des HBG pourrait significativement diminuer avec les changements climatiques à venir et ce qui va augmenter le nombre de cas de résistance à ceux-ci si les HBG demeurent l'approche de gestion des adventices la plus fréquente (Fernando *et al.*, 2016; Varanasi *et al.*, 2016).

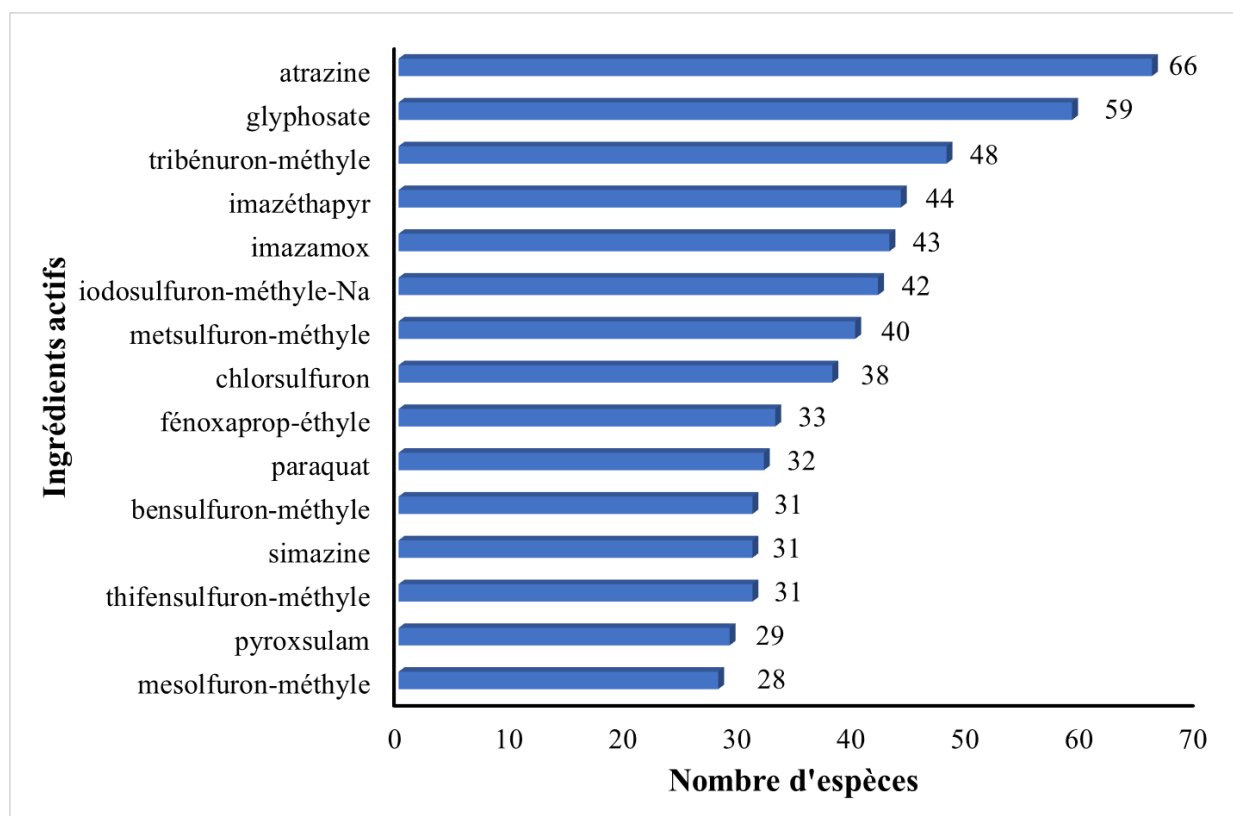


Figure I.2 Top 15 des ingrédients actifs présents dans les hecudues avec le plus grand nombre d'espèces d'adventice résistantes à ces derniers. Les données sont tirées de weedscience.org (3 avril 2024)

II. UN PREMIER PAS VERS LA DURABILITÉ DES CULTURES : L'AVÈNEMENT DES PRATIQUES AVEC TRAVAIL RÉDUIT DU SOL ET LE DÉVELOPPEMENT DES PRATIQUES DE SEMIS DIRECT

Des premières remises en question des fondements scientifiques et historiques du labour ont eu lieu dans les années 1930, suite aux événements du bassin de poussière (*Dust Bowl*) qui s'est produit aux États-Unis lors de la Grande Dépression (Derpsch, 1998; Triplett et Dick, 2008). Ces réflexions

avaient pour but de freiner les problématiques occasionnées par le travail mécanique intensif du sol et son impact sur le sol et l'environnement. Au début des années 1940, les prémices du semis direct (SD), ont vu le jour à travers l'œuvre de Edward Faulkner intitulé *Plowman's Folly* (1943) et ses essais préliminaires. Le SD se définit comme une agriculture sans labour et où les semis sont effectués sans avoir travaillé mécaniquement le sol (Fanning et Brady, 1963; Triplett et Dick, 2008). Toutefois, l'idée de semer directement dans le sol sans l'avoir travaillé préalablement remonte à plusieurs centaines d'années soit à l'Égypte antique et au temps des Incas (Derpsch, 1998). Ces derniers utilisaient un bâton pour créer un trou dans le sol afin de semer manuellement dans celui-ci (Derpsch, 1998).

Le développement du SD s'est accentué lors des années 1950 dans des systèmes de production de plantes fourragères grâce, entre autres, à la participation de l'entreprise *John Deere*® dans la conception de nouvelles machineries agricoles. Lors des premières années de développement, plusieurs expérimentations (ex. développement de semoirs adaptés à un sol non labouré, homogénéité des semis, développement de nouveaux herbicides, etc.) ont été nécessaires avant de pouvoir proposer une approche en semis direct applicable aux grandes cultures (Derpsch, 1998; Kassam *et al.*, 2019; Triplett et Dick, 2008). C'est seulement autour des années 1960 que l'approche du SD est devenue adéquate pour une adoption en grandes cultures (Derpsch, 1998; Shear, 1968; Triplett et Dick, 2008). La mise en marché des premiers herbicides tels que le 2,4-D a nettement favorisé le succès du SD. Par la suite, d'autres herbicides tels que l'atrazine et le dicamba ont facilité son adoption notamment au Brésil, pays pionnier pour l'adoption du SD, à grande échelle lors des années 1970. Lors de cette période, l'adoption du SD fut considérable dans les productions brésiliennes de soja et maïs ce qui occasionna une modification de l'occupation des sols (déforestation, déplacement des zones en pâturage et dégradation de plusieurs milieux naturels dans les états du sud du pays) (Derpsch, 1998; Triplett et Dick, 2008). Malgré que plusieurs pays aient suivi le pas par la suite avec l'adoption du SD, son implantation reste toutefois très marginale comparativement à l'agriculture conventionnelle (Derpsch, 1998). C'est vraiment lors des années 1990 avec une utilisation plus marquée des HBG et l'arrivée des semences TG, que l'adoption du

SD par les producteurs enregistra une croissance rapide (Derpsch, 1998; Kassam *et al.*, 2019; Triplett et Dick, 2008). L'utilisation des semences TG dans les grandes cultures de maïs-soya aux États-Unis va passer de 17% en 1997 à 90% en 2018 (USDA et ERS, 2021) ce qui va entraîner une adoption des pratiques SD à plus de 80% dans ces cultures (Yu, Z. *et al.*, 2020).

Les bénéfices du SD sont surtout liés à la conservation des fonctions du sol à travers un maintien de sa structure et de sa fertilité (Derpsch *et al.*, 2010; Kassam *et al.*, 2022; Triplett et Dick, 2008). Premièrement, la perte d'eau par évaporation moins importante que ceux en labour ce qui peut influencer positivement la productivité des cultures. De plus, une meilleure rétention de l'eau dans les sols peut procurer un avantage non négligeable durant des périodes plus sèches (Pimentel *et al.*, 1995; Robertson *et al.*, 2014; Troeh *et al.*, 2003). Un autre bénéfice important de l'approche en SD est incontestablement lié au maintien de la matière organique dont l'appauvrissement est plus élevé en système avec labour dû à une oxydation et décomposition plus rapide (Catania *et al.*, 2018; Grandy et Robertson, 2007; Liu *et al.*, 2016). Il est largement reconnu que la matière organique permet une meilleure structure et une agrégation plus grande des sols ce qui permet de limiter grandement son érosion, et ainsi, assurer leur durabilité (Grandy et Robertson, 2007; Liu *et al.*, 2016). L'implantation de systèmes en SD dans les grandes cultures de maïs-soya aux États-Unis a permis en 10 ans de réduire de 51,7% la perte de carbone du sol sur 12 Mha, passant de $14,7 \times 10^9$ t C an⁻¹ en 1998 à $7,1 \times 10^9$ t C an⁻¹ en 2008 (Yu, Z. *et al.*, 2020) (Figure I.3).

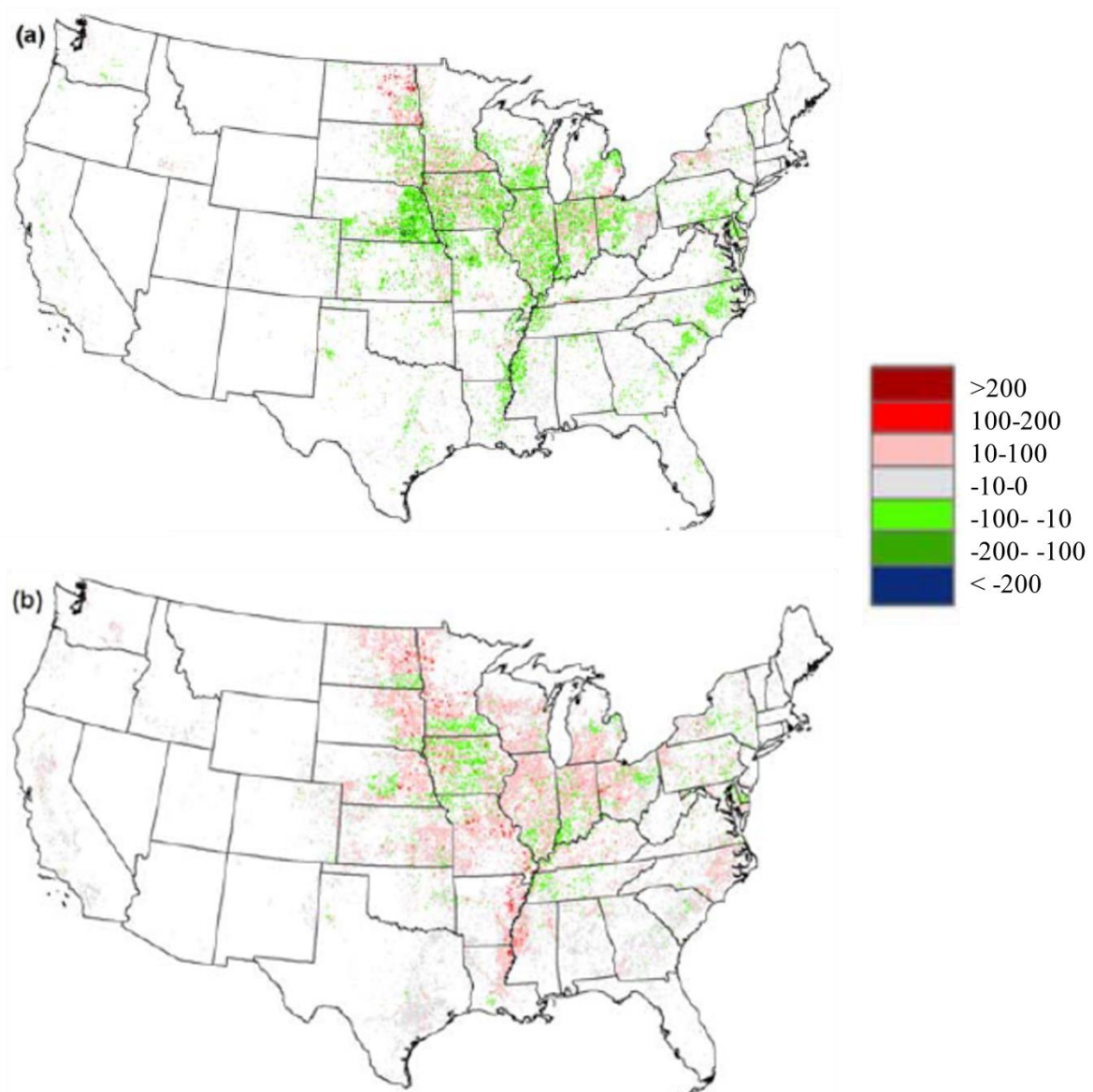


Figure I.3 Variations des stocks de carbone du sol (g cm^{-2}) induites par le changement d'intensité du travail du sol dans le système de culture maïs-soya américain durant a) 1998 à 2008 et b) 2008 à 2016. Tirée de Yu *et al.*, 2020

Les pratiques de SD permet de conserver la couche arable (*topsoil*) généralement perturbée par le labour (Pimentel *et al.*, 1995; Vanwalleghem *et al.*, 2017; Yu, Z. *et al.*, 2020) et favorise la présence de la matière organique, des nutriments et des minéraux disponibles pour les cultures et autres organismes du sol (Robertson *et al.*, 2014; Troeh *et al.*, 2003). Une grande part de la fertilité des sols est liée à la matière organique considérant qu'en plus d'être la principale source carbone (C), environ 95% de l'azote (N) et 25 à 50% du phosphore (P) du sol se retrouve dans celle-ci (Pimentel *et al.*, 1995). Moins de perte en N représente un bénéfice agronomique considérable puisque celui-ci est généralement un facteur limitant au développement des cultures (Vidal *et al.*, 2020). Il a été observé sur plusieurs années, qu'un système agricole avec SD perdait près de 20% moins de N comparativement à un système avec labour (Robertson *et al.*, 2014). Les systèmes SD peut aussi contribuer à diminuer les émissions de gaz à effet de serre (GES) associé à l'agriculture (Pimentel *et al.*, 1995; West et Marland, 2002; Yu, Z. *et al.*, 2020). Il a été estimé qu'en moyenne ces émissions représentent 1,7 g C m⁻² dans les systèmes SD comparativement à 4,9 g C m⁻² avec labour soit une diminution d'environ 65,3% (West et Marland, 2002).

Les pratiques de SD n'apportent pas que des bénéfices et certaines limitations qu'on retrouve dans ce système peuvent avoir une influence considérable sur les pratiques des producteurs et sur la durabilité des cultures. L'absence de travail de sol peut occasionner un durcissement de la couche supérieure du sol (Magdoff, 2007) ce qui peut compliquer le travail des semoirs, mener à des profondeurs de semis non homogènes, retarder la germination de certaines semences et empêcher une levée uniforme des plantules ce qui ultimement peut se traduire par des rendements plus faibles (Derpsch, 1998; Derpsch *et al.*, 2010). L'absence de travail du sol peut également favoriser la présence d'inoculum pathogènes tels que le *Fusarium* et la *Sclerotinia* généralement maîtrisés par le labour (Venter *et al.*, 2016). De plus, la majorité des superficies cultivées en semis direct sont des monocultures ce qui les rend aussi vulnérables aux épidémies de ravageurs (ex. le puceron du soja, le nématode kyste du soja, la pyrale du maïs) (Ekroth *et al.*, 2019; Wu *et al.*, 2022).

Le plus gros défi des systèmes SD reste toutefois le contrôle des adventices et limiter le potentiel compétitif de celles-ci avec les cultures d'intérêt (Juraimi *et al.*, 2013; Sen *et al.*, 2020). En fait, le succès des pratiques SD dépendent fortement de l'utilisation d'herbicides, ce qui peut favoriser directement l'augmentation du nombre d'adventices résistantes à ces derniers (Colbach et Cordeau, 2022; Maheswari, 2021; Triplett et Dick, 2008). Afin de pallier à cette problématique, certains producteurs ont décidé de revenir vers une approche plus conventionnelle ce qui a de nouveau eu comme conséquence une augmentation de la perte de carbone dans des sols qui étaient devenus des puits de carbone lors des dernières années (Yu, Z. *et al.*, 2020). Au cours des dernières années, la quantité de HBG utilisée dans les pratiques en semis direct a aussi fortement augmenté pour réussir à maîtriser les adventices. Cela peut avoir des répercussions directes sur la rentabilité et l'intégrité des cultures. Les HBG sont largement associés à l'utilisation de semences TG telle que mentionnée plus haut. Malgré que ces semences aient été développées pour être tolérantes aux HBG, les cultures TG restent toutefois sensibles à une utilisation excessive de ces derniers. Plusieurs études ont pointé du doigt le fait que l'utilisation d'HBG peut avoir des impacts négatifs sur certains processus physiologiques des cultures TG (Albrecht *et al.*, 2014; Albrecht *et al.*, 2011; Bernier Brillon *et al.*, 2023; Bernier Brillon *et al.*, 2022; Duke *et al.*, 2012; Hall *et al.*, 2014; Krenchinski *et al.*, 2017). Ces impacts peuvent aboutir à une perturbation de l'activité des stomates et des échanges gazeux (Albrecht *et al.*, 2014; Albrecht *et al.*, 2011; Bernier Brillon *et al.*, 2023; Bernier Brillon *et al.*, 2022; Krenchinski *et al.*, 2017; Smedbol *et al.*, 2019; Zobiolo *et al.*, 2010), une diminution de la photosynthèse (Krenchinski *et al.*, 2017; Zobiolo *et al.*, 2010) et une réduction de la nodulation qui permet aux légumineuses de fixer de l'azote atmosphérique (Zobiolo *et al.*, 2010; Zobiolo *et al.*, 2011). Plusieurs impacts peuvent être directement observables sur les cultures tels que le jaunissement brusque (*yellow flashing*) sur les feuilles de soja et de canola quelques heures après l'application de HBG (Duke *et al.*, 2012; Hall *et al.*, 2014). L'AMPA impacte aussi fort probablement les cultures TG, non tolérantes à ce produit de dégradation du glyphosate (Albrecht *et al.*, 2014; Gomes *et al.*, 2014; Krenchinski *et al.*, 2017). En fait, l'ajout des gènes bactériens CP4 et GOX (glyphosate oxydoréductase) permet aux cultures TG d'être tolérantes aux HBG, mais pas à l'AMPA (Gomes *et al.*, 2014; Pollegioni *et al.*, 2011; Smedbol *et al.*, 2019).

Selon ce qui a été proposé, l'impact de l'AMPA se fait principalement au niveau des chloroplastes et des mitochondries où il provoque un stress oxydatif important dans ces compartiments cellulaires (Gomes *et al.*, 2014; Krenchinski *et al.*, 2017; Reddy *et al.*, 2004). D'autres impacts des HBG sur les cultures TG ont été observés sur la qualité des grains de certaines cultures telles que le soja et sur leur contenu en protéine et en acide linoléique (Smedbol *et al.*, 2020).

Au-delà du fait que les HBG aient mauvaise presse ces dernières années, une diminution ou un arrêt complet de leur usage dans des pratiques de semis direct représente un défi colossal (Beckie *et al.*, 2020a; Brookes, 2019; Brookes *et al.*, 2017). De ce fait, une transition agroécologique est nécessaire afin de diminuer à la fois le travail du sol et l'utilisation d'herbicides tels que les HBG tout en maintenant des rendements intéressants en grandes cultures (Beckie *et al.*, 2020b; Beckie *et al.*, 2020a; Lemessa et Wakjira, 2015; Moss *et al.*, 2019).

III. LA TRANSITION AGROÉCOLOGIQUE ET L'UTILISATION DES CULTURES DE COUVERTURE

La motivation à réduire les impacts agronomiques et environnementaux des grandes cultures et de l'agriculture conventionnelle fait de plus en plus son chemin dans le monde scientifique et auprès des différents acteurs du milieu agricole (DeLonge *et al.*, 2016). La transition agroécologique a pour but d'augmenter la productivité des sols agricoles tout en permettant une diminution considérable de l'usage d'intrants (DeLonge *et al.*, 2016; Magdoff, 2007). Cette transition a aussi comme objectif l'implantation de systèmes agricoles durables et résilients face à différentes sources de perturbations (ex. sécheresse, froid, maladies et la présence d'adventices) (Altieri *et al.*, 2017; Brooker *et al.*, 2016; DeLonge *et al.*, 2016; Liebman *et al.*, 2016). Un aspect important de la transition agroécologique dans les grandes cultures repose en grande partie sur l'implantation des différents principes de l'agriculture de conservation (AC). La FAO a défini l'AC comme un système agricole qui a pour but de limiter la perte de surface arable ou la régénération des zones

dégradées (FAO, 2023; Kassam *et al.*, 2019). L'AC renforce la biodiversité et les processus biologiques naturels au-dessus et au-dessous de la surface du sol, ce qui contribue à accroître l'efficacité de l'utilisation de l'eau et des éléments nutritifs et à améliorer ou maintenir la production agricole (FAO, 2023; Kassam *et al.*, 2019). L'AC se caractérise par trois pratiques agricoles combinées ou piliers fondamentaux (FAO, 2023; Kassam *et al.*, 2019; Kassam *et al.*, 2022; Scopel *et al.*, 2013) (Figure I.4). Le premier pilier repose sur un minimum de perturbation mécanique du sol dans lequel s'intègre le SD présenté auparavant (Kassam *et al.*, 2019; Scopel *et al.*, 2013) (Figure I.4). Le second pilier vise le maintien d'un couvert végétal permanent via soit la présence de résidus de cultures et/ou la présence de cultures de couverture (Kassam *et al.*, 2019; Scopel *et al.*, 2013) (Figure I.4). Ce couvert végétal doit avoir un taux de recouvrement minimal de 30% afin qu'il soit jugé adéquat (FAO, 2023; Kassam *et al.*, 2022). Le troisième pilier mise sur l'intégration d'une biodiversité culturale plus élevée, notamment à travers des rotations de cultures plus diversifiées (Kassam *et al.*, 2019; Scopel *et al.*, 2013) (Figure I.4). Une diversification des cultures peut permettre de briser le cycle des maladies (Venter *et al.*, 2016) ce qui peut s'avérer être un atout dans un système en semis direct vulnérables à celles-ci. De plus, l'empreinte positive de l'implantation de cultures différentes peut également être observée sur la quantité de résidus racinaires (Bowsher *et al.*, 2018; Ma *et al.*, 2012; Magdoff, 2007; Sun *et al.*, 2019; Venter *et al.*, 2016).

Minimum mechanical soil disturbance

(i.e. no tillage) through direct seed and/or fertilizer placement.



Permanent soil organic cover

(at least 30 percent) with crop residues and/or cover crops.



Species diversification

through varied crop sequences and associations involving at least three different crops.



Figure I.4 Les trois piliers fondamentaux de l'agriculture de conservation. Tirée de la page web de FAO (www.fao.org; septembre 2023)

En théorie, ces trois pratiques agricoles doivent être combinées pour constituer *de facto* l'AC (FAO, 2023; Kassam *et al.*, 2022). En réalité, la combinaison totale des trois approches est rarement adoptée par les producteurs et l'AC ne mentionne absolument pas la réduction de l'usage de d'herbicides en particulier, ce qui peut limiter les bénéfices attendus (Callaci Trottier, 2019; Takam Fongang *et al.*, 2023).

Le second pilier représente le fer de lance de cette thèse qui repose sur les bénéfices potentiels liés à l'utilisation des CC dans les grandes cultures avec des pratiques de semis direct.

IV. OBJECTIFS ET HYPOTHÈSES DU PROJET DE THÈSE

L'utilisation de plantes vivantes comme CC est proposée comme un complément important au SD. La présence de plantes devrait pallier à diverses limitations observées dans les systèmes agricoles avec des pratiques de semis directs telles que limiter les impacts des HBG sur les processus physiologiques des plantes, diminuer la présence d'adventices dans les champs et favoriser la biodiversité du microbiote du sol. Toutefois, ces bénéfices semblent différer selon les études et semblent dépendre étroitement du contexte environnemental et des pratiques culturales. Cette thèse a pour but de cerner les bénéfices d'implanter des CC dans un contexte de grandes cultures de soja et maïs TG basées sur l'utilisation d'HBG. Les trois éléments d'impacts à l'étude (influence des HBG sur les cultures TG, la présence accrue d'adventices et la perte de biodiversité dans les sols) sont indissociables dans un contexte où l'on cherche à augmenter la résilience des grandes cultures afin d'assurer leur durabilité par l'entremise de la transition agroécologique. De plus, l'usage de CC pourrait s'avérer être une avenue intéressante pour rentrer en compétition avec les adventices et ainsi réduire l'usage des herbicides, en particulier les HBG.

IV.1 Objectif 1 : Déterminer l'influence de pratiques de semis direct avec ou sans cultures de couverture sur la sensibilité des stomates du soja TG au déficit de pression de vapeur (V_{pd}).

L'utilisation des HBG peut avoir un impact négatif sur certains processus physiologiques des plantes tels que les échanges gazeux effectués par les stomates. Les stomates sont de petits pores qu'on retrouve sur les feuilles et sont les sites principaux pour l'assimilation de CO_2 nécessaire pour le cycle de Calvin et dans la transpiration (ou perte de H_2O) des plantes. Ces échanges gazeux représentent un compromis d'une importance capitale pour les cultures, surtout pour les plantes avec un métabolisme en C_3 telles que le soja. En temps normal, une molécule de CO_2 assimilé peut correspondre à la perte de 400 molécules d' H_2O vers l'atmosphère pour les plantes en C_3 (Taiz *et al.*, 2015). Dans un contexte de limitation en eau, les plantes ferment leurs stomates pour limiter la perte excessive d' H_2O ce qui a des répercussions directes la conductance stomatique (ou le

potentiel d'échange gazeux). Une conductance stomatique plus faible représente une quantité de CO₂ entrant plus faible dans la plante et nécessaire pour subvenir à des processus physiologiques essentiels pour celle-ci telles que la photosynthèse (Zobiolo *et al.*, 2010). Avec les changements climatiques, les périodes plus sèches seront plus fréquentes et auront une influence considérable sur la teneur en eau des sols pendant les périodes clés de la croissance des plantes cultivées (Seager *et al.*, 2015; Zhao *et al.*, 2017). De plus, le stress hydrique des plantes de culture occasionné lors des périodes de sécheresse peut être exacerbé par un second stress provoqué par l'application d'HBG sur les cultures TG (Albrecht *et al.*, 2014; Reddy *et al.*, 2004). Toutefois, certains impacts des HBG observés sur les stomates ou sur l'activité physiologique des plantes proviennent d'études qui ont utilisé des quantités de HBG qui dépassent celles généralement utilisées par les producteurs. Ici, les doses d'HBG appliquées pour lutter contre les adventices sont similaires à celles utilisées par les producteurs en grandes cultures au Québec (voir objectif 2). Ce design expérimental permettra d'avoir un regard réaliste sur l'influence de l'interaction entre les CC et les HBG sur les cultures TG. L'implantation de CC peut apporter des avantages agronomiques tels que la limitation de la compaction du sol en surface, l'augmentation de la porosité du sol et de l'infiltration de l'eau, et la limitation de l'évaporation de l'eau du sol (Scholberg *et al.*, 2010; Wagg *et al.*, 2021; Wittwer *et al.*, 2017). Nous émettons l'hypothèse que les CC peuvent influencer positivement le potentiel d'échange gazeux et permettre aux cultures TG de maintenir leur activité lors d'épisodes de stress comme la sécheresse manifestée par des conditions de déficit de pression de vapeur (Vpd) plus élevée dans un contexte où les plantes subissent déjà un stress causé par une exposition aux HBG (Lobell *et al.*, 2014; Seager *et al.*, 2015; Zhao *et al.*, 2017). Le Vpd représente la différence entre la quantité de vapeur d'eau que l'air peut contenir à saturation et la pression de vapeur réelle dans l'air (Driesen *et al.*, 2020; Grossiord *et al.*, 2020; Ocheltree *et al.*, 2014). Le Vpd est considéré comme une mesure directe du pouvoir de dessiccation de l'atmosphère, un facteur important qui influence la productivité des plantes (Driesen *et al.*, 2020; Grossiord *et al.*, 2020; Ocheltree *et al.*, 2014) et augmente l'évapotranspiration et la sensibilité à d'autres facteurs de stress abiotiques. Cette étude vise à déterminer si l'utilisation de CC dans les grandes cultures peut représenter une piste pour réduire la sensibilité des plantes cultivées et exposées aux HBG face aux variations de Vpd et

aux périodes de sécheresse. Malgré qu'il a été avancé que des valeurs élevées de V_{pd} semblent influencer la conductance stomatique (Bernier Brillon *et al.*, 2022) peu d'études ont considéré observer l'influence des HBG dans la gestion en eau des cultures TG.

IV.2 Objectif 2 : Utiliser les cultures de couverture pour lutter contre les mauvaises herbes et réduire la quantité d'herbicide à base de glyphosate appliquée dans le soja et le maïs TG en semis direct.

La présence d'adventices continue de représenter un des défis les plus importants en grandes cultures. Avec l'augmentation du nombre de cas de résistance aux herbicides en particulier aux HBG, la problématique des adventices a engendré un dogme d'incertitude pour la soutenabilité des systèmes cultivés avec des pratiques de semis direct. La gestion des adventices en grandes cultures représente un défi de grande envergure (Beckie *et al.*, 2020b; Beckie *et al.*, 2020a; Brookes *et al.*, 2017), ce qui nécessite le développement d'alternatives pour contrôler les mauvaises herbes afin de diminuer l'utilisation d'HBG et d'autres herbicides, voire de les substituer complètement.

Le deuxième objectif de cette étude est de déterminer si l'utilisation de CC constitue une option envisageable pour le contrôle des adventices et si leur utilisation est une alternative intéressante pour réduire l'utilisation d'HBG dans les cultures de maïs et de soja TG avec des SD. La première hypothèse est que la présence de CC pourrait permettre de réduire l'implantation et la densification des adventices dans les champs. En occupant potentiellement l'espace, les CC réduisent la durée ou la superficie du sol mise à nu ce qui limite l'envahissement du champ par les adventices entre les périodes de production (Gerhards et Schappert, 2020; Osipitan *et al.*, 2019). Notre seconde hypothèse est que le contrôle partiel des adventices par la compétition avec les CC sera possible avec des doses d'application réduites de HBG et ainsi pourrait permettre une réduction de leur usage en grandes cultures TG. L'usage de CC reste encore marginal pour maîtriser les adventices dans les systèmes avec des SD dans les grandes cultures de soja et de maïs. Toutefois, des résultats positifs pourraient aider à convaincre certains producteurs réticents à adopter les CC,

sans que ces dernières représentent une charge opérationnelle plus élevée ou puissent concurrencer les cultures d'intérêt (Lemessa et Wakjira, 2015). Dans le cadre de cette étude, il sera possible à la fois d'observer les bénéfices de jumeler l'usage de CC avec trois doses d'application différentes d'HBG dont la plus élevée représente la dose couramment utilisée par les producteurs. Les deux autres doses utilisées sont plus faibles ce qui permettra de voir s'il est envisageable de réduire les doses de HBG généralement recommandées par les manufacturiers.

IV.3 Objectif 3 : Déterminer l'influence des cultures de couverture sur le contenu en prokaryotes et eukaryotes du sol dans les cultures de soja et maïs TG exposées à différents taux d'application d'herbicides à base de glyphosate.

Le troisième objectif de cette thèse vise à observer si l'utilisation de CC combinée à différentes doses d'application d'HBG peut influencer le microbiote du sol. Notre hypothèse est que malgré les impacts négatifs des HBG sur le microbiote des sols (notamment sur les procaryotes et protozoaires), l'usage de CC peut avoir une influence positive sur la richesse, l'abondance et la composition de certaines communautés de microorganismes du sol de grandes cultures de soja et maïs TG. Les CC pourraient également limiter l'impact des HBG sur les microorganismes des sols selon les doses appliquées. Selon les doses d'applications, les HBG ont la propriété d'avoir un impact non sélectif sur la microfaune et macrofaune dans les sols et à la surface dans les champs (Gomes *et al.*, 2014; Kaur Gill *et al.*, 2018). Les HBG ont été développés pour inhiber la voie shikimate et la synthèse d'acides aminés dans les plantes (Gomes *et al.*, 2014). Toutefois, les procaryotes possèdent aussi cette voie de synthèse, ce qui peut les rendre également vulnérables aux applications d'HBG (Zobiolo *et al.*, 2011). En contrepartie, l'utilisation de CC a la propriété d'augmenter la diversité racinaire présente dans les sols (Amsili et Kaye, 2021; Magdoff, 2007). Cette diversité racinaire favorise un plus grand éventail de produits provenant de l'exsudation et de la sénescence des plantes ce qui offre une plus grande diversité de ressources pouvant être utilisées par des organismes du sol (Bowsher *et al.*, 2018). La présence de CC devrait aussi promouvoir une agrégation du sol plus élevée, ce qui peut également amener à une plus grande

diversité du microbiote (Lehmann *et al.*, 2018; Liu *et al.*, 2005; Morugan-Coronado *et al.*, 2022). Certains auteurs ont proposé que l'utilisation de CC a le potentiel de capter une partie des HBG appliqués ce qui permettrait de réduire leur présence dans les sols, et ainsi mitiger leur impact sur les diverses populations de procaryotes présents dans les sols (Locke *et al.*, 2008). Toutefois très peu d'études ont porté sur l'effet combiné des CC et l'application de HBG sur la biodiversité du microbiote édaphique.

Le microbiote est un excellent indicateur de la santé des sols et même, de la qualité des aliments produits (Ferris et Tuomisto, 2015; Hirt, 2020; Pervaiz *et al.*, 2020). Un faible microbiote du sol peut entraîner des carences dans les aliments destinés à la consommation humaine, ce qui a été observé dans certaines régions du monde (Vargas Rojas *et al.*, 2016). La majorité des organismes (ex. arthropodes et champignons non pathogènes), des relations symbiotiques entre les organismes et les plantes et de l'activité biologique (ex. accès et recyclage des nutriments) se retrouve dans les horizons supérieurs du sol généralement perturbés par le travail mécanique du sol (Bowsher *et al.*, 2018; Ferris et Tuomisto, 2015; Habig et Swanepoel, 2015). Lorsque les trois piliers de l'AC sont adoptés en complémentarité, cette pratique devrait permettre d'assurer la biodiversité et les processus naturels présents dans les sols, ce qui favorise leur durabilité et leur productivité (Kassam *et al.*, 2019; Kassam *et al.*, 2022).

CHAPITRE I

INFLUENCE OF NO-TILL SYSTEM WITH OR WITHOUT COVER CROPS ON STOMATA SENSITIVITY OF GLYPHOSATE-TOLERANT SOYBEANS TO VAPOR PRESSURE DEFICIT

JÉRÔME BERNIER BRILLON¹, MATTHIEU MOINGT¹ AND MARC LUCOTTE¹

¹ GEOTOP (Research Centre in Earth System Dynamics) & Institute of Environmental Sciences,
University of Quebec at Montreal, 201, Avenue du Président Kennedy, Montréal, QC H2X 3Y7,
Canada

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1.1 ABSTRACT

Soybeans are vulnerable to drought and temperature increases potentially induced by climate change. Hydraulic dysfunction and stomatal closure to avoid excessive transpiration are the main problems caused by drought. The vulnerability of soybeans to drought will depend on the intensity and duration of water stress. The purpose of this study was to determine if the use of cover crops (CCs) can influence the gas exchange potential of glyphosate-tolerant soybeans when the vapor pressure deficit (Vpd) increases. This two-year study was conducted in an open experimental field comprising direct seeding plots with or without CCs. Stomatal conductance (Gs) was measured five times on the same identified leaves following glyphosate-based herbicide application. These leaves were then collected in order to observe the stomata and foliar traits with a scanning electron microscope. The Vpd was calculated concomitantly to Gs measurements at the leaf surface. The results suggest that the use of CCs promotes phenotypic change in soybean leaves (more elaborate venation and a higher abaxial stomatal density), which in turn may enhance their tolerance to drier conditions. In 2019, Gs could be up to 29% higher in plots with CCs compared to those without CCs with similar Vpd values. This study shows that the benefits of using CCs can be observed via the morphological development strategies of the crop plants and their higher tolerance to drought.

Keywords: stomatal conductance; stomatal density; stomatal size; vapor pressure deficit; vein density; foliar traits; glyphosate-based herbicide

1.2 INTRODUCTION

Over the recent years, alternative cropping systems have been proposed to challenge the negative environmental impacts of conventional field cropping caused by intensive mechanical soil disturbance and use of synthetic pesticides and fertilizers (Carlson et Stockweel, 2013; DeLonge *et al.*, 2016; FAO et ITPS, 2015; Magdoff, 2007; Oerke, 2006; Triplett et Dick, 2008). Direct seeding (DS) systems have been put forward to reduce mechanical tillage and more than 70% (Lee *et al.*, 2021) of the incidence of soil erosion. DS systems allow reducing carbon and nitrate leaching from soils or emissions to the atmosphere, and maintaining soil organic carbon (SOC) and soil functions (Kassam *et al.*, 2019; Triplett et Dick, 2008; Yu, Z. *et al.*, 2020). The use of glyphosate-based herbicides (GBH) in combination with glyphosate-tolerant plants has promoted the adoption of DS systems on a larger scale (Kassam *et al.*, 2019; Kassam *et al.*, 2022; Yu, Z. *et al.*, 2020). During 1998 and 2008, DS area have increased by 71.6% in soybean production in the United States of America (Yu, Zhen *et al.*, 2020). Although DS systems aim at maintaining SOC and soil functions, they tend to provoke surface soil compaction, in turn limiting water infiltration, seed germination and the development of crop plants (Triplett et Dick, 2008). Reduced water infiltration into the soil can result in water limitation for crops, which can influence their physiological activities and their gas exchange potential (Domec *et al.*, 2009; Driesen *et al.*, 2020). Stomata present on leaves constitute the main sites for CO₂ assimilation by the plants (Tanaka *et al.*, 2010; Zeiger *et al.*, 1987). Stomata also play an important role in plant transpiration, since for the uptake of CO₂ corresponds to water release, i.e. a significant trade-off for the metabolic management of the plant (Driesen *et al.*, 2020; Krober et Bruelheide, 2014). Along with climate change, the

occurrence of drier periods will be more frequent and will have a considerable influence on the water content of soils during key periods of crop plants growth.

Direct seeding on cover crops (DSCC) imply implementing catch crop between field crop production periods or intercropping during the field crop production periods (Hartwig et Ammon, 2002; Woolford et Jarvis, 2017). The addition of cover crops (CCs) may bring agronomic benefits such as limiting surface soil compaction, increasing soil porosity and water infiltration and limiting soil water evaporation (Amsili et Kaye, 2021; Hartwig et Ammon, 2002; Liu *et al.*, 2005; Robertson *et al.*, 2014; Wagg *et al.*, 2021). We hypothesize that CCs can influence gas exchange potential and enable plants to maintain their activity during stressful drought episodes. We also hypothesize that plants from plots without CCs will be more sensitive to an increase in Vpd, which will induce stomatal closing and reduce stomatal conductance. C3 plants such as soybean are sensitive to abiotic factor such as temperature, air humidity, light intensity in turn influencing the gas exchange potential that contributes to water management and photosynthesis of the plant (Driesen *et al.*, 2020; Roche, 2015). In the coming years, climate change will have strong repercussion on the temperature and air humidity that will cause more frequent and longer drought period (Seager *et al.*, 2015; Zhao *et al.*, 2017). It has been reported that the increase of vapor pressure deficit (Vpd) has a negative impact on field crop production (Lobell *et al.*, 2014; Seager *et al.*, 2015; Zhao *et al.*, 2017). It is estimated that an increase in Vpd will reduce crops yields by more than 30% over the next 50 years, with a more drastic impact from 2050 onwards (Lobell *et al.*, 2014). Vpd represents the difference between the amount of water vapor that air can contain at saturation and the actual vapor pressure in the air. Vpd can thus be considered as a direct measurement of the atmospheric desiccation power, an important factor influencing plants productivity, enhance evapotranspiration and sensitivity to other abiotic stressors (Grossiord *et al.*, 2020; Ocheltree *et al.*, 2014). Because Vpd is highly dependent upon temperature, it will increase with global warming and thus raise questions regarding field crops water management. To our knowledge, few studies have yet reported the influence of CCs on stomatal conductance while Vpd values are raising. It has been observed that higher Vpd values seemed to have a particular influence

on soybean stomatal conductance (Bernier Brillon *et al.*, 2022). This 1-year study also pointed out the potential mitigation effect of CCs on crop plants on experiencing higher V_{pd} values (Bernier Brillon *et al.*, 2022). However, there is an urgent need to understand the processes that may impact cash crop production, and to put in place practices that will increase the plasticity and resilience of crops in the face of future climate change. This study aims at determining if the use of CCs in row crops may represent a clue for reducing soybean sensitivity to variation of V_{pd} and to drought periods. In addition, it also aims at integrating physiological activity data by complementing them with leaf drought tolerance traits data (ex. foliar size and vein architecture).

1.3 MATERIALS AND METHODS

1.3.1 Description of the experimental design

We conducted a two years field study aiming at determining how the implementation of CCs may influence the gas exchange potential of glyphosate-tolerant soybean in DS systems. Experiments were conducted in 2019 and 2020 in an open field located at the Grain Research Center (CEROM) in St-Mathieu-de-Beloeil (Quebec, Canada, (45.5828 N, -73.2374 W). Soil cores collected with an auger were taken from the experimental site in order to obtain a characterization of the soils prior to the implementation of the different plots. The experimental plots were established in 2018 on a humic Gleysol (AAFC, 1998) with a heavy clay texture (average and standard deviation percentage of clay: 72.6 ± 0.9 %, loam: 27.4 ± 0.9 % and sand: 0%). The soil mineral total content of the 0-20 cm horizon is presented in Table 1.1. The Mehlich-3 extraction method have been used to obtain the metal element contain (Mehlich, 1984) The size of each plot was 9 m x 20 m and each treatment was replicated four times. The experimental design relied on the use of glyphosate-tolerant soybean (Altitude R2®) on two Direct Seeding practices: direct seeding without CCs (DS) and with CCs (DSCC) with The choice to work with glyphosate-tolerant soybean is based on the desire to reproduce as closely as possible the conditions of direct seeding production in North and South America where this type of crops is widely used (Sims *et al.*, 2018).

In addition, the choice of cultivar is based on the suggestions of agronomists from the Ministry of Agriculture, Fisheries and Food of Quebec (MAPAQ) and on regional specificities. In each plot, soybean was seeded on previous year corn residues at a rate of 90.8 kg ha⁻¹, May 18th 2019 and May 26th 2020. During the 2018 autumn, winter rye (200 kg ha⁻¹) was also sown as cover crops in those plots between annual productions. No cover crops were sown in the DS plots. Herbicide treatment (Roundup WheaterMax® with glyphosate a.i. at 540 g L⁻¹) has been applied twice at rates of 902 g ha⁻¹ of glyphosate in DS and DSCC plots. The first application was done May 18th 2019 and June 2nd 2020. The second application was done post-emergence June 24th 2019 and July 3rd 2020 at V2 soybean growth stage. Soybean was harvested October 15th 2019 and October 31th 2020. The field meteorological data including total daily precipitation (mm) and minimum, maximum and average daily temperatures were recorded for the 2019 and 2020 production period with a weather station located on the CEROM main building (Figure 1.1a and 1.1b).

Table 1.1 Soil mineral total content at the depth 0-20 cm of the experimental site

Elements	Content
P	12.87 ± 2.51 mg kg ⁻¹
K	313.50 ± 20.84 mg kg ⁻¹
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	24.92 ± 5.72 mg kg ⁻¹
Mn	2.33 ± 0.18 mg kg ⁻¹
Na	47.60 ± 3.06 mg kg ⁻¹

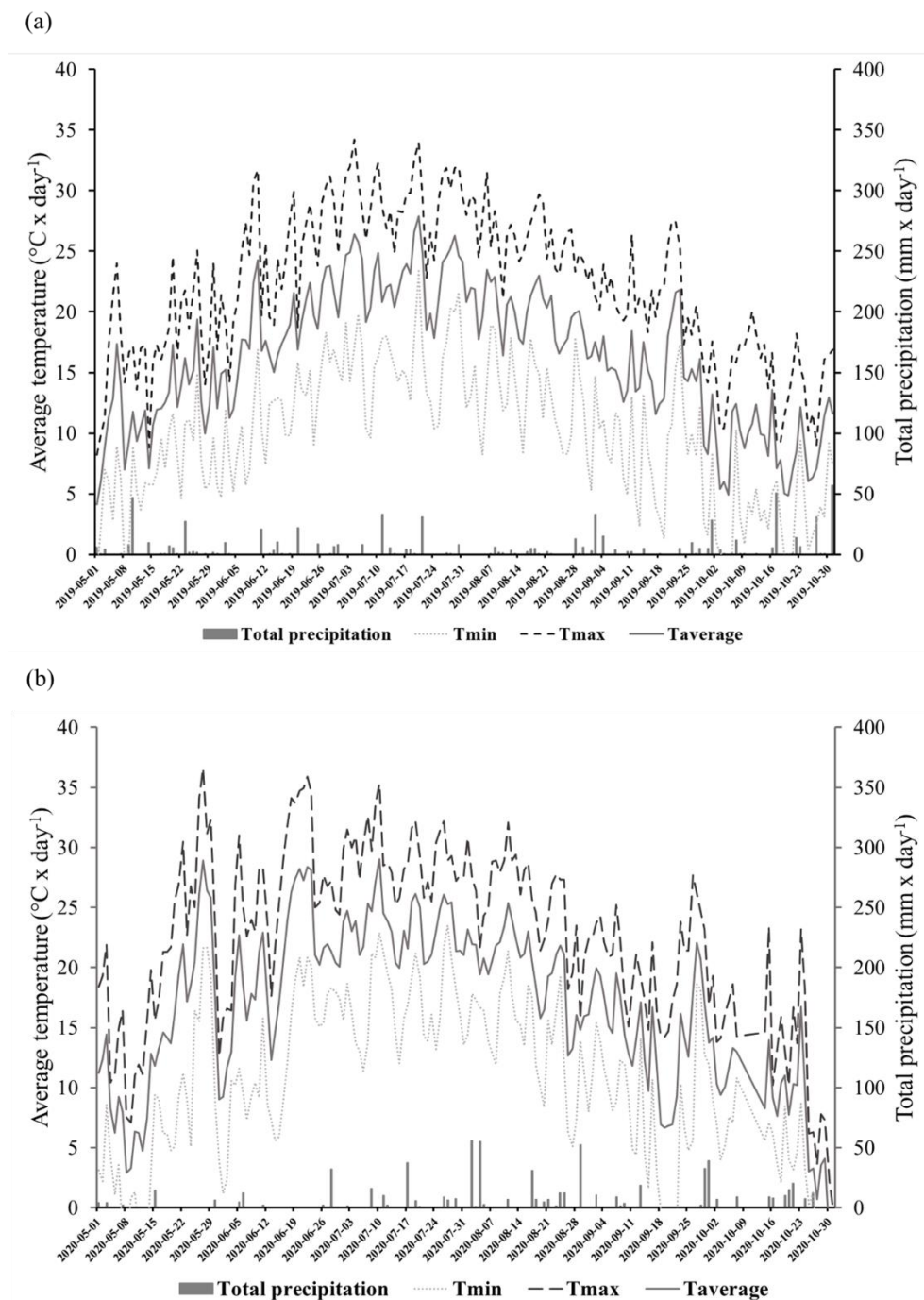


Figure 1.1 Total daily precipitation (mm) and minimum, maximum and average daily temperatures ($^{\circ}\text{C}$) at the experimental field during the growth period (May to October) in 2019 (a) and 2020 (b).

1.3.2 Sampling and measurements

Stomatal conductance and stomatal traits

At each plot, stomatal conductance (G_s expressed as $\text{mmol m}^{-2} \text{s}^{-1}$) was measured with a steady-state diffusion porometer (SC-1 Leaf porometer, Decacon Devices[®]) using one leaflet from three different plants similarly arranged and with initially the same growth stage (V2). All plants and leaves were identified the V2 growth stage in order to follow the same plants and leaflets throughout the study period (from the V2 to the R2-R3 growth stages). The stomatal conductance was measured around midday on abaxial foliar surfaces during five fields sampling campaigns (48h and 7, 14, 21 and 28 days following the second GBH application). In-situ calibration of the porometer was carried out on all sampling days before measurements began. The measurements began at 11 am and ended around 2 pm. Leaves temperature and air relative humidity were also recorded using a portable psychrometer (REED instrument[®], model#8706) at the leaf surface. The corresponding V_{pd} at the leaf surface was calculated according to the August-Roche Magnus formula, where $V_{pd} = 6.1094^{17.625 * T / T + 243.04}$ (Alduchov et Eskridge, 1996).

After measuring the stomatal conductance 28 days after the second GBH application, each identified leaf (R2-R3 growth stage) was collected in order to calculate the stomatal size, density and index. On each leaf, three locations on one fresh leaflet were observed for the stomatal density (StoDen) calculation on the abaxial surface with a scanning electron microscope (Hitachi S-3400N) at a magnification of 400x (Figure 1.2a). The three observed locations came from a section of the fresh leaflet ($\pm 1 \text{ cm} \times 2.5 \text{ cm}$) which has been mounted on a microscope slide using a transparent double-sided adhesive tape (Figure 1.2b). Pictures of those observations with the SEM were taken and the stomatal sizes (StoSize), width (StoWidth) and length (StoLength) were measured with ImageJ[®] software (NIH). StoSize represent the total area of the stomata, StoWidth is the distance between the two opposite outer thin wall of the guard cell and StoLength is the maximum length of the guard cell. The stomatal index (StoIndex) was calculated by multiplying the stomatal density by the stomatal size (Kim *et al.*, 2021). An average of these measurements was calculated following

observation of the three locations on the abaxial surface, providing the information needed to interpret the results.

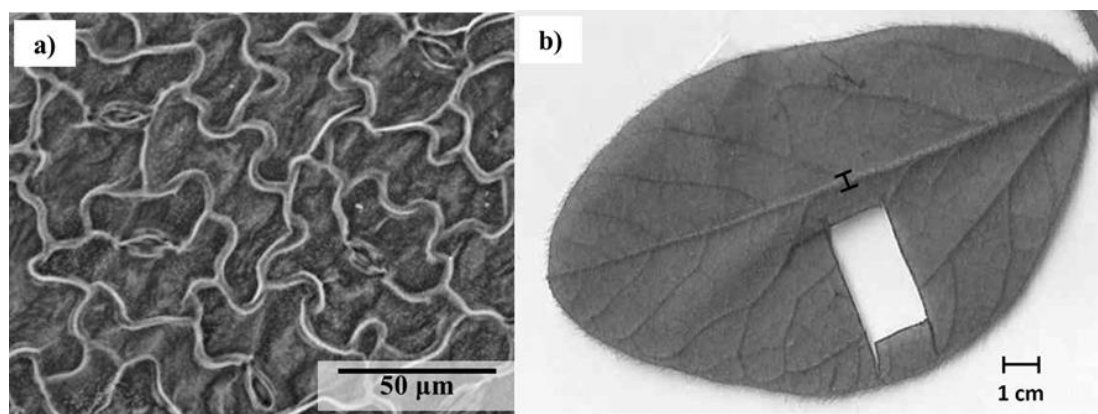


Figure 1.2 Observation of a) soybean stomata with a scanning electron microscope SEM at magnification of 400x and b) leaflet trait with an imagery software.

Foliar trait

One of the leaflets from each leaf collected in the field for stomatal trait measurements (28 days after second GBH application) were stored in a decolorizing solution (70% aqueous ethanol solution (Krober et Bruelheide, 2014)). Once decolorized, these leaves were dipped in a safranin solution (4% v/v) for 30 minutes or until we obtained a sufficient staining of the foliar veins. This vein staining increased color contrast and allow better accuracy of leaf trait measurements. The colored leaflets were then scanned and the measurements were made using an imagery software (ImageJ[®] software).

In this study, distance between veins has been used to obtain a proxy of the venation density (Uhl et Mosbrugger, 1999). An average of 11 measurements has been taken between secondary

vein for each leaflet. The midrib width and the leaves size of those leaflet have also been measured with the imagery software (ImageJ[®] software) (Figure 1.2b)

1.3.3 Statistical analyses

The G_s/G_{smax} values from all plants and for both sampled years were used to obtain a generalized linear model (GLM) in function of the corresponding V_{pd} with beta distribution using a *logit* link function) (Figure 1.3) (Bernier Brillon *et al.*, 2022; Krober et Bruelheide, 2014). Here, G_{smax} represents the highest G_s value measured during the two years, which was $1110.5 \text{ mmol m}^{-2} \text{ s}^{-1}$. The inflexion point was calculated for each curve and considered as the optimal condition for gas exchange (Bernier Brillon *et al.*, 2022; Krober et Bruelheide, 2014). The V_{pd} values for these optimum points were calculated to determine if there is a difference in plant sensitivity to V_{pd} between DS systems with or without CCs. Figure 1.3 shows an example of the fitted curves from the GLM as a function of V_{pd} centered value for each system and for the years 2019 and 2020. Since the logit function has only one rising point of inflection, the optimal gas exchange points were calculated from the second derivative for each curve. The confidence interval (95% CI) was calculated for those points to take into consideration the interval on the values for the stomatal conductance (the y-axis interval) and the V_{pd} (the x-axis interval).

An ANOVA analysis was carried out to assess whether there is a significant difference ($p \leq 0.05$) in G_s values between years for the stomatal traits and foliar traits from different cultivation

systems. Also, a Chi square test analysis was also carried out to evaluate the influence of both the year of production and the combination of year-agricultural management.

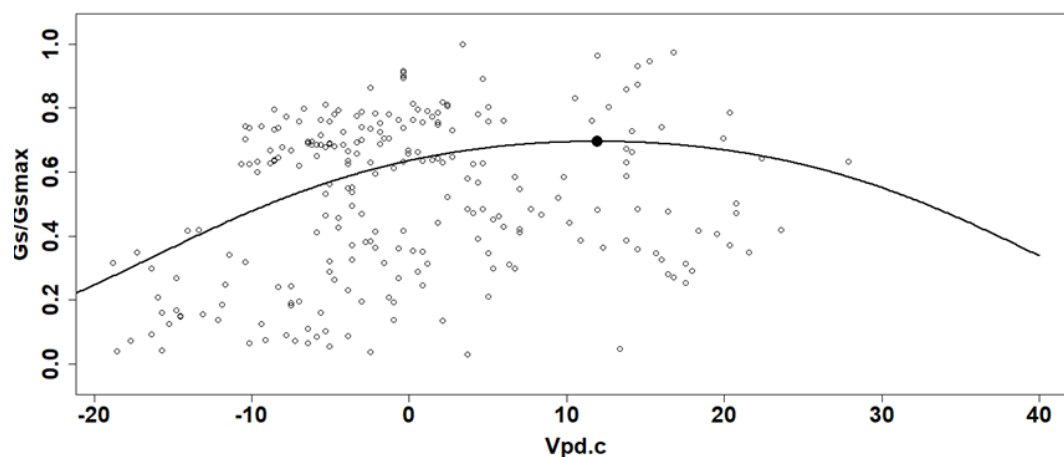


Figure 1.3 Example of Gs/Gsmax data (curve and point of inflexion) generated by the general linear model in function on centered values of vapor pressure deficit (Vpd.c).

1.4 RESULTS

1.4.1 Stomatal conductance and vapor pressure deficit

Combining DS and DSCC, significant differences in Gs between years are observed ($p = 0.0006$) where the means \pm SE values were $635.15 \pm 23.80 \text{ mmol m}^{-2}\text{s}^{-1}$ for 2019 and $516.14 \pm 24.51 \text{ mmol m}^{-2}\text{s}^{-1}$ in 2020. On the opposite, the mean \pm SE values of Vpd are significantly higher ($p < 0.0001$) in 2020 ($58.68 \pm 0.84 \text{ hPa}$) than in 2019 ($51.33 \pm 0.75 \text{ hPa}$).

By modelling the relation between Gs and the raising values of Vpd, we observe that the calculated inflexion points from Gs values in 2019 is similar between DS and DSCC plots (Figure 1.4 and Table 1.2). However, we observe significant difference in 2019 between DSCC and DS Vpd values based on the 95% confidence interval (Figure 1.4 and Table 1.2) where DSCC have similar Gs

values at higher Vpd values. No difference is observed on the calculated inflexion points from Gs values and on Vpd values in 2020 between DSCC and DS. The large variation around the inflexion points values in 2020 do not allow to observe difference with 2019.

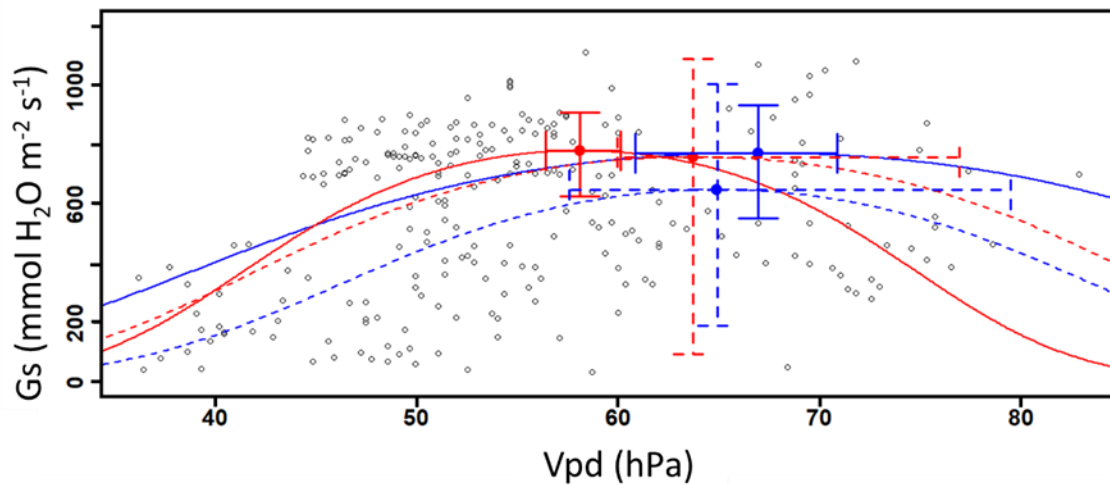


Figure 1.4 Abaxial stomatal conductance as a function of raising vapor pressure deficit in soybean ($n=240$). The curves represent the DS (Red) and DSCC (Blue) plots for 2019 (solid line) and 2020 (dashed line). The optimal points are defined here as the mean Gs and Vpd values with their associated confidence interval (95% CI).

Table 1.2 Calculated optimal gas exchange points following glyphosate-based herbicide application on soybean fields with DSCC and DS plots.

	2019		2020	
	DSCC	DS	DSCC	DS
<i>Gs</i> ($mmol\ m^{-2}\ *s^{-1}$)				
moyenne	772.45	780,00	649.15	759.07
95% IC [LL]	553.56	627.52	191.77	96.61
95% IC [UL]	932.89	909.13	1004.51	1088.46
<i>Vpd</i> (hpa)				
moyenne	66.94	58.10	64.92	63.73
95% IC [LL]	60.87	56.41	57.58	59.95
95% IC [UL]	70.86	60.14	79.49	76.95

Note: Data are presented as mean and their confidence interval (95% CI) with the lower limit (LL) and upper limit (UL) for the stomatal conductance (*Gs*) and the vapor pressure deficit (*Vpd*) values ($r^2 = 0.2447$).

1.4.2 Stomatal traits

Abaxial stomatal traits analysis (Table 1.3) shows that *StoDen* is significantly higher in DSCC plots compared to DS ones in both 2019 ($p = 0.0581$) and 2020 ($p = 0.0247$). *StoLength*, *StoWidth* and *StoSize* have higher values in DS plots in 2019 when compared to DSCC but no significant difference between the system is observed in 2020. In the case of *StoIndex*, no significant difference between DS and DSCC is observed for either year. However, different results on the stomatal traits are observed between both years where *StoDen* and *StoIndex* values for both systems are lower in 2020 compared to 2019. *StoWidth* and *StoSize* are significantly higher in DS

plots in 2019 than in 2020 whereas no significant differences on those traits are observed in DSCC plots between 2019 and 2020.

Table 1.3 Soybean stomatal traits measurements in direct seeding on cover crops (DSCC) and direct seeding (DS) for the years 2019 and 2020.

Stomatal traits	2019			2020		
	DSCC	DS	<i>p</i> value	DSCC	DS	<i>p</i> value
StoLength	15.91 ± 0.23 ^{bB}	16.78 ± 0.23 ^{aA}	0.0068*	16.18 ± 0.23 ^{aAB}	16.12 ± 0.24 ^{aAB}	0.8379
StoWidth	6.75 ± 0.15 ^{bB}	7.60 ± 0.13 ^{aA}	<0.0001*	6.93 ± 0.10 ^{aB}	6.97 ± 0.09 ^{aB}	0.7506
StoSize	109.34 ± 3.54 ^{bB}	128.72 ± 3.22 ^{aA}	<0.0001*	112.60 ± 2.35 ^{aB}	113.43 ± 2.88 ^{aB}	0.8230
StoDen	307.0 ± 8.3 ^{aA}	282.6 ± 9.5 ^{bB}	0.0481*	265.3 ± 9.2 ^{aBC}	247.0 ± 7.9 ^{bC}	0.0247*
StoIndex	0.0343 ± 0.0023 ^{aAB}	0.0364 ± 0.0017 ^{aA}	0.4481	0.0297 ± 0.0011 ^{aBC}	0.0277 ± 0.0010 ^{aC}	0.2129

Note: Stomatal traits consists in stomatal length (StoLength) (µm), stomatal width (StoWidth) (µm), the stomatal size (StoSize) (µm²), the stomatal density (StoDen) and stomatal index (StoIndex) on the abaxial leaf surface. Data are presented as mean ±SE from three leaves (with 3 observations by leaf) collected on all plots for each agricultural management replicates (four times) (n=72). The * and different lower-case letters indicate that mean values are significantly different between DS and DSCC plots ($p < 0.05$) according to a univariate test (ANOVA) and t-Student test respectively for each variable measured. Bold capital letters indicate significant difference between 2019 and 2020 according to a Chi square test ($p < 0.05$).

1.4.3 Foliar traits

No difference on the leaf size values between DSCC and DS is observed in 2019 and 2020 (Table 1.4). Also, the average leaf sizes are similar between years. Concerning the midrib width, significant differences exist between DSCC and DS in 2019 but not in 2020 (Table 1.4). In 2019, the midrib width values are higher in DSCC compared to DS plots. Also, significant smallest DistVein values are observed in DSCC for the year 2019 and 2020.

Table 1.4 Soybean foliar traits measurements in direct seeding on cover crops (DSCC) and direct seeding (DS) for the years 2019 and 2020.

	2019			2020		
	DSCC	DS	<i>p</i> value	DSCC	DS	<i>p</i> value
Leaf size (cm ²)	11.83 ± 0.48 ^{aA}	11.39 ± 0.43 ^{aA}	0.4912	11.77 ± 0.32 ^{aA}	12.47 ± 0.47 ^{aA}	0.2166
Midrib Width (mm)	9.13 ± 0.28 ^{aA}	7.88 ± 0.22 ^{bAB}	0.001*	7.25 ± 0.35 ^{aB}	6.88 ± 0.29 ^{aC}	0.4201
DistVein (cm)	0.63 ± 0.02 ^{bB}	0.80 ± 0.02 ^{aA}	0.001*	0.59 ± 0.01 ^{aB}	0.86 ± 0.02 ^{aB}	0.0442*

Note: Foliar traits consists in leaf size (cm²), midrib width (mm) and distances between secondary vein (DistVein) (J.W. *et al.*). Data are presented as mean ± standard error on means from three leaves (with 3 observations by leaf for Leaf size and Midrib Width (n=72) and 11 measures by leaf for DistVein (n=264) collected on all plots for each agricultural management replicates (four times). The * and different lower-case letters indicate that mean values are significantly different between DS and DSCC plots ($p < 0.05$) according to a univariate test (ANOVA) and t-Student test respectively for each variable measured. Bold capital letters indicate significant difference between 2019 and 2020 according to a Chi square test ($p < 0.05$).

1.5 DISCUSSION

The observed correlation between Vpd and Gs confirms that Vpd influences the physiological activity of plants, which is consistent with other publications (Bernier Brillon *et al.*, 2022; Driesen *et al.*, 2020; Ocheltree *et al.*, 2014). Here, a positive relation between Vpd and Gs is observed until the gases exchange potential reaches an inflexion point defined as an optimal point (Figure 1.4). Once this value is reached, Gs values decrease along with higher Vpd values. Higher Vpd values promote the ascension of water in the xylem, enhancing water accumulation in the sub-stomatal cavities and its exit through plant transpiration (Driesen *et al.*, 2020; Sinclair *et al.*, 2017). The decreasing Gs values observed in this study can be explained by the fact that plants close their stomata in order to limit excessive water loss when their ambient environment becomes drier (higher Vpd values) (Bernier Brillon *et al.*, 2022; Driesen *et al.*, 2020; Krober et Bruelheide, 2014). Here, it was not possible to observe significantly higher abaxial Gs values in plants present in plots without CCs compared with those in plots with CCs, which invalidates our first hypothesis. However, DSCC plots in 2019 appear less sensitive to Vpd while plants maintained their physiological activities and stomata opening during a less favorable periods (i.e. drought and hydric stress episodes). This result corroborated our second hypothesis, which stated that plants from plots without plant cover would be more sensitive to an increase in Vpd. Gs values can be up to 29% higher in DSCC plots compared to DS ones for the same Vpd values in 2019 (Figure 1.4 and Table 1.2).

Interestingly, we are able to observe differences in morphological traits of plants grown in DS and DSCC plots (Table 1.3 and Table 1.4). This observation corroborates other research, which proposed that a difference in physiological activity and gas exchange can be explained by different leaf morphological traits. Those differences in leaf can be explained by morphological plasticity to optimize plant performance according to growth conditions (Carins Murphy *et al.*, 2014; Franks *et al.*, 2009; Puglielli *et al.*, 2017; Xiong *et al.*, 2017). These morphological differences can occur in

stomata and other foliar traits such as the main vein structure (Carins Murphy *et al.*, 2014; Scoffoni *et al.*, 2011). Stomatal activity could be explained by the stomata density considering that it can be positively correlated with stomatal conductance (Chen *et al.*, 2016; Driesen *et al.*, 2020; Gaskell et Pearce, 1983; Qi et Torii, 2018; Roche, 2015; Tanaka *et al.*, 2010; Tanaka et Shiraiwa, 2009; Tanaka *et al.*, 2008). StoDen is a good drought proxy and is an indicator of the strategy that plants adopt to develop their stomata while reducing stressful conditions (Bernier BrillonLucotte, *et al.*, 2023; Bernier Brillon *et al.*, 2022; Driesen *et al.*, 2020; Tanaka et Shiraiwa, 2009). These strategies closely influence the number and size of stomata, which in combination represent the potential for foliar epidermal cells surface allocation for gas exchange and optimal stomatal conductance potential (Franks *et al.*, 2009). In this study, the epidermal cells surface allocation for gas exchange is represented by the StoIndex value, which shows no significant differences between agricultural managements and years (Tableau 1.2). We observe that in 2019, StoDen of soybean growing in DSCC plots is higher than that of plants growing in DS plots (Table 1.3). This could explain the differences in stomatal behavior and gas exchange where DSCC StoDen values are significantly different from those for plants growing in DS plots in 2020 (p value = 0.0247) (Table 1.3). Moreover, the abaxial stomata of plants growing in DSCC plots were significantly smaller than those in DS plots (Table 1.3). This was expected considering that it has been largely demonstrated that a negative relationship generally exists between stomatal size and number of stomata (Franks *et al.*, 2009). However, smaller and more numerous stomata allows soybean to quickly adapt their stomatal aperture for optimal conductance or closing them in order to avoid excessive transpiration (Aasama *et al.*, 2001; Bernier Brillon *et al.*, 2022; Franks *et al.*, 2009). This can be a significant short-term advantage, especially for crops that have to react quickly without allocated epidermal cell for the development of new stomata. For short-lived crops like soybean, plants tends to optimize resource acquisition by minimizing construction cost (Correia et Ascensão, 2017; Puglielli *et al.*, 2017). In the case of DSCC plants in 2019, a higher StoDen allowed physiological plasticity, which allows to maintain gas exchange in a context where V_{pd} values were higher.

In addition, the different stomatal traits on the abaxial surface between agricultural managements can also be explained by the morphological differences of the foliar veins, i.e. another indicator of drought tolerance of the plants (Scoffoni *et al.*, 2011). In our case, it was observed that soybean growing in DSCC plots in 2019 had a significantly wider midrib and a significantly lower DistVein, which represents a higher venation density. Higher venation density can be an indicator of willingness and resilience of the plants growing in plots with CCs during high V_{pd} or drought episode (Carins Murphy *et al.*, 2014). A more elaborated venation may be linked to a better water management (Uhl et Mosbrugger, 1999). Scoffoni *et al.* (2011) have proposed that large midrib and small distance between secondary veins allows a more important number of stomata which is also consistent with our observations. Higher major vein density would thus have lower hydraulic vulnerability allowing a larger number of stomata (Scoffoni *et al.*, 2011). Also, the presence of CCs can have a positive influence on soil functions, which can explain the willingness that facilitate phenologic plasticity of plants in DSCC plots. It has been shown that CCs can increase the number and diversity of root systems in the field which can improve soil porosity, aggregation and fertility (Amsili et Kaye, 2021; Liu *et al.*, 2005). These soil functions can facilitate the accessibility and the uptake of water by crop plants, in turn favoring gas exchange and transpiration with less restriction.

1.6 CONCLUSION

This study suggests that CCs contribute to maintain gas exchange potential in a context of soybean exposed to higher V_{pd} values. The implementation of CCs would thus favor a higher resilience to potential combined stress of drought and GBH application by increasing crop plasticity in glyphosate-tolerant soybean field crops. At similar V_{pd} values, the stomatal conductance on the abaxial leaf surface of plants growing in DSCC plots was significantly higher than that of plants growing in DS plots. This can be explained by a higher tolerance under

conditions that can cause water limitation to plants. This tolerance is expressed by a more elaborate venation and higher StoDen in plants growing in DSCC plots. Through the response of the plants and their development strategy, the benefits of CCs on crops could be observed in the short term in this study. Finally, CCs seem to represent, in part, a sustainable solution to fight against drought and future climate changes. CCs also seem to be a promising alternative to minimize the reduction of gas exchange of soybean triggered by herbicides spraying during a drought period.

1.7 ACKNOWLEDGEMENTS

We wish to acknowledge the implication of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) and Le Centre de recherche sur les grains (CEROM) on this project.

1.8 CONFLICT OF INTEREST

The authors declare no conflict of interest.

CHAPITRE II

USING COVER CROPS AS MEANS FOR CONTROLLING WEEDS AND REDUCING THE APPLIED QUANTITY OF GLYPHOSATE-BASED HERBICIDE IN NO-TILL GLYPHOSATE TOLERANT SOYBEAN AND CORN

Jérôme Bernier Brillon¹, Marc Lucotte¹, Ariane Bernier¹, Myriam Fontaine¹ and Matthieu
Moingt¹

¹GEOTOP & Institute of Environmental Sciences, University of Quebec at Montreal, 201, avenue
du Président-Kennedy Montréal, Québec Canada H2X 3Y7 Qc, Canada

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2.1 ABSTRACT

Weeds represents a serious drawback affecting field crops productivity worldwide. While the most common approach to control weeds in no-till practices is the use of glyphosate-based herbicides (GBH), reducing their use represents a major challenge. This two-year field study aims at evaluating whether the use of cover crops (CC) in transgenic soybean and corn productions can 1) help control weeds and 2) reduce the amount of GBH needed for managing weeds. Sampling was carried out in 32 experimental field plots (4 crop managements with 4 replicates on both crops). Crop managements consisted in GBH applications at 0.84, 1.67 and 3.3 L ha⁻¹ rates in plots in direct seeding with CC (DSCC) and at 3.3 L ha⁻¹ rates in plots without CC (DS). Weeds cover rates, plant parameters (fresh and dry weights and heights), grain yields, water and cation contents in soil were considered as indicators of interspecific competition. Results obtained in both years show that it is possible to reduce GBH use by 50% in plots with CC compared to plots without CC using 3.33 L ha⁻¹ rate of GBH application (DS 3.3). However, weeds had a large impact on water content in soil which was reflected by smaller plants and lower yields in plots with only 0.84 L ha⁻¹ of GBH applied. In the context of the study, the use of CCs seems to facilitate the development of more sustainable agriculture, while reducing the quantities of GBH generally used.

Keywords: cover crops; grain yield; biomass production; plant height; interspecific competition; glyphosate-based herbicide

2.2 INTRODUCTION

Despite technological, genetic and chemical advances in recent decades, weed control remains one of the greatest challenges in field crops operations (Perotti *et al.*, 2020). Weeds currently represents the greatest threat to yields compared to other pests in agriculture (Clay, 2021; Oerke, 2006). Weeds can compete directly with crops of interest for access to resources, which contributes to yield loss and profitability for producers (Harlan et de Wet, 1965; Sharma *et al.*, 2021). It is estimated that the presence of weeds represents losses up to 50-52% in soybeans and corn yield in Canada and the United States, which represents billions of dollars in losses annually (Soltani *et al.*, 2016, 2017). Mechanical tillage and herbicides used as burnout before crops implantation have historically been the most widely used conventional approach to weed control in North America (Timmons, 2005). Since the 1950s and the Green Revolution, herbicide use has increased dramatically due to the low cost of the products, the ease of use, and the ability to reduce the amount of labor required to control weeds (Clay, 2021; Timmons, 2005). Currently, 60% of the herbicide market is attributed to four mechanisms of action (EPSP synthase, auxin, acetolactate synthase, acetyl-CoA carboxylase) (Fernando *et al.*, 2016; Heap et Duke, 2018). In counterpart, the intensive use of herbicides with these mechanisms of action has contributed significantly to the actual 530 cases of herbicide resistance globally (Heap, 2024) and this problem may increase with the impact of climate change (Fernando *et al.*, 2016; Varanasi *et al.*, 2016).

During the last decades, direct seeding (DS) has been put forward to limit the degradation and loss of fertility of agricultural soils caused by compaction and soil erosion caused by intensive mechanical tillage (Triplett et Dick, 2008). DS systems help maintaining soil carbon content and soil functions (Liu *et al.*, 2016; West et Marland, 2002; Yu, Z. *et al.*, 2020). DS systems have widespread in the world since 1996 and their adoption by producers has been greatly enhanced after the introduction of genetically modified herbicide-resistant seeds such as glyphosate tolerant

(GT) cultivars (Derpsch *et al.*, 2010; Kassam *et al.*, 2019). Currently, glyphosate-based herbicides (GBH), with glyphosate ($C_3H_8NO_5P$; N-(phosphonomethyl)glycine) as the main active ingredient, are the most widely used herbicides (Beckie *et al.*, 2020a; Duke *et al.*, 2018). The combination of GBH and GT seeds in direct seeding has helped reducing excessive tillage and related rapid decline in soil quality in many agroregions around the world (Farooq *et al.*, 2011). The great popularity surrounding GBH has greatly limited the research and development of herbicides with new mechanisms of action and no history of weed resistance (Duke, 2017; Duke *et al.*, 2018). However, reduced tillage in DS limits the number of weeds typically controlled mechanically, making DS vulnerable to weeds and herbicide resistance (Yu, Z. *et al.*, 2020). Also, despite the effectiveness of GBH, there are now 59 weeds resistant to GBH (Heap, 2024). Producers are now forced to use larger quantities of GBH, to combine GBH with different types of herbicides or to increase to mechanical tillage (Heap et Duke, 2018; Yu, Z. *et al.*, 2020). Also, despite the current criticism and debate surrounding the use and impact of GBH and its impact on human and environmental health, a ban on its use does not seem feasible without significant economic and environmental repercussions (Beckie *et al.*, 2020a; Brookes, 2019; Brookes *et al.*, 2017; Kanissery *et al.*, 2019). Weed management in field crops is currently at an impasse, which requires the development of alternatives to control weed and to potentially substitute GBH and other herbicides use.

The purpose of this two-year field study is to determine if the use of cover crops (CC) is an option for weed control and if their use is a good alternative to reduce the use of GBH in transgenic corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) in DS crops. CCs allow to occupy space and reduce the time the soil is bare and limit the establishment and densification of weeds between production periods (Osipitan *et al.*, 2019). However, this approach is still marginal in DS management in soybean and corn crops. Furthermore, many producers believe that CC represent more labor and can compete with crops of interest, which limits their adoption (Lemessa et Wakjira, 2015). This study also aims at understanding if interspecific competition may exist between CC, weeds and crops of interest. Few studies have combined CCs and the use of GBH

before, making this study suitable for outlining the benefits of using CCs to reduce the use of GBH in soybean and corn crops.

2.3 MATERIALS AND METHODS

2.3.1 Site description and Experimental design

The study was conducted in an open field over two years, 2019 and 2020, at the Grain Research Center (CEROM) located in St-Mathieu-de-Beloeil, Quebec, Canada. The plots were established in 2018 on a humic Gleysol soil type represented by a heavy clay texture (mean \pm standard deviation percentage of clay: $72.625 \pm 0.916\%$, loam: $27.375 \pm 0.916\%$ and sand: 0%). The soil mineral content on the 0-20 cm horizon was measured when the plots were implanted (12.87 ± 2.51 mg kg⁻¹ for P, 313.50 ± 20.84 mg kg⁻¹ for K, 2943.42 ± 219.62 mg kg⁻¹ for C, 803.17 ± 48.27 mg kg⁻¹ for Mg, 1056.71 ± 19.32 mg kg⁻¹ for Al, 11.00 ± 0.47 mg kg⁻¹ for Cu, 217.54 ± 13.92 mg kg⁻¹ for Fe, 24.92 ± 5.72 mg kg⁻¹ for Mn, 2.33 ± 0.18 mg kg⁻¹ for Zn and 47.60 ± 3.06 mg kg⁻¹ for Na). The experimental design was a randomized complete block design consisting of 48 experimental plots of 9 m x 20 m (Figure 2.1). The plots were in three different crop rotations (wheat-corn; corn-soybean; soybean-wheat). Only 32 plots were sampled, corresponding to soybean and corn plots in crop rotations (plots from the soybean-wheat and corn-soybean rotation in 2019 and plots from the corn-soybean and wheat-corn rotation in 2020). According to figure 2.1, this represents 12 crop managements but only 8 of these were considered in the study. Those 8 crop managements were T5 (corn DS 3.3), T6 (corn DSCC 0.84), T7 (corn DSCC 1.67), T8 (corn DSCC 3.3), T9 (soybean DS 3.3), T10 (soybean DSCC 0.84), T11 (soybean DSCC 1.67), T12 (soybean DSCC 3.3) for 2019 and T1 (corn DS 3.3), T2 (corn DSCC 0.84), T3 (corn DSCC 1.67), T4 (corn DSCC 3.3), T5 (soybean DS 3.3), T6 (soybean DSCC 0.84), T7 (soybean DSCC 1.67), T8 (soybean DS 3.3) for 2020 (Figure 2.1). As shown in figure 1, the design includes four rows in order to have experimental replicates of each crop managements (Row 1, Row 2, Row 3, Row 4). The distance between rows

illustrated in figure 2.1 is 12 m. The distance between plots arranged in the same row is 2.5 m. The plots were planted to either wheat (Hoffman HRF: May 8th 2019 and April 25th 2020), corn GT (P9188AM: May 8th 2019 and May 15th 2020) or soybean GT (Altitude R2: May 18th 2019 and May 26th 2020). Within the plots, wheat and soybean were sown at 19 cm (7.5 inches) and the corn at 76 cm (30 inches) apart following the recommendation of agronomists from the ministry of agriculture of Quebec (MAPAQ).

Experimental design

Row#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
Row#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
Row#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
Row#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
	2019 Soybean	2020 Wheat	GBH application (Roundup WheaterMax)									
	Com	Soybean	3.3 = 3.3 L ha ⁻¹ in 2 applications									
	Wheat	Com	1.67 = 1.67 L ha ⁻¹ in 2 applications									
			0.84 = 0.84 L ha ⁻¹ in 2 applications									

Figure 2.1 Randomized complete block design for the twelve different crop managements with direct seeding and cover crops (DSCC) or without cover crops (DS) at St-Mathieu-de-Beloeil.

Fertilization was made in wheat plots (90 kg of N added June 6th 2019; 90 kg of N and 65 kg of P added June 4th 2020) and in corn plots (90 kg of N and 60 kg of P added June 28th 2019; 50 kg of N and 80 kg of P were added May 14th with an extra 120 kg of N July 2nd 2020). No fertilization was done in soybean plots.

Two sequential applications of GBH (Roundup WeatherMax® with glyphosate a.i. at 540 g L⁻¹) were sprayed on each experimental plots cultivated with direct seedling either without CC (DS) or with CC (DSCC). The first GBH applications were made pre-sowing on April 12th 2019 and April 24th 2020 in corn plots and on May 18th 2019 and June 2nd 2020 in soybean plots. The second GBH applications were made at post-emergence (V2 for soybean plots and V3 in corn plots) according to the recommendations of agronomists and ministry of agriculture (OMAFRA, 2017). The dates of these second applications were June 13th 2019 and June 15th 2020 in corn plots and June 24th 2019 and July 3rd 2020 in soybean plots. Three different doses of GBH were applied in different DSCC plots: 0.84 L ha⁻¹ (DSCC 0.84 with total of 454 g a.i.), 1.67 L ha⁻¹ (DSCC 1.67 with total of 902 g a.i.) and 3.3 L ha⁻¹ (DSCC 3.3 with total of 1782 g a.i.) (Figure 2.1). Only 3.3 L ha⁻¹ of GBH were applied in DS plots (DS 3.3 with total of 1782 g a.i.). Weed control between plots was carried out using a rototiller in order to avoid contamination and weed pressure on the plot edges. No GBH application were carried out in the wheat plots. In wheat, Infinity® was applied (at 0.83 L ha⁻¹) in 2019 and 2020.

No CCs was sowed in DS plots and only residues of previous crops were present on the ground. In each plot, soybean was seeded on previous year corn residues, corn was seeded on previous wheat residues and wheat was seeded on previous soybean residues.

In DSCC plots, different CCs were sown depending upon the main crops (Table 2.1). The CCs were sown by manually broadcasting the seeds in the plots before emergence of crop of interest. A Great Plains® seed drill was used later in the season when the crop of interest is present (Table

2.1). The species sown in CCs and the rates of seed per ha used are shown in Table 2.1. During the growing season, CCs were not controlled with pesticides or mechanical work. All CCs were terminated by frost during the winter. Only autumn wheat planted in the soybean plots had the property of surviving the winter.

Table 2.1 Cover crops mix sown and rates applied in the different type of crop of interest in 2019 and 2020.

Years	Cover crops mix sown		
	Wheat	Corn	Soybean
2019	May 12th: berseem (5 kg ha ⁻¹) and crimson clover (5 kg ha ⁻¹). Sown manually.	June 19th: crimson clover (5 kg ha ⁻¹), tillage radish (3 kg ha ⁻¹) and tillage turnips (2 kg ha ⁻¹). Sown manually.	September 6th: autumn wheat (225 kg ha ⁻¹). Sown with a Great Plains® seed drill.
	August 27th: buckwheat (5 kg ha ⁻¹), sunflower (5 kg ha ⁻¹), faba bean (15 kg ha ⁻¹), tillage radish (3kg ha ⁻¹), phacelia (1 kg ha ⁻¹), pea (25 kg ha ⁻¹) and oats (20 kg ha ⁻¹). Sown with a Great Plains® seed drill.	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.	
2020	June 1st: berseem (5 kg ha ⁻¹) and crimson clover (5 kg ha ⁻¹). Sown manually.	June 28th: crimson clover (5 kg ha ⁻¹), tillage radish (3 kg ha ⁻¹) and tillage turnips (2 kg ha ⁻¹). Sown manually.	October 6th: autumn wheat (200kg ha ⁻¹). Sown with a Great Plains® seed drill.
	August 14th: buckwheat (5 kg ha ⁻¹), sunflower (5 kg ha ⁻¹), faba bean (15 kg ha ⁻¹), tillage radish (3kg ha ⁻¹), phacelia (1 kg ha ⁻¹), pea (25 kg ha ⁻¹) and oats (20 kg ha ⁻¹). Sown manually.	September 8th: autumn rye (200 kg ha ⁻¹). Sown with a Great Plains® seed drill.	

Field meteorological data were recorded in 2019 and 2020 by a weather station located at the CEROM main building and approximately 1.1 km from the sampling site (Bernier Brillon, Moingt, *et al.*, 2023). Those data included total precipitations and temperatures (minimum, maximum and average) recorded hourly each day (Bernier Brillon, Moingt, *et al.*, 2023).

2.3.2 Sampling and measurements

Weeds cover rates

Weed cover rates were obtained by visually estimating the percent cover in a quadrat of each broadleaf and grass species present. Only the weed cover rates in the soybean and corn phase of the rotation were estimated. Two quadrats (size: 1 m x 0.5 m) were randomly used in each plot. For corn and soybean, data were obtained during three sampling periods. The first sampling periods were pre-sowing May 9th 2019 and May 20th 2020 in upcoming soybean and corn plots. Apart from winter wheat, the other CCs were not present at that time. Winter wheat was easily identifiable and distinguishable from weeds in the plots. The second one was at post emergence and after the second GBH application at V2 growth stage for soybean (July 2nd 2019 and July 14th 2020) and V3 for corn (June 20th 2019 and June 26th 2020). The third sampling periods were September 19th 2019 and September 24th 2020 in soybean and corn plots.

Crops biomass and height

In each plot, three plants were harvested at R2-R3 growth stages for soybean and at V8 growth stages for corn. Fresh weight (FW) and height of the above ground part of each plant were measured at the time of harvest. Corn and soybean plants were both measured from ground to the extended leaf tip. The collected plants were placed later on in an oven at 60° C for a minimum of 4 days in order to obtain their dry weight (DW).

Grain yields

Grain yields were measured by each harvester shift in the plots. In order to avoid border bias, the grain harvest used to estimate yields was carried out on the two rows in the center of the plots. The soybean plots were harvested October 15th 2019 and October 31st 2020 and corn plots, October 29th and October 18th 2020. Subsequently, grain yields (t ha^{-1}) were adjusted to 13.5% moisture for soybean and to 14.5% for corn in order to obtain a comparative database with others studies.

Soil water content

The soil volumetric water content (VWC) defined as the ratio of the volume of water to the unit volume of soil (Datta *et al.*, 2017) was obtained by time domain reflectometry (Fieldscout TDR 150©). In all plots during 2019 and 2020, measurements with a TDR probe were carried out five time on the 0-20 cm horizon and at within 30 cm of the crop stem. Those measurements were realized after the second GBH application at V2 stage for in soybean and V3 stage for corn) (OMAFRA, 2017).

Soil physicochemical analyses

In each plot, three soil cores were collected at 0-20 cm horizon and pooled together. Soil sampling was executed at the same three different periods as weed cover rates measurements. The elementary contents were obtained following the Mehlich-3 extraction 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) (Mehlich, 1984). All elementary contents were quantified using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Optima 4300DV).

2.3.3 Statistical analyses

All statistical analyses were carried out using the software jmp16 developed by SAS Institute. A Shapiro-Wilk good-fit test was performed to determine the normal distribution of the residuals. A

two-way ANOVA with interaction between the two categorical variables (crop managements and years) was carried out for the continuous quantitative variables (weed cover rates, FW, DW, grain yield and plant height).

When no significant interaction was observed with year*crop managements and when significant differences were observed ($p \leq 0.05$), a Student's paired test (parametric) was performed on the means between the crop management for each year. Otherwise, a student's paired test was made on each group of observations (4 crops management x 2 years = 8 groups) if the interaction was significant. If the distribution of the residuals was not normal, a Wilcoxon test and a Steel-Dwass non-parametric multiple means comparisons test was performed to compare variable responses between the four crop managements by years. The use of Student's paired test and Steel-Dwass was justified considering that each crop managements group had the same number of observations.

Also, simple linear regressions were executed in order to confirm relationships between weed cover rates and soil elementary contents, and also between weed cover rates and soil VWC. Both AICC and ICC values was carried out to determine which correlation to consider.

2.4 RESULTS

The results obtained from the two-way ANOVA are shown in Table 2.2. Crop managements has a significant effect on most crop parameters for soybean GT and for corn GT (Table 2.2). Differences between years 2019 and 2020 are observed in soybean plots (plant height) and corn plots (plant FW, plant height and grain yield) (Table 2.2). The interaction between years and crop managements has significant effect on grain yield in the corn plots (Table 2.2).

Table 2.2 Effect of fixed factor (years and crop managements) and their interaction on soybean GT and corn GT crops parameters (weed cover rates, plant fresh weight (FW), plant dry weight (DW), plant height and grain yield).

	Crop parameters	Years	Crop managements	Years*Crop managements
Soybean	Weed cover rates	0.053	< 0.0001*	0.9911
	Plant FW	0.642	0.0261*	0.6794
	Plant DW	0.0182	< 0.0001*	0.183
	Plant height	< 0.0001*	0.1113	0.9751
	Grain yield	0.067	< 0.0001*	0.0848
	Crop parameters	Years	Crop managements	Years*Crop managements
Corn	Weed cover rates	0.8695	0.0013*	0.8186
	Plant FW	< 0.0001*	0.0001*	0.1152
	Plant DW	0.2107	0.0068*	0.3269
	Plant height	0.0013*	0.0001*	0.0842
	Grain yield	< 0.0001*	< 0.0001*	0.027*

2.4.1 Weeds cover rates

In soybean plots, the highest weed cover rates were obtained in DSCC 0.84 plots in 2019 ($23.5 \pm 2.9\%$) and DSCC 0.84 plots in 2020 ($23.2 \pm 1.6\%$) (Figure 2.2a). No significant difference was observed between DSCC 1.67 plots (2019: $13.5 \pm 1.7\%$ and 2020: $17.8 \pm 1.9\%$) and DS 3.3 plots (2019: $14.5 \pm 2.1\%$ and 2020: $17.3 \pm 2.4\%$) (Figure 2.2a). In 2020, DSCC 3.3 plots ($9.5 \pm 1.1\%$) had the lowest weed cover. (Figure 2.2a).

In corn plots, DSCC 0.84 had significantly higher weed cover compared to DSCC 1.67 and DSCC 3.3 plots in 2019 ($26.4 \pm 5.3\%$) and 2020 ($25.3 \pm 3.7\%$) (Figure 2.2c and figure 2.2d). Weed cover rates in DS 3.3 and DSCC 0.84 corn plots were similar in 2019 and 2020 (Figure 2.2c and figure 2.2d). Weed cover rates in DSCC 1.67 plots (2019: $15 \pm 3.7\%$ and 2020: $16.3 \pm 1.6\%$), DSCC 3.3 plots (2019: $14.4 \pm 2.6\%$ and 2020: $12.0 \pm 2.1\%$) and DS 3.3 plots were similar in both study years. However, weed cover rates in DSCC 3.3 plots were significantly lower than in DS 3.3 plots in 2020 ($p = 0.0330$) (figure 2.2d).

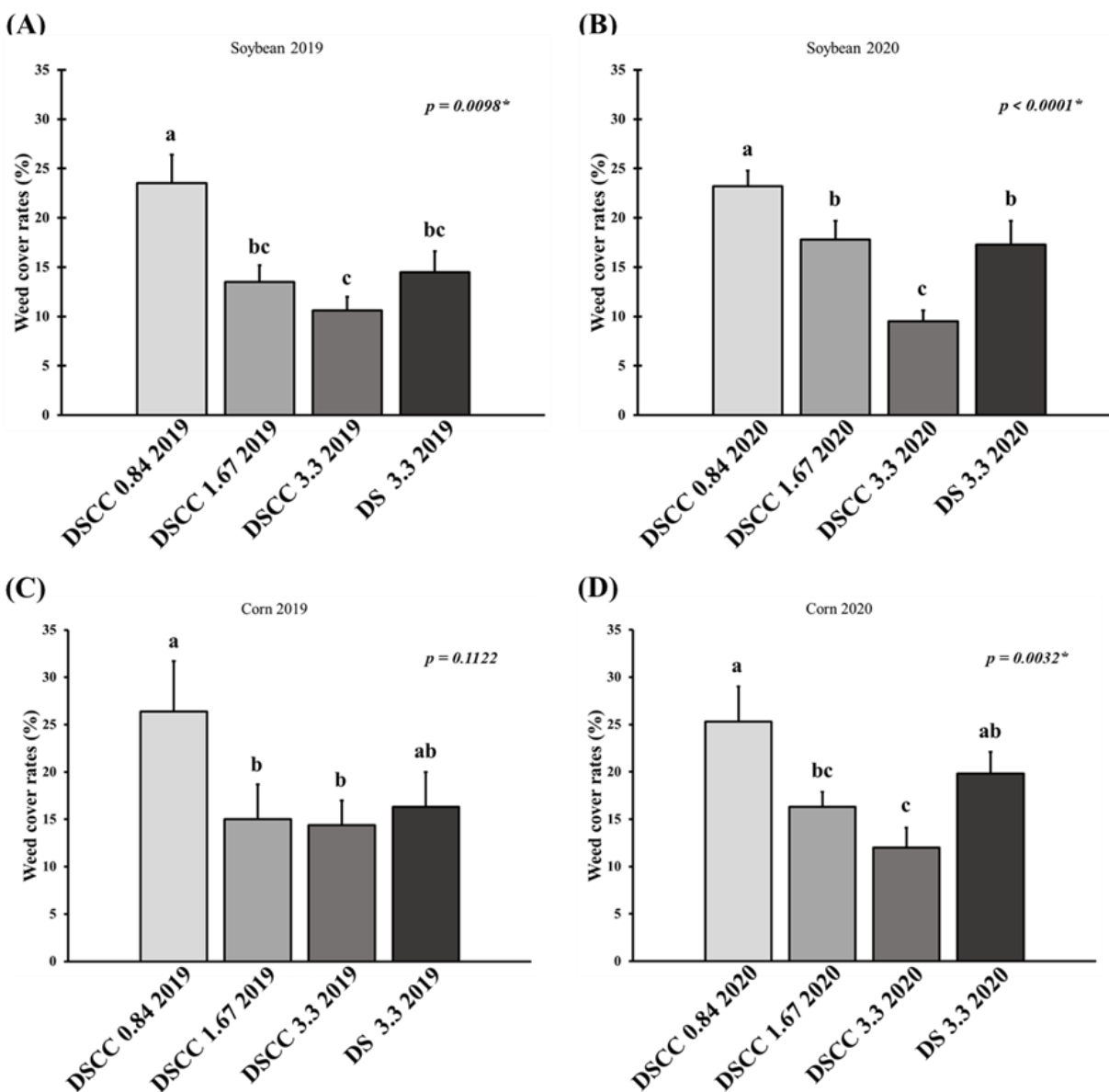


Figure 2.2 Average and standard error of broadleaf and grassy weed cover rates in (A) soybean plots 2019 (B) soybean plots 2020 (C) corn plots 2019 and (D) corn plots in 2020 (n = 96). All crop managements used direct seeding without CC (DS) and with CC (DSCC) with different doses of glyphosate-based herbicide (0.84, 1.67 and 3.3 L ha⁻¹).

2.4.2 Crops weights, heights and grain yields

Soybean plants

Soybean FW and DW were not significantly different between crop managements in 2019 (Table 2.3). Similarly, no significant difference was observed for the DW values between crop managements in 2020 (Table 2.3). For all crop managements combined, DW values are significantly different between 2019 and 2020 (Table 2.3).

Soybean plants in DSCC 3.3 plots were significantly taller compared to all other crop managements in 2019. Plants in DSCC 0.87 plots were smaller during both years, compared to plants from the than those growing under other crop managements plots (Table 2.3).

Corn plants

No significant differences were observed for DW and FW between crop managements in 2019 (Table 2.3). In 2020, FW and DW are similar between plants from DSCC 1.67, DSCC 3.3 and DS 3.3 plots. However, plants in DSCC 0.84 plots had the lowest FW and DW values in 2020 (Table 2.3). In general, FW values were significantly lower in 2020 compared to 2019 ($p < 0.0001$) but no difference in DW values was observed between these years.

In 2019, plant heights in DS 3.3 plots were significantly taller compared to the three DSCC crop managements (Table 2.3). In 2020, plants growing in DSCC 0.84 plots were smaller compared to plants in other crop managements (Table 2.3).

A strong positive correlation was observed between corn plant height and FW (r^2 adjusted = 0.874) and DW (r^2 adjusted = 0.842). A weaker positive correlation was also observed between soybean heights and FW (r^2 adjusted = 0.361) and DW (r^2 adjusted = 0.127) as compared to corn heights.

Grain yields

Grain yields for soybean and corn were the lowest in DSCC 0.84 plots in 2019 and 2020 (Table 2.3). No difference was observed for soybean yields between DSCC 1.64, DSCC 3.3 and DS 3.3 plots in 2019 and 2020 (Table 2.3). Corn yields in DSCC 3.3 plots were significantly lower compared to yields measured in DS 3.3 plots in 2019 (Table 2.3). However, yields were similar in DSCC 3.3 and DS 3.3 plots in 2020 (Table 2.3). Corn yields were not different in DSCC 1.67 and DS 3.33 plots in 2020 (Table 2.3).

Table 2.3 Biomass production (Fresh weight (FW) and Dry weight (DW)), height and grain yield of soybean and corn plants in 2019 and 2020 (n=36).

		2019					2020				
Plant parameters		DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	p value	DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	p value
Soybean	Fresh weight (FW) (g)	31.4 ± 3.6 ^a	33.8 ± 2.94 ^a	37.8 ± 3.5 ^a	35.9 ± 2.7 ^a	0.5352	26.0 ± 4.6 ^a	31.9 ± 3.4 ^a	41.1 ± 4.2 ^a	35.3 ± 3.2 ^a	0.0597
	Dry weight (DW) (g)	7.5 ± 0.8 ^a	7.9 ± 0.7 ^a	9.1 ± 0.9 ^a	8.0 ± 0.5 ^a	0.516	3.9 ± 0.9 ^a	4.6 ± 0.6 ^a	5.9 ± 0.7 ^a	5.0 ± 0.6 ^a	0.2564
	Heights (cm)	49.3 ± 1.6 ^c	53.7 ± 1.2 ^b	54.5 ± 3.1 ^b	58.4 ± 5.8 ^a	0.0004*	48.3 ± 2.7 ^b	56.5 ± 1.6 ^a	60.5 ± 0.9 ^a	61.2 ± 1.2 ^a	< 0.001*
	Grain yield (t ha ⁻¹)	1.6 ± 0.1 ^b	2.4 ± 0.1 ^a	2.6 ± 0.1 ^a	2.5 ± 0.1 ^a	< 0.001*	1.2 ± 0.4 ^b	2.8 ± 0.3 ^a	3.3 ± 0.3 ^a	3.1 ± 0.1 ^a	0.0008*
		2019					2020				
		DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	p value	DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	p value
Corn	Fresh weight (FW) (g)	116.7 ± 15.4 ^a	118.1 ± 10.1 ^a	140.6 ± 18.8 ^a	173.4 ± 20.9 ^a	0.0962	51.7 ± 4.8 ^b	91.1 ± 12.3 ^a	111.7 ± 14.4 ^a	101.5 ± 11.2 ^a	0.0004*
	Dry weight (DW) (g)	11.8 ± 1.5 ^a	11.2 ± 0.9 ^a	14.0 ± 1.7 ^a	16.0 ± 1.7 ^a	0.1162	8.8 ± 0.7 ^b	12.3 ± 1.3 ^a	14.1 ± 1.5 ^a	12.8 ± 1.2 ^a	0.0231*
	Heights (cm)	93.9 ± 3.7 ^b	94.8 ± 2.9 ^b	100.5 ± 4.0 ^b	111.8 ± 4.2 ^a	0.0054*	80.3 ± 2.3 ^b	92.0 ± 3.0 ^a	99.5 ± 4.3 ^a	94.8 ± 4.5 ^a	0.0042*
	Significant interaction year*weed managements										
		2019					2020				
		DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33		DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	p value
Grain yield (t ha ⁻¹)		6.5 ± 0.5 ^D	8.8 ± 0.2 ^B	8.7 ± 0.4 ^B	10.2 ± 0.6 ^A		4.0 ± 0.4 ^E	6.1 ± 0.3 ^D	8.1 ± 0.2 ^{BC}	7.1 ± 0.6 ^{CD}	< 0.027*

Note: Data are presented as means ± standard error of mean for each weed management. When no interaction year*crop managements was observed, the * and different small letters indicate that mean values are significantly different between crop managements ($p < 0.05$) for each year according to a multiple means comparison (post-hoc test with letters). Capital letters indicate significant differences between years* crop managements when an interaction was observed ($p < 0.05$) (only for grain yield in corn plots).

2.4.3 Soil water content

Soil VWC in soybean plots were significantly different at the first sampling period between 2019 (June 28th) and 2020 (July 7th) (Figure 2.3a and 2.3b). On June 28th 2019, VWC were lower in DSCC 3.3 plots ($18.95 \pm 0.81\%$) compared to DS 3.3 plots ($20.79 \pm 0.37\%$) (Figure 2.3a). On July 7th 2020, VWC were lower in DSCC 0.84 plots ($14.88 \pm 1.13\%$) plots compared to other crop managements ($18.64 \pm 0.80\%$ in DSCC 1.67 plots, $19.25 \pm 0.70\%$ in DSCC 3.3 plots and $17.53 \pm 0.73\%$ in DS 3.3 plots (Figure 2.3b).

VWC in corn plots were lower in DS 3.3 plots ($19.74 \pm 0.57\%$) compared to DSCC 1.64 plots ($21.48 \pm 0.54\%$) on June 20th 2019 (Figure 2.3c). On June 27th 2020, VWC were lower in DS 3.3 plots ($18.96 \pm 0.75\%$) compared to DSCC 0.84 plots ($21.37 \pm 0.61\%$) and DSCC 1.64 plots ($21.68 \pm 0.90\%$) (Figure 2.3c). DSCC 3.3 corn plots had higher values compared to DSCC 0.84 plots ($11.05 \pm 0.44\%$) on June 17th and June 20th 2020 ($12.50 \pm 0.45\%$ for DSCC 3.3 plots, $11.80 \pm 0.31\%$ for DS 3.3 plots, $10.98 \pm 0.46\%$ for DSCC 1.67 plots and $10.42 \pm 0.46\%$ for DSCC 0.84 plots) (Figure 2.3d). On July 6th 2020, VWC in DSCC 3.3 plots ($14.87 \pm 0.52\%$) and DS 3.3 plots ($14.84 \pm 0.51\%$) were similar but higher than in DSCC 1.64 plots ($12.24 \pm 0.32\%$) and DSCC 0.84 plots ($12.25 \pm 0.35\%$) (Figure 2.3d). Moreover, DSCC 0.84 plots ($13.18 \pm 0.51\%$) and DSCC 1.64 plots ($13.15 \pm 0.32\%$) exhibited lower VWC compared to DSCC 3.3 plots ($16.13 \pm 0.69\%$) and DS 3.3 plots ($15.59 \pm 0.95\%$) on July 14th 2020 (Figure 2.3d). Moreover, no correlation was observed in soybean plots between weed cover rates and VWC in 2019 (r^2 adjusted = 0.002) and in 2020 (r^2 adjusted = 0.074). In corn plots, no correlation was observed between those two variables in 2019 (r^2 adjusted = 0.054). However, a negative correlation was observed in corn plots (r^2 adjusted = 0.413) in 2020.

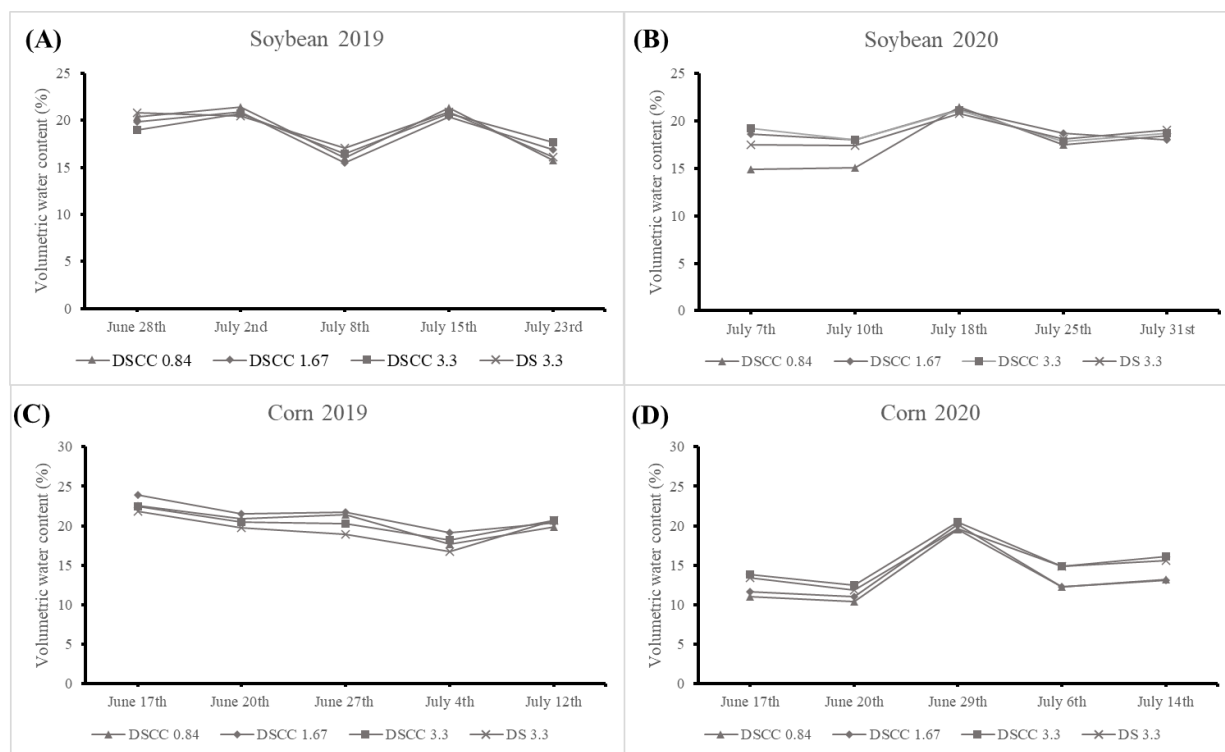


Figure 2.3 Soil volumetric water content in GT soybean plots (a and b) and GT corn plots (c and d) in 2019 and 2020 (n=60).

2.4.4 Soil cation content nutriment

Significant differences were observed for Mg ($p = 0.05$), and Ni ($p = 0.0452$) contents in soil between crop managements (Table 2.4). Mg contents were lower in DS 3.3 plots ($780.35 \pm 13.3 \text{ mg kg}^{-1}$) and DSCC 1.64 plots ($787.6 \pm 6.7 \text{ mg kg}^{-1}$) compared to DSCC 0.84 plots ($820.7 \pm 25.9 \text{ mg kg}^{-1}$) but similar to DSCC 3.3 plots ($794.9 \pm 15.0 \text{ mg kg}^{-1}$) (Table 2.4). Also, Ni contents were lower in DSCC 0.84 plots ($1.29 \pm 0.04 \text{ mg kg}^{-1}$) compared to DSCC 1.64 plots ($1.45 \pm 0.03 \text{ mg kg}^{-1}$) but similar to DSCC 3.3 plots ($1.36 \pm 0.03 \text{ mg kg}^{-1}$) and DS 3.3 plots ($1.39 \pm 0.05 \text{ mg kg}^{-1}$) (Table 2.4). In corn plots, a linear regression showed that weed cover rates had a positive correlation with B (r^2 adjusted = 0.227), Mn (r^2 adjusted = 0.118) and Zn (r^2 adjusted = 0.103) contents and a negative correlation with Al (r^2 adjusted = 0.213) content. In soybean plots, a linear regression showed that weed cover rate have a negative correlation with B (r^2 adjusted = 0.170), Cu (r^2 adjusted = 0.10) and Cr (r^2 adjusted = 0.331) contents.

Table 2.4 Elementary contents between crop managements with different applied quantity of glyphosate-based herbicide (n=72).

Metal elements (mg kg ⁻¹)	Crop managements				<i>p</i> value
	DSCC 0.84	DSCC 1.67	DSCC 3.33	DS 3.33	
P	13.95 ± 2.54 ^A	12.74 ± 0.82 ^A	11.03 ± 0.68 ^A	16.90 ± 4.66 ^A	0.4797
K	320.8 ± 5.0 ^A	331.0 ± 7.0 ^A	320.6 ± 5.1 ^A	318.6 ± 5.2 ^A	0.4051
Ca	2897.8 ± 25.2 ^A	2787.5 ± 48.4 ^A	2950.9 ± 55.7 ^A	2940.9 ± 51.3 ^A	0.0603
Mg	820.7 ± 25.9 ^A	787.6 ± 6.7 ^B	794.9 ± 15.0 ^{AB}	780.35 ± 13.3 ^B	0.023*
Al	1043.0 ± 5.6 ^A	1053.2 ± 5.2 ^A	1050.5 ± 4.4 ^A	1049.3 ± 5.1 ^A	0.5423
B	0.700 ± 0.012 ^A	0.702 ± 0.016 ^A	0.740 ± 0.014 ^A	0.718 ± 0.020 ^A	0.2438
Cu	10.94 ± 0.12 ^A	11.20 ± 0.10 ^A	11.38 ± 0.18 ^A	11.12 ± 0.17 ^A	0.2198
Fe	219.0 ± 4.8 ^A	221.7 ± 2.9 ^A	219.2 ± 3.4 ^A	217.4 ± 3.1 ^A	0.8632
Mn	21.82 ± 1.00 ^A	20.72 ± 1.07 ^A	23.14 ± 1.06 ^A	22.95 ± 1.54 ^A	0.4503
Zn	2.36 ± 0.07 ^A	2.53 ± 0.05 ^A	2.51 ± 0.07 ^A	2.53 ± 0.11 ^A	0.3698
Na	44.26 ± 0.84 ^A	44.06 ± 0.94 ^A	45.13 ± 0.78 ^A	44.51 ± 1.03 ^A	0.8519
Ni	1.29 ± 0.04 ^B	1.45 ± 0.03 ^A	1.36 ± 0.03 ^{AB}	1.39 ± 0.05 ^{AB}	0.0455*
Cd	0.086 ± 0.002 ^A	0.089 ± 0.001 ^A	0.089 ± 0.002 ^A	0.089 ± 0.002 ^A	0.4941
Cr	0.289 ± 0.004 ^A	0.284 ± 0.003 ^A	0.291 ± 0.004 ^A	0.295 ± 0.005 ^A	0.3184
Co	0.446 ± 0.017 ^A	0.451 ± 0.018 ^A	0.468 ± 0.018 ^A	0.475 ± 0.024 ^A	0.6918
Pb	3.49 ± 0.09 ^A	3.73 ± 0.07 ^A	3.85 ± 0.12 ^A	3.63 ± 0.11 ^A	0.0787

Note: The 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). Data are presented as means ± standard error of mean for each elementary content. Data is presented for corn and soybean plots of the three crop rotations in 2019 and 2020 respectively. The * and different bold small letters indicate that mean values are significantly different ($p < 0.05$) between the different crop managements according to a multiple means comparison and post-hoc test with letters.

2.5 DISCUSSION

2.5.1 The use of cover crop to control weeds

In this study, DSCC 3.3 plots present the lowest weed pressure compared to the other crop managements. These results are interesting and demonstrate that by combining cover crops with commonly applied rates of GBH (two application of 1.64 L ha^{-1}), it is possible to significantly reduce the presence of weed. Although weed cover rates are similar in DS 3.3 and DSCC 3.3 soybean and corn plots in 2019, weed presence is lower up to 57% in DSCC 3.3 soybean plots and up to 55% in DSCC 3.3 corn plots in 2020 (Figure 2.2a and Figure 2.2b). These results are consistent with those observed in other studies, where the use of CCs reduced weed biomass by 40% to 95% (Nichols *et al.*, 2020; Osipitan *et al.*, 2019). This weeds rate reduction may be explained by interspecific competition between CCs species and weeds (Osipitan *et al.*, 2019; Petit *et al.*, 2018; Teasdale, 1996; Woolford et Jarvis, 2017), which provides additional weed control on top of GBH impact. Although weed cover rates are lower in DSCC 3.3 plots, biomass production (FW and DW) and grain yields are similar between the DSCC 3.3 and DS 3.3 maize and soybean plots in both study years (Table 2.3). These results show that the presence of CCs does not appear to compete with the integrity of the crop of interest and grain yields. This observation is particularly interesting in corn plots, where CCs were present as intercrops during the production period. These results support other observations where the use of CCs such as sunflower and buckwheat as intercrops could provide weed control without reducing soybean yields (Cheriere *et al.*, 2020; Sharma *et al.*, 2021). The variation of soil VWC and elementary contents can also be used as indicator of interspecific competition. No trace of water competition between CCs and crops seems to be observed, whereas soil VWC values are similar between DSCC 3.3 plots and DS 3.3 plots (Figure 2.3). Similarities are also observed between those plots based on elementary contents (Table 2.4). This may encourage field crop producers to plant CCs without fearing that they will negatively impact grain yields.

Corn plots are more severely impacted by the presence of weeds than soybean plots. This is demonstrated by a high correlation index between weed cover and soil water content (r^2 adjusted = 0.413) in 2020. The scarcity of soil water subsequently has repercussions on corn plants development, with the smallest plants observed in plots with the lowest VWC values (Table 2.3 and Figure 2.3). The weeds competition for water is probably higher in 2020, given that soil VWC are generally much lower ($p < 0.001$) in 2020 ($14.4 \pm 0.2\%$) compared to 2019 ($20.4 \pm 0.2\%$). The average size of maize plants is also significantly lower in 2020 than in 2019 ($p = 0.0038$), more so in plots where weeds are more prevalent. For example, FW of corn plants in 2020 are on average 56% lower ($p < 0.001$) and corn plant heights 14% smaller ($p = 0.0046$) in the DSCC 0.84 plots (Table 2.3). However, as suggested by the correlation index, the presence of weeds does not appear to be the only factor influencing soil water content. It was observed in fields under climatic conditions identical to this study, that vapour pressure deficit (Vpd) in crop plants were higher in 2020 (Bernier Brillon, Moingt, *et al.*, 2023). It is well known that Vpd is an indicator of air dryness and that it strongly influences soil evapotranspiration (Grossiord *et al.*, 2020; Seager *et al.*, 2015; Sinclair *et al.*, 2017). This can partly explain why lower FW production ($p = 0.0041$) and smaller plants ($p = 0.0111$) were also observed in the DS 3.3 plots in 2020 (Table 2.3), despite the fact that the presence of weeds in these plots was not the highest (Figure 2.2b). In plots without CCs, bare soil is more exposed and vulnerable to rising soil temperatures and greater water loss through soil evaporation (Pimentel *et al.*, 1995; Robertson *et al.*, 2014; Scopel *et al.*, 2013). An interesting observation is that, although DSCC 1.64 and DSCC 3.3 plots exhibit lower VWC in 2020, no difference in plant variables is observed between 2019 and 2020 (Table 2.3 and Figure 2.3). It seems that the agronomic conditions in these plots have made them more resilient to the presence of weeds and the lack of water.

Among CC species used in this study, some are known to have allelopathic properties which may partly explain the effectiveness of CCs in controlling weeds. As such, rye, sunflower, oats and various Brassicaceae (e.g. tillage radish and tillage turnips) are recognized for their allelopathic properties (Baraibar *et al.*, 2021; Jabran *et al.*, 2015). Rye and sunflower are recognized for their

ability to produce over 16 different allelopathic compounds, including benzoxazinones [2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA) and 2(3H)-benzoxazolinone (BOA), phenyllactic acid and phenolic acids (Abbas *et al.*, 2021; Alsaadawi *et al.*, 2012; Schulz *et al.*, 2013). Those species can continue to offer allelopathic properties after plant senescence (e.g. after harvesting or freeze death) (Jabran *et al.*, 2015; Scavo et Mauromicale, 2021). Residues of Brassicaceae species are also known to have similar properties through the production of glycosinolate, an important allelopathic compound (Bachheti *et al.*, 2019; J.W. *et al.*, 2001).

2.5.2 Influence of CC on GBH doses of application

This study suggests that it is possible to reduce GBH doses for weed control when using CCs between crops or as intercrops under no-till practices. In corn and soybean cultivations, DSCC 1.64 plots share many similarities with DS 3.3 plots, such as weed cover rates, grain yields, FW, DW and plants heights. Weed cover rates in DSCC 1.64 plots were not different from those in DS 3.3 plots in 2019 and 2020 (Figure 2.2a and Figure 2.2b). The use of CCs combined with a reduction of GBH doses connects to the vision of integrated weed management (IWM), which recommends a diversification of approaches to weed control and a reduction in herbicide doses in order to limit the development of herbicide resistance (Blackshaw *et al.*, 2008; Moss *et al.*, 2019). In IWM, the ecological approach is to be prioritized as often as possible with respect to mechanical or chemical treatments. Use of machinery should be sparing and superficial, only when necessary or needed to implement CCs (e.g. stubble ploughing, strip-till, make furrows to increase sowing success) or to control them (e.g. harvester, roller-crimpers, knife-roller) (Beckie *et al.*, 2020a; Clements *et al.*, 1994; Davis, 2010; Harker et O'Donovan, 2013; Mirsky *et al.*, 2011). Also, the use of chemical herbicides must be seen as last resort and be applied carefully following the herbicide resistance risk matrix (Moss *et al.*, 2019).

It has also been observed in this study that reducing GBH doses to 0.84 L ha⁻¹, i.e. below the minimal dose recommended by manufacturers, already entails risks and visible impacts on crop

integrity. DSCC 0.84 plots present the highest weed infestation in soybean and corn cultivation in 2019 and 2020 (Figure 2.2a and Figure 2.2b). The high presence of weeds in DSCC 0.84 plots seems to have had an impact on plant development and grain yield in both study years more critically so in 2020 (Table 2.3). In 2020, we observed that VWC values are lower in DSCC 0.84 plots during the first and second sampling campaigns, which means interspecific competition for water with weeds may have occurred and impacted the subsequent development of the plants. Soybean and maize plants were indeed statistically smaller with lower biomass (Table 2.3). A longer implantation period of CCs during spring may probably help to achieve satisfactory weed control with minimum GBH application. However, this study shows that without a significant presence of CCs during the intercrop period, it is not practicable to reduce up to 75% the use of GBH along with CCs.

2.6 CONCLUSION

This two years field study showed that the use of CCs combined with GBH may represent an interesting alternative for limiting weeds in field crops. The lowest weed cover rates were observed in the plots where CCs were paired with the 3.3 L ha⁻¹ application of GBH compared to plots without CCs with the same GBH dose applied. The differences between crop managements are more striking in 2020 which can be explained by greater interspecific competition between crops and weeds compared to 2019. It has been observed that smallest plants were located in plots with the lowest water content. Compared to elementary content where no correlation exists with weeds cover, a strong correlation was observed between water content and weed cover in these plots. Moreover, this correlation was strongly attributable to the presence of broadleaf weeds. Finally, many similarities are observed between DS 3.3 plots and DSCC 1.87 plots on weed cover rates, crop plants parameters and grain yield during both years. These results suggest the possibility of reducing the use of GBH by up to 50% with the use of CCs in soybean cultivation after harvest and as intercrop in corn cultivation. However, without an efficient CCS implantation, it does appear

feasible to reduce the use of GBH below 1.87 L ha⁻¹ doses without having negative repercussions on crops.

2.7 ACKNOWLEDGMENTS

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2.8 CONFLICTS OF INTEREST

The authors declare no conflict of interest.

CHAPITRE III

CROSS-EFFECT BETWEEN COVER CROPS AND GLYPHOSATE-BASED HERBICIDE APPLICATION ON MICROBIOTE COMMUNITIES IN FIELD CROPS SOILS

Jérôme Bernier Brillon^{1*}, Marc Lucotte¹, Blandine Giusti¹, Gilles Tremblay² and Matthieu Moingt¹

¹ GEOTOP & Institute of Environmental Sciences, University of Quebec at Montreal, 201, avenue du Président-Kennedy Montréal, Québec Canada H2X 3Y7 Qc, Canada

² Quebec Ministry of Agriculture, Fisheries and Food (MAPAQ), 1355, rue Daniel-Johnson Ouest, bureau 3300, Saint-Hyacinthe, Québec, Canada J2S 8W7

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3.1 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

3.2 INTRODUCTION

Soil degradation remains a major issue worldwide whereas approximately 33% of agricultural lands are currently in a precarious situation (FAO et ITPS, 2015; Ferreira *et al.*, 2022). Soil degradation can be linked to compaction, loss of fertility, limitation of bioavailable nutrients needed by crop plants, poor water infiltration and increased salinity (FAO et ITPS, 2015; Ferreira *et al.*, 2022; Mason *et al.*, 2023). The FAO emphasizes that the loss of soil resources and functions can be avoided with sustainable practices (FAO et ITPS, 2015). Conventional agriculture with mechanical tillage such as ploughing is still a widely used soil management practice and is largely responsible for soil degradation (Strudley *et al.*, 2008; Vanwallegem *et al.*, 2017; Yu, Z. *et al.*, 2020). To challenge this issue, conservation agriculture (CA) has been put forward (Kassam *et al.*, 2019; Kassam *et al.*, 2022; Scopel *et al.*, 2013). CA is designed to assure biodiversity and natural biological processes in order to assure soil sustainability and to increase agricultural productivity (Kassam *et al.*, 2019). Limiting tillage and maintaining a permanent vegetal cover are two out of the three pillars of this approach (Derpsch *et al.*, 2010; Scopel *et al.*, 2013). 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) (Kassam *et al.*, 2022; Scopel *et al.*, 2013). By considerably reducing tillage, DS remain vulnerable to surface soil compaction, limiting field operations and influencing soil structure (de Moura *et al.*, 2021; Triplett et Dick, 2008). The problems of soil compaction observed in DS could also influence soil microbiota (Lauber *et al.*, 2008; Lehmann *et al.*, 2018; Trivedi *et al.*, 2016). Healthy, fertile soil provides greater resilience to biotic and abiotic stresses, while sustaining high productivity over the long term (Habig et Swanepoel, 2015). Soil productivity is closely linked to the biodiversity of its biota (Lehmann *et al.*, 2018; Morugan-Coronado *et al.*, 2022). Prokaryotes and Eukaryotes organisms are known to be the cornerstone for maintaining soil functions and availability of essential nutrients for crops (Trivedi *et al.*, 2016; Zak *et al.*, 2003). 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 (Habig et Swanepoel, 2015).

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 (Amsili et Kaye, 2021; Liu *et al.*, 2005; Magdoff, 2007), 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 (Morugan-Coronado *et al.*, 2022). The presence of CC also promotes higher soil aggregation, which can further contribute to increased microbiota diversity (Lehmann *et al.*, 2018; Liu *et al.*, 2005; Morugan-Coronado *et al.*, 2022). On the other hand, non-selective herbicides such as glyphosate-based ones are frequently used in no-till farming (Derpsch, 1998; Yu *et al.*, 2020). At certain application rates, their use may have a negative impact on soil microorganisms (Duke *et al.*, 2012; Kremer et Means, 2009; Locke *et al.*, 2008), 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 (Gomes *et al.*, 2014), 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 (Locke *et al.*, 2008). 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.

3.3 MATERIALS AND METHOD

3.3.1 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 (Mehlich, 1984) (Table 3.1). The soil type at the site is a humic Gleysol with a heavy clay texture (Table 3.1). The experimental design included three crops in rotation (soybean-corn-wheat). Four different weed management with application of GBH (Roundup Ready Wheatermax®) were applied [DS 3.3: direct seeding without CC + 3.3 L ha⁻¹ in 2 applications (1,804 g a.i), DSCC 3.3: direct seeding with CC + 3.3 L ha⁻¹ in 2 applications (1,804 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 3.1). The weed 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 x 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 3.1 and have been defined according to the recommendations of the Quebec Ministry of Agriculture, Fisheries and Food (MAPAQ) agronomists.

Experimental design												
Bloc #4	T12	T10	T6	T7	T1	T8	T11	T5	T2	T9	T4	T3
	DSCC	DSCC	DSCC	DSCC	DS	DSCC	DSCC	DS	DSCC	DS	DSCC	DSCC
	3.3	0.84	0.84	1.67	3.3	3.3	1.67	3.3	0.84	3.3	3.3	1.67
Bloc #3	T9	T3	T4	T5	T11	T2	T7	T8	T1	T6	T10	T12
	DS	DSCC	DSCC	DS	DSCC	DSCC	DSCC	DS	DSCC	DSCC	DSCC	DSCC
	3.3	1.67	3.3	3.3	1.67	0.84	1.67	3.3	3.3	0.84	0.84	3.3
Bloc #2	T10	T8	T12	T3	T6	T5	T4	T2	T9	T7	T1	T11
	DSCC	DSCC	DSCC	DSCC	DSCC	DS	DSCC	DSCC	DS	DSCC	DS	DSCC
	0.84	3.3	3.3	1.67	0.84	3.3	3.3	0.84	3.3	1.67	3.3	1.67
Bloc #1	T3	T1	T7	T9	T8	T10	T12	T11	T2	T5	T4	T6
	DSCC	DS	DSCC	DS	DSCC	DSCC	DSCC	DSCC	DS	DSCC	DSCC	DSCC
	1.67	3.3	1.67	3.3	3.3	0.84	3.3	1.67	0.84	3.3	3.3	0.84
2018	2019	GBH application (Roundup Wheat®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 sown		
				1st seeding date	2e seeding date	
2018	Corn GT: Elite E65GE2R		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.	
				October 6th: autumn wheat (200 kg ha ⁻¹). Sown with a Great Plains® seed drill.	n/a	
	Soybean GT: Altitude R2		May 11th 2018	May 10th: berseem (10 kg ha ⁻¹) and crimson clover (10 kg ha ⁻¹). Sown manually.	n/a	
2019	Corn GT: P918SAM		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.	
				September 6th: autumn wheat (225 kg ha ⁻¹). Sown with a Great Plains® seed drill.	n/a	
	Soybean GT: Altitude R2		May 18th	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.	
	Wheat	Hoffman HRF	May 8th			

Figure 3.1 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

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

Corn plots were fertilized with 95 kg ha⁻¹ of N added June 29th, 2018, and 90 kg ha⁻¹ of N and 60 kg ha⁻¹ of P added June 28th 2019. In wheat plots, 50 kg ha⁻¹ of N and 65 kg ha⁻¹ of P were added May 9th with an additional 60 kg ha⁻¹ of N June 20th, 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: August 10th 2018 and August 15th 2019). The autumn cover crops were sown a few weeks before the harvest of soybean (soybean harvest: October 5th 2018 and October 15th 2019) and corn (corn harvest: November 12th 2018 and October 27th). No cover crop was sown in DS plots.

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 November 4th, 2018, and October 24th, 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.

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) (Mehlich, 1984). All elemental contents were quantified using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin Elmer Optima 4300DV, Perkin Elmer Inc., Waltham, Massachusetts, USA).

DNA extraction

All soil samples were then ground and sieved through a 2mm screen after having been dried at room temperature for 72h. 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 μ l (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 (Giusti *et al.*, 2023).

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). 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% ($R^2 = 0.99$) for total bacteria and 91.7% ($R^2 = 1$) 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 (Jeanne *et al.*, 2019). 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, Canada).

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 (Bolyen *et al.*, 2019; Callahan *et al.*, 2016). 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 (DeSantis *et al.*, 2006; Guillou *et al.*, 2012; Quast *et al.*, 2013). Sequences were rarefied to 10,000 per sample prior to statistical analyses (Giusti *et al.*, 2023).

Statistical analyses

All analyses on metagenomic data were performed with the R software (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 (McMurdie et Holmes, 2013). The microorganisms composition was defined through an ordination using nonmetric multidimensional scaling of the Bray-Curtis (Figure 3.2). Then, the eukaryotic and prokaryotic composition were assessed with permutational multivariate analysis of variance (PERMANOVA) and the adonis function (Anderson, 2001). The vegan package was used to performing the adonis function (Anderson, 2001). The abundance of the main genus between managements were identified with the ampvis2 package (Andersen *et al.*, 2018) and the Operational Taxonomic Units (Hanh Le *et al.*). 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.

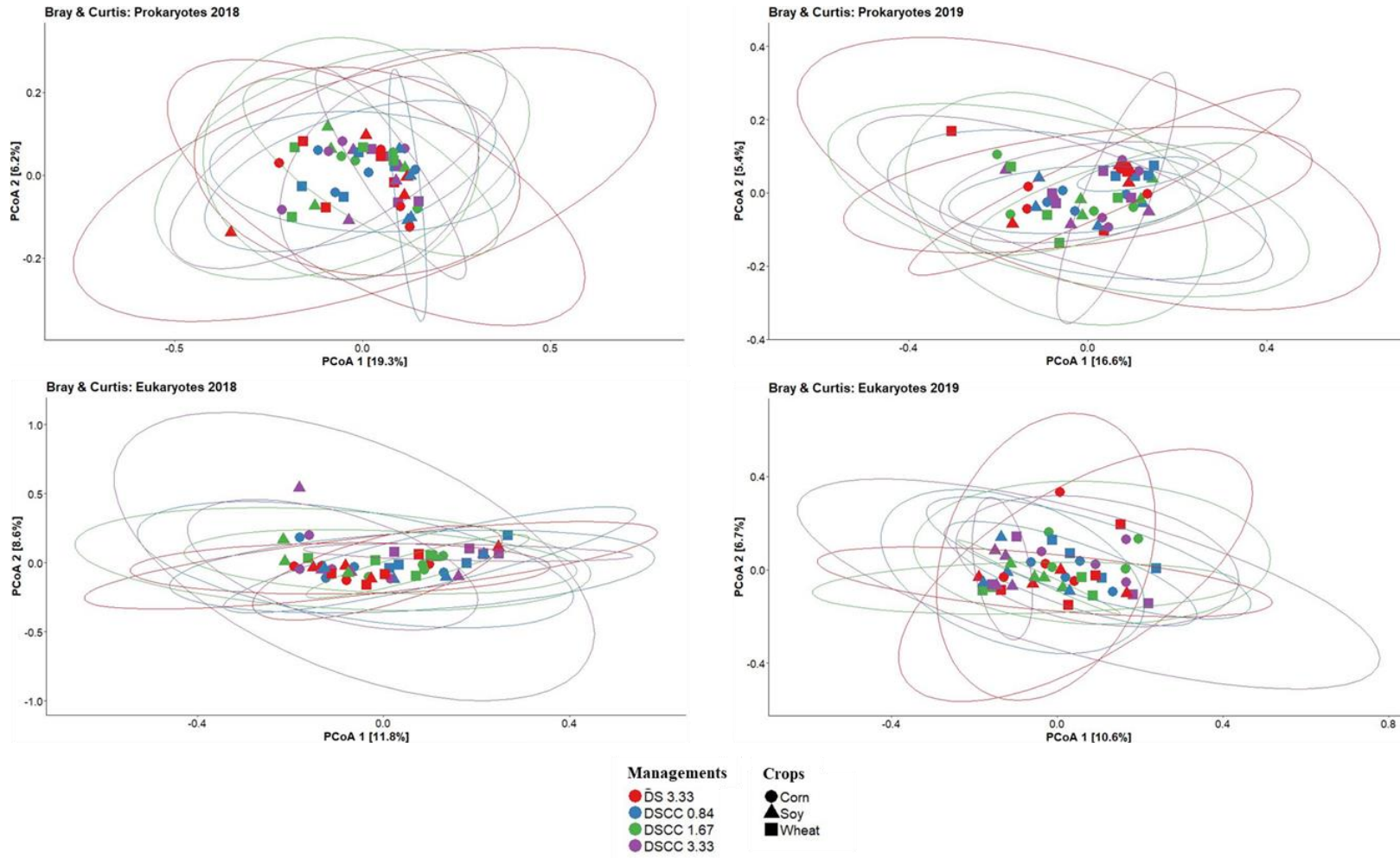


Figure 3.2 Principal coordinates analysis (PCoA) using Bray-Curtis dissimilarity test on procaryotes and eucaryotes composition in soil between the different crop managements in corn, soy and wheat.

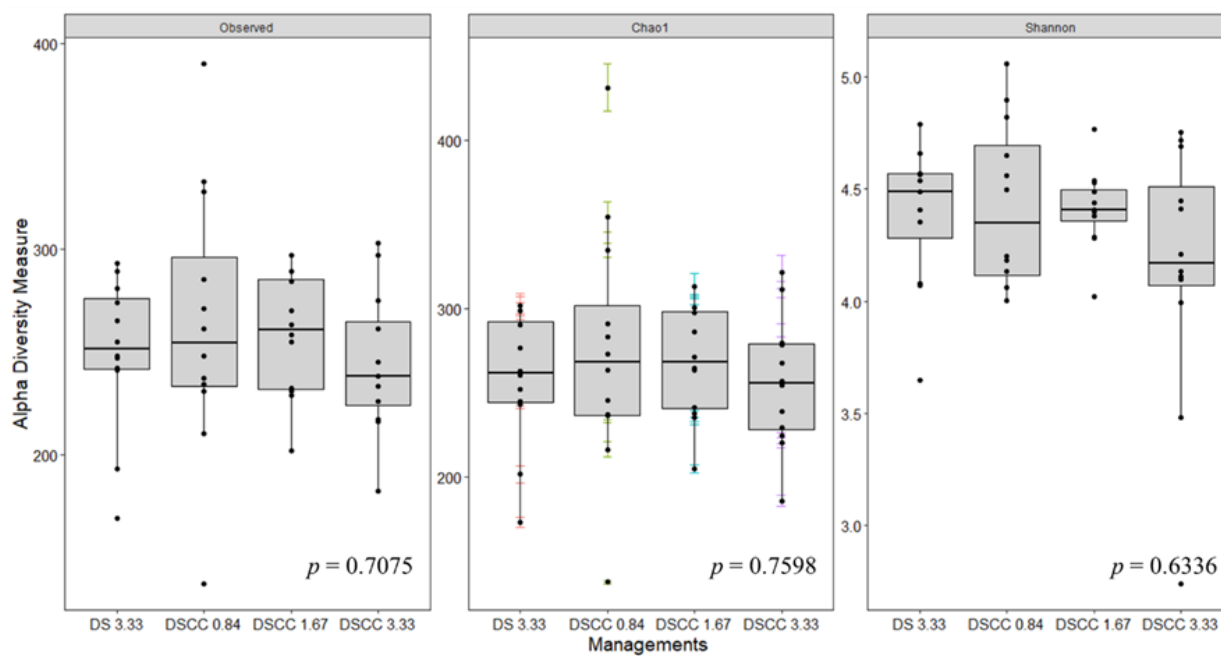
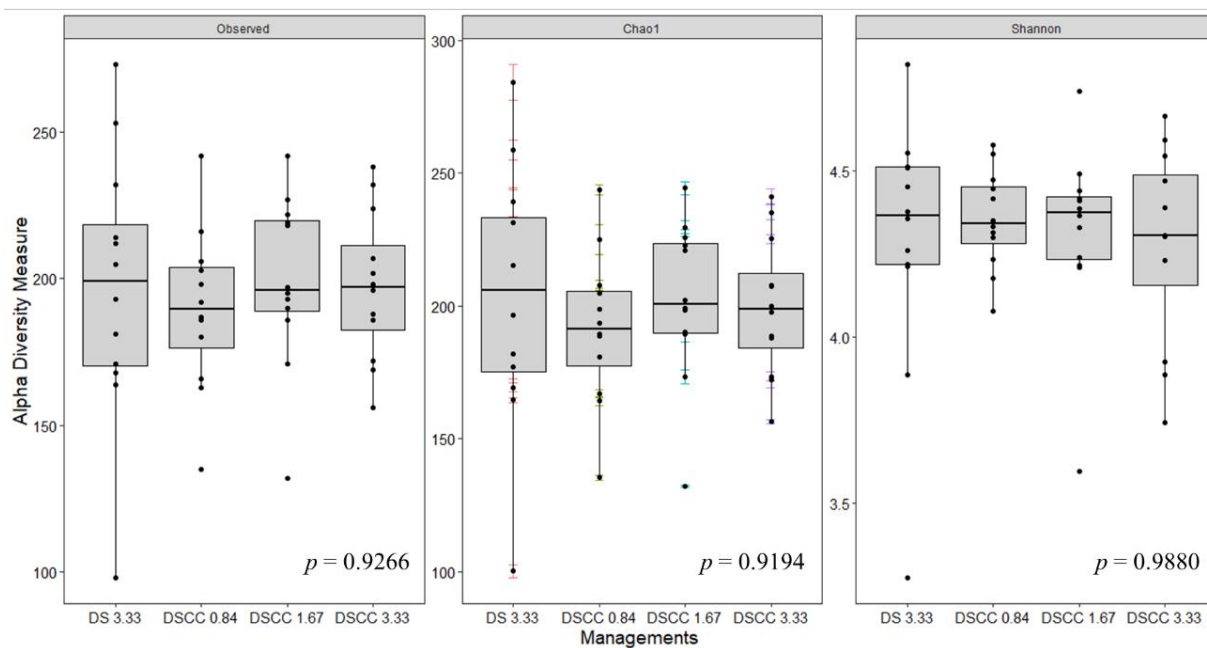
An analysis with contrast was performed to assess significant difference between DS and DSCC plots. Also, a univariate analysis was performed to assess significant difference between all crops managements. A threshold of 0.05 was used to assessed statistical significance for all statistical analyses. 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.

3.4 RESULTS

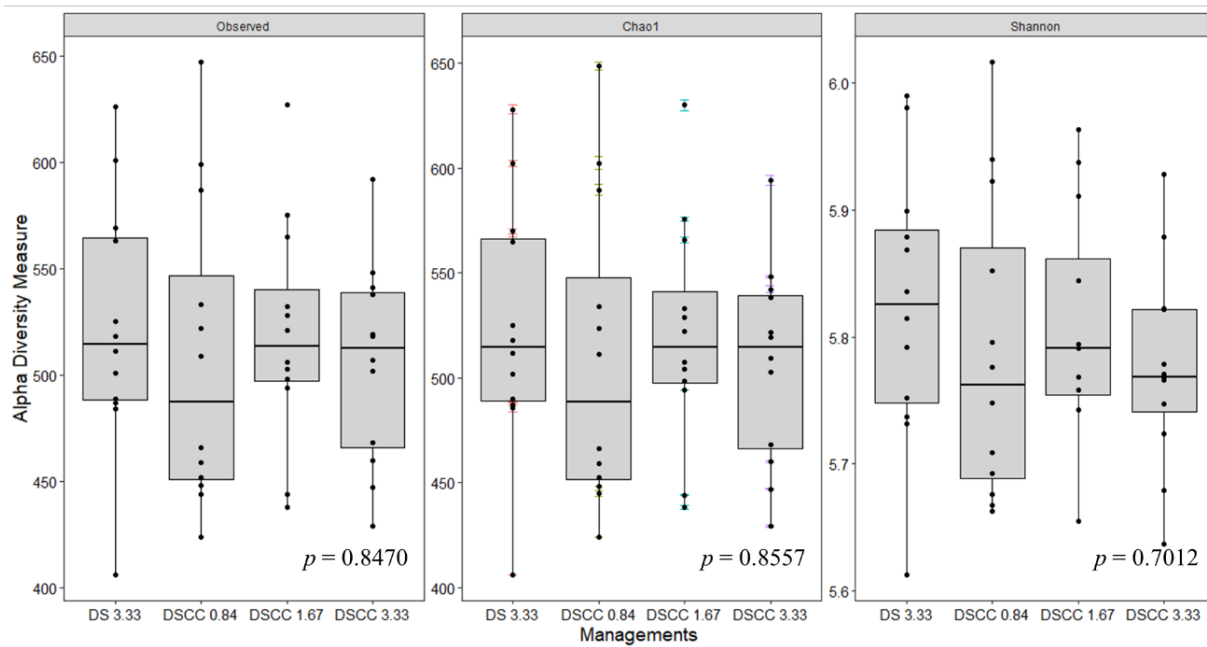
3.4.1 Soil organisms content index values

In 2018, no significant difference was observed for the eukaryotic richness and diversity between managements according to total eukaryotes Observed index, the Shannon index and the Chao 1 index (Figure 3.3a). No significant differences were assessed based on the total eukaryotes Observed index, the Shannon index and the Chao 1 index in 2019 (Figure 3.3b).

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 3.3c). Also in 2019, no significant difference was observed based on the prokaryotic Observed index, Shannon index and Chao 1 index (Figure 3.3d).

(A) Eukaryotes 2018**(B) Eukaryotes 2019**

(C) Prokaryotes 2018



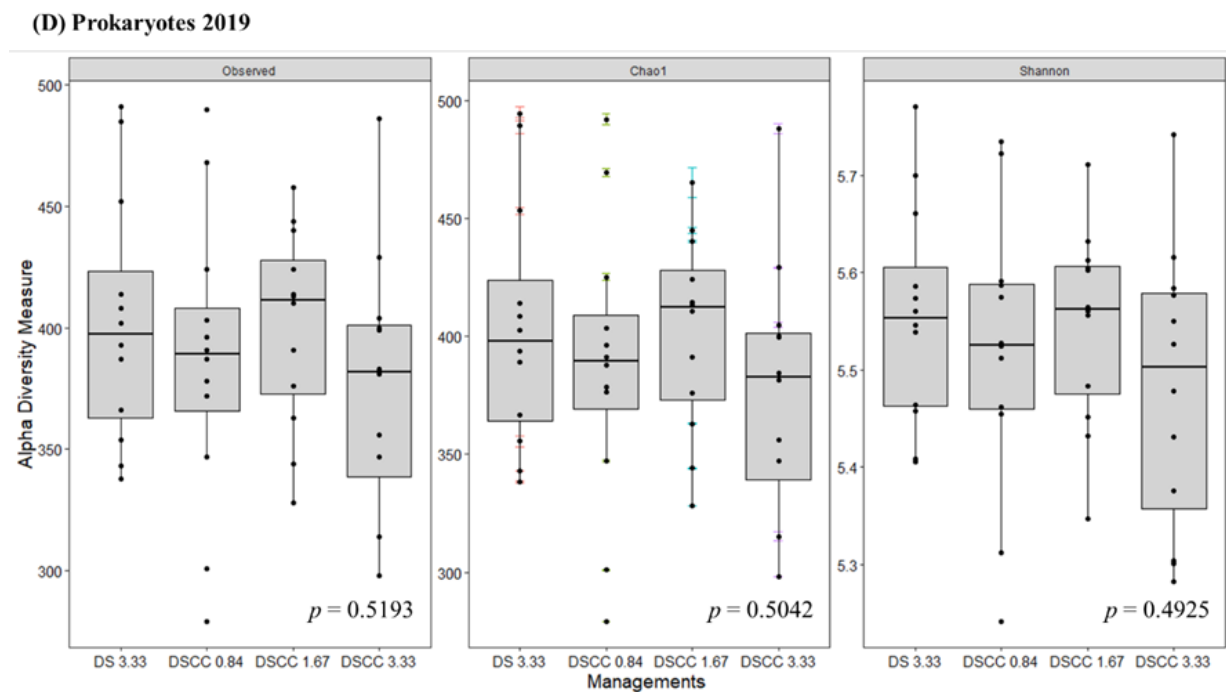


Figure 3.3 Values of eukaryotic (A) (B) and prokaryotic (C) (D) richness index in soil (n=96) between managements (DS 3.33, DSCC 0.84, DSCC 1.67 and DSCC 3.33).

In 2018 and 2019, no significant difference was observed between managements based on Evenness index for eukaryotes and prokaryotes (Figure 3.4).

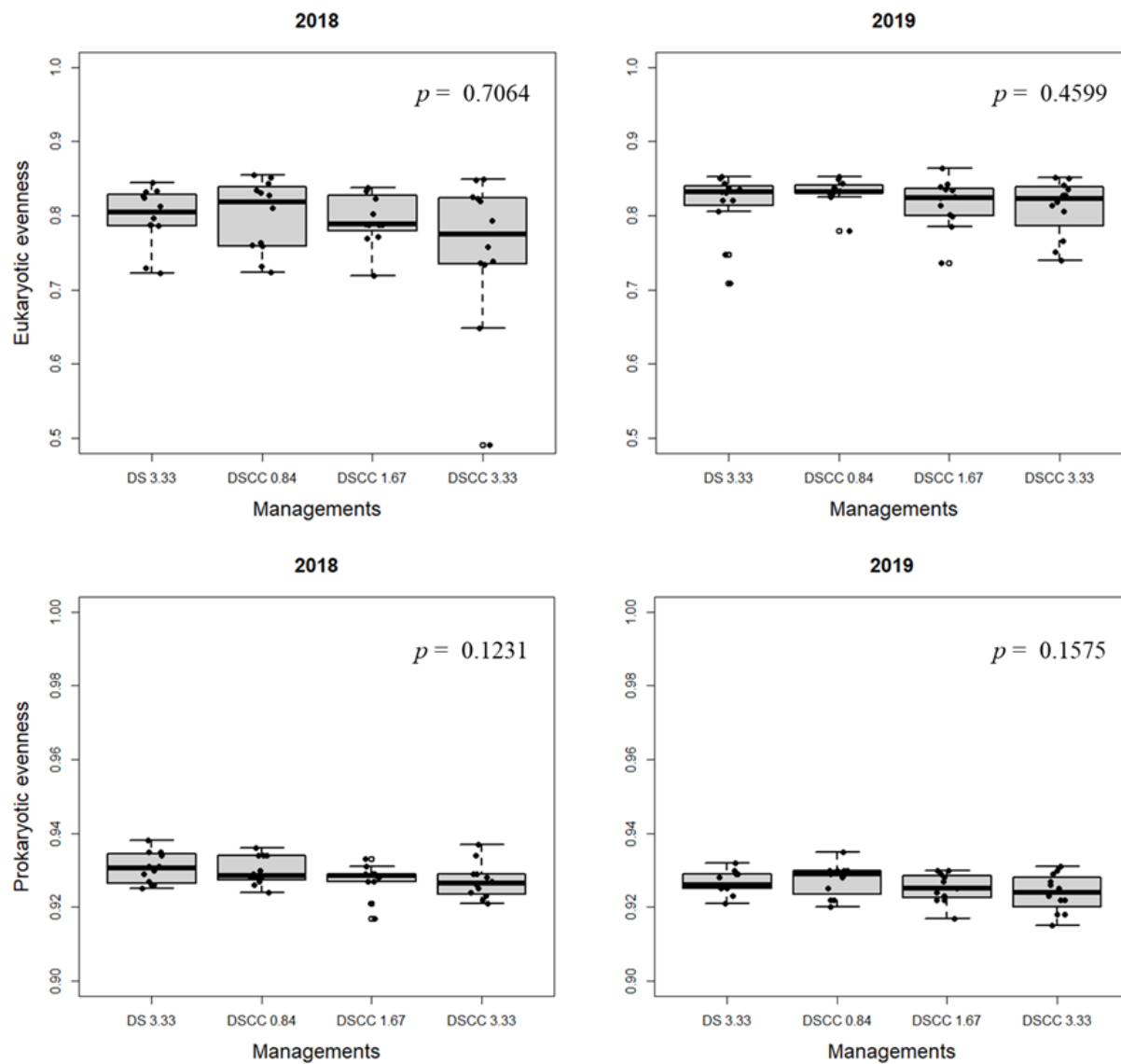


Figure 3.4 Eukaryotic and prokaryotic evenness between crop managements in 2018 and 2019

3.4.2 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 3.3). 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 3.1). 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 3.1).

Tableau 3.1 Permutational multivariate analysis of variance (PERMANOVA) of the eukaryotic and prokaryotic composition between crops management systems 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, managements systems or the mixed effect of crops*management systems are significant based on the p value threshold ($p < 0.05$).

3.4.3 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 3.2). 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 3.2).

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 3.2).

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 3.3). For the prokaryotes in 2019, the result of the analysis with contrast showed significant differences for 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 3.2 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
Saccharomycetes		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 ($p < 0.05$).

Table 3.3 Contrast analysis of prokaryotic content between plots with (DSCC) or without cover crops (DS) for 2018 and 2019.

Taxon		2018				2019			
		DS vs DSCC	Sovbean DS vs DSCC	Wheat DS vs DSCC	Corn DS vs DSCC	DS vs DSCC	Sovbean 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
Arthroacter	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
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
Soiirubrobacter	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 managements (Table 3.5). For the prokaryotes, one difference was observed for the abundance of the *Anaeromyxobacter* genus between managements ($p = 0.0331$) (Table 3.6). 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) (Table 3.6).

In 2019, significant differences were observed for the Enoplea family ($p = 0.0494$) and the Agaricomycetes class ($p = 0.0341$) (Table 3.5). 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 ($p = 0.0050$) (Table 3.5). Also, one difference was observed for the prokaryotes (Table 3.6). 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 ($p = 0.0099$).

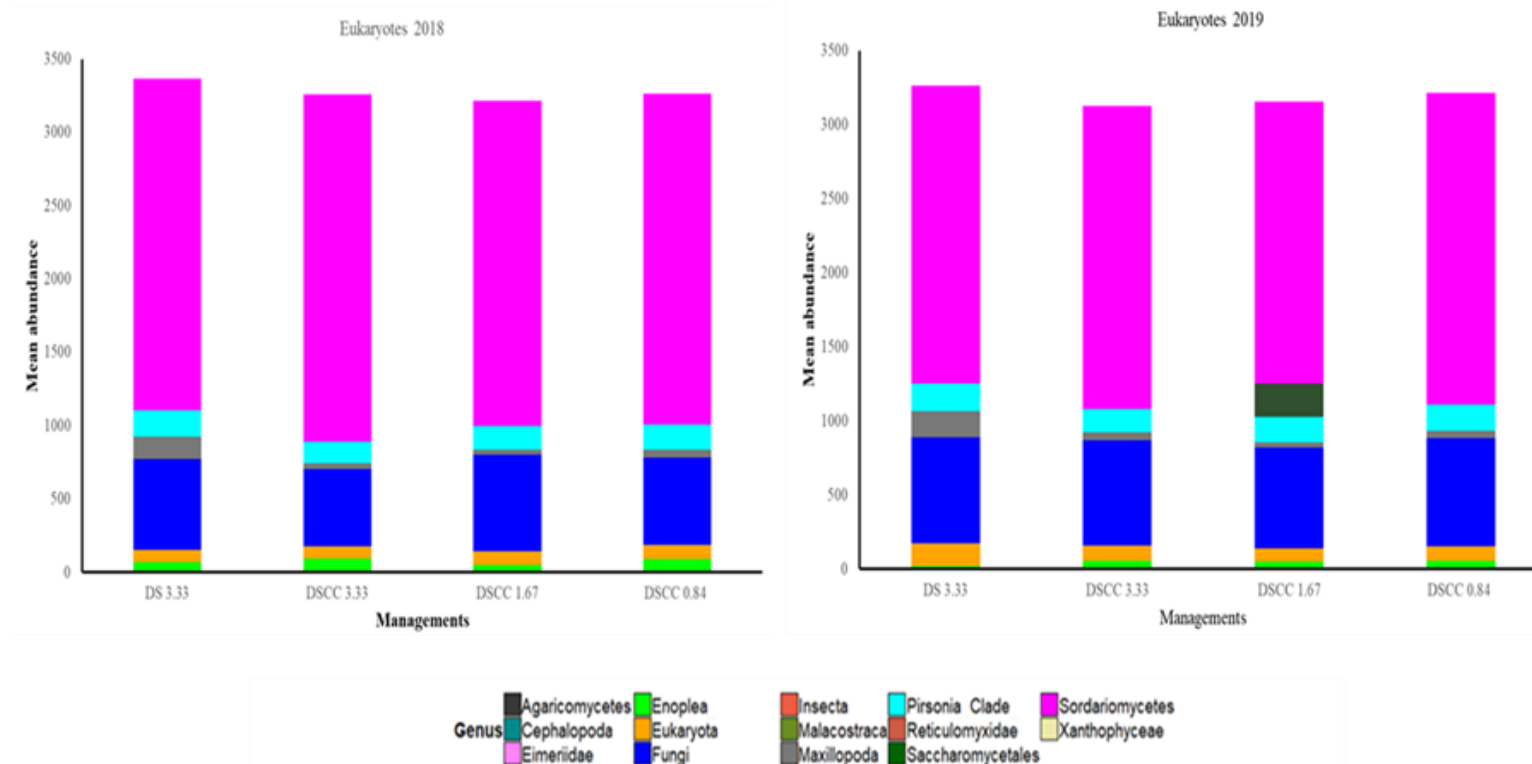


Figure 3.5 Abundance of eukaryotes between crop managements in 2018 and 2019

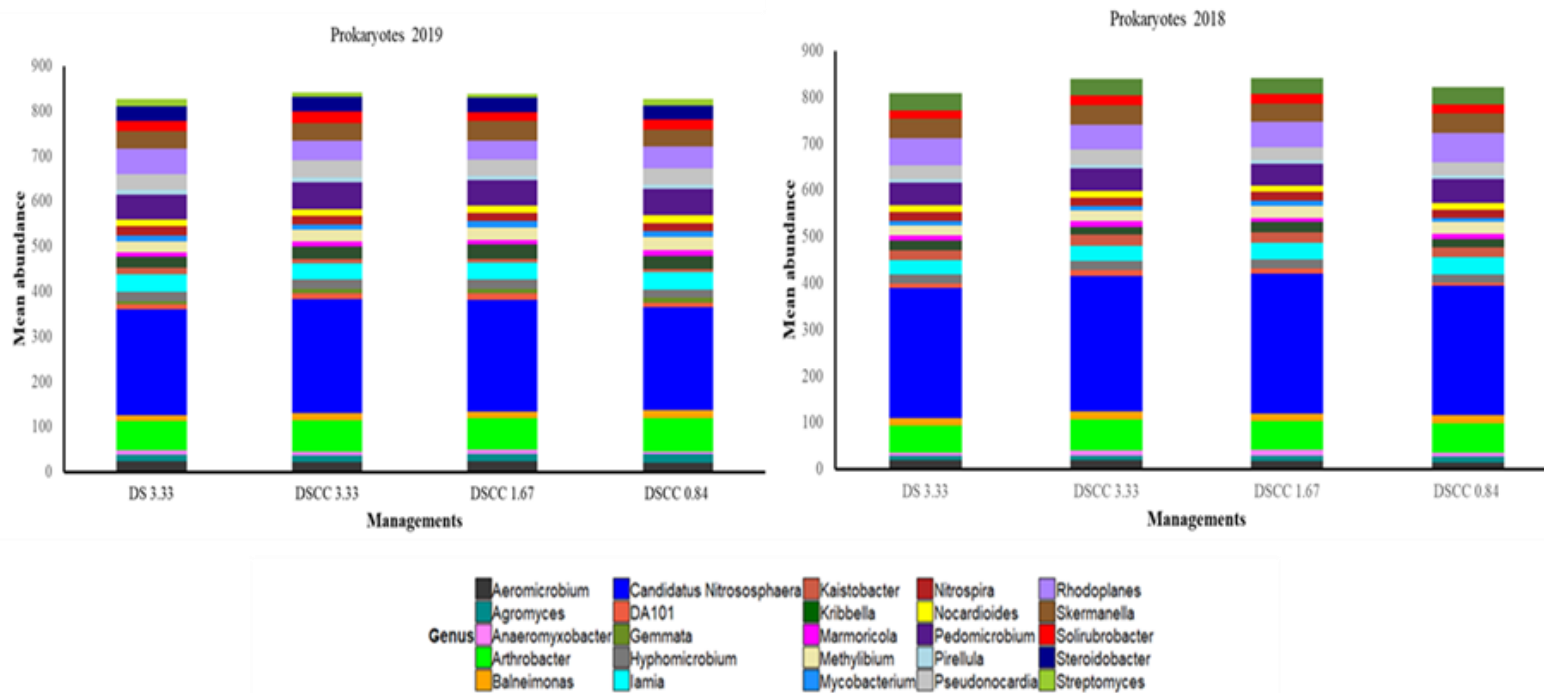


Figure 3.6 Abundance of prokaryotes between crop managements in 2018 and 2019

Over 2018 and 2019 years, the relative abundance of each taxon was calculated for the different crops (Figure 3.7a). In the case of eukaryotes, the highest proportion is attributed to the fungus group where the genus Sordariomycetes is the more abundant ($67.3 \pm 0.1\%$) followed by other unidentified fungi ($20.3 \pm 0.5\%$) and Agaricomycetes ($0.22 \pm 0.02\%$). The other eukaryotes represented less than 6% of the total eukaryotic composition in soil and where the more abundant taxon is Pirsonia Clade ($5.2 \pm 0.2\%$) followed by other unidentified Eukaryota ($3.0 \pm 0.1\%$). 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 managements 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 ($0.41 \pm 0.02\%$) and the lowest values in DSCC 3.33 plots ($1.43 \pm 0.35\%$) and DS 3.33 plots ($1.04 \pm 0.19\%$) (Figure 3.7a). In wheat plots, one difference was observed between crop managements for the Sordariomycetes genus ($p = 0.0241$) (Figure 3.7a). In wheat plots, the highest relative abundance values were measured in the DSCC 3.33 plots ($70.6 \pm 4.5\%$) and the lowest in the DS 3.33 plots ($63.7 \pm 1.9\%$).

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 3.7b). Among them, Candidatus Nitrososphaera have the higher relative abundance value ($30.9 \pm 0.4\%$), followed by Arthrobacter ($7.5 \pm 0.2\%$), Rhodoplanes ($7.2 \pm 0.1\%$) and Pedomicrobium ($6.7 \pm 0.1\%$). 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 ($7.6 \pm 0.4\%$) and the lowest values in the DS 3.33 plots ($5.6 \pm 1.7\%$) (Figure 3.7b).

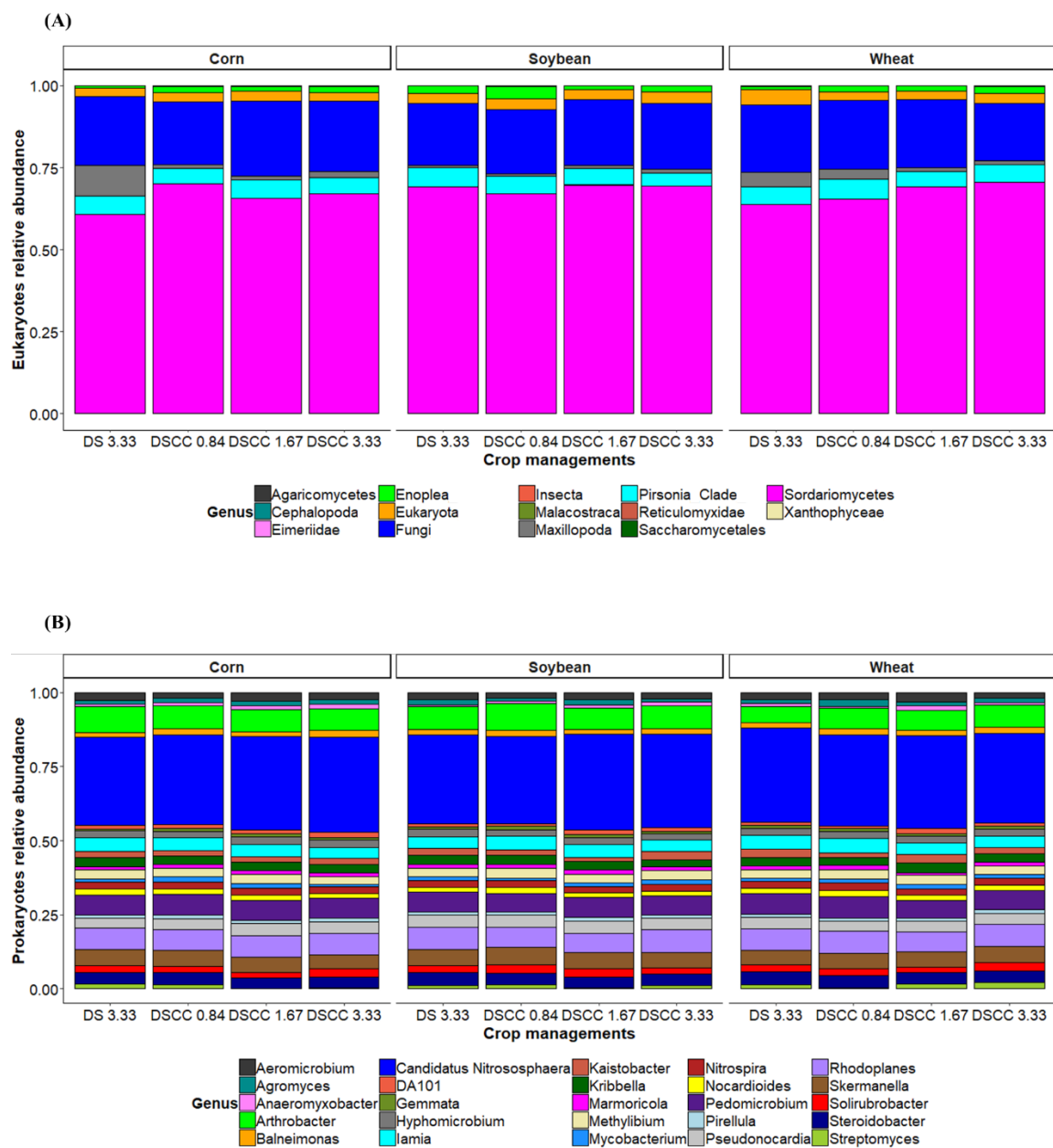


Figure 3.7 Relative abundance of eukaryotes (A) and prokaryotes (B) taxa and comparison between crops managements.

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 3.4), and higher Co content in DSCC 1.67 plots (Table 3.4). In wheat, Mg content was lower in DSCC 1.67 plots (Table 3.4). Mn content was also lower in DSCC 1.67 plots, but also in DSCC 0.84 plots (Table 3.4). The only lower content observed in DS 3.3 plots was that of Ni (Table 3.4). 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 3.4).

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 3.5). These differences were observed for only three elements: P, K, and Zn (Table 3.5). 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 3.5).

Tableau 3.4 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 * 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).

Tableau 3.5 Contrast analysis of elementary contents between crop managements for 2018 and 2019.

Element (mg*kg ⁻¹)	DS vs DCC		Corn DS vs DSCC		Soybean DS vs DSCC		Wheat DS vs DSCC	
	F value	<i>p</i> value	F value	<i>p</i> value	F value	<i>p</i> value	F value	<i>p</i> 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 ($p < 0.05$).

3.5 DISCUSSION

3.5.1 No significant difference in richness and evenness along with management systems

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 the management systems (DS vs. DSCC) in both 2018 and 2019 (Figures 3.3a-d and Figure 3.4). 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 at similar GBH rates (Giusti *et al.*, 2023). This could indicate that other human, environmental or agricultural factors such as ploughing, climate or crop type, play a more dominant role in the short term (Kim *et al.*, 2020; Schmidt *et al.*, 2018). 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 (Kelly *et al.*, 2021; Schmidt *et al.*, 2018).

3.5.2 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 (Benitez *et al.*, 2021; Khmelevtsova *et al.*, 2022; Matus-Acuna *et al.*, 2021). 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 3.5). 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 (Su *et al.*, 2017). 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 (Matus-Acuna *et al.*, 2021). 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 (Säle *et al.*, 2015). 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.

3.5.3 Notable effects on specific taxonomic groups

Prokaryotes

Significant differences are observed in certain prokaryotic genera, such as *Anaeromyxobacter* in 2018 or *Nitrospira* and *Rhodoplanes* in 2019 (Table 3.3), highlighting that specific bacterial groups may be sensitive to differences between DS and DSCC systems. 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* (Duan *et al.*, 2023; Vasiljevic *et al.*, 2024). *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 (Masuda *et al.*, 2020; Pitombo *et al.*, 2016). 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* (Hou *et al.*, 2024; Onley *et al.*, 2018).

Similarly, significant differences for *Nitrospira* and *Rhodoplanes* in 2019 may also be linked to changes in soil pH, nitrogen availability, or organic matter dynamics under different management practices (Hou *et al.*, 2024; Zhang *et al.*, 2022). 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 (Ouverson *et al.*, 2022).

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 3.2). 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 (Kelly *et al.*, 2021).

3.5.4 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 (Newman *et al.*, 2016; Wolmarans et Swart, 2014; Zobiolo *et al.*, 2010). 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 (Nguyen *et al.*, 2016; Roslycky, 1982). 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 (Busse *et al.*, 2001; Nguyen *et al.*, 2016). 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 (Duke *et al.*, 2012).

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 3.4). 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 3.4), as well as certain soil microorganisms (Hemkemeyer *et al.*, 2021). Although it has been shown that the use of CC alone does not explain the differences in elementary contents in soybean plots (Table 3.5), the variations in these contents do not either follow a GBH application rate-dependent relationship (Table 3.4). 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 (Mertens *et al.*, 2018), 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 (Mertens *et al.*, 2018). 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 (Busse *et al.*, 2001; Duke *et al.*, 2012; Kremer et Means, 2009). 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 (Duke *et al.*, 2012; Locke *et al.*, 2008). In 2018, the abundance of Anaeromyxobacter was higher in DSCC 3.33 and DSCC 1.67 plots (Figure 3.6). As mentioned earlier, Anaeromyxobacter 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 (Bernier Brillon *et al.*, 2024).

Interestingly, higher abundance of Marmoricola was observed in DSCC 0.84 plots (Figure 3.6). Marmoricola is a Gram-positive and chemoorganotrophic 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 (Evtushenko, 2015; Qiu *et al.*, 2022; Urzı *et al.*, 2000). 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 (Lijuan, 2018; Trivedi *et al.*, 2016). 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 (van Bruggen *et al.*, 2021; van Bruggen *et al.*, 2018). 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 3.5) 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 (Gutiérrez-Gutiérrez *et al.*, 2020; Singh *et al.*, 2013). Longidorus abundance is higher in plots with CC and significantly more present in DSCC 0.84 plots (Figure 3.5). The lowest abundance of this type of nematode was observed in the DS 3.33 plots (Figure 3.5). 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 (Zabaloy *et al.*, 2022). It has been observed that even at low GBH application rates, glyphosate can induce oxidative stress in nematodes (Kronberg *et al.*, 2014), and that the persistence of glyphosate in the environment can influence the structure, abundance, and recovery of various nematode communities in the long term (McQueen *et al.*, 2024). 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 (Anwar *et al.*, 2009; Gowda *et al.*, 2019; Klingen *et al.*, 2002; Klingen et Haukelan, 2006; Thomas, 1969). 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 (Bernier Brillon *et al.*, 2024).

Agaricomycetes are the other eukaryote group where significant differences were observed between crops managements (Figure 3.5). This class of fungi comprises almost 36,000 species, some closely associated with wood rot (Sánchez-García *et al.*, 2020; Takemoto *et al.*, 2010). Schizophyllum abundance was highest in DSCC 3.33 plots compared to DSCC 1.67 plots (Figure 3.5). Fungi, particularly arbuscular mycorrhizal fungi, can be sensitive to glyphosate and their response to different application rates could vary (Druille *et al.*, 2013; Wilkes *et al.*, 2020). However, their abundance was very low here and it is known that the influence of crop managements on Schizophyllum varies greatly according to the species known in this genus (Bongiorno *et al.*, 2016; Takemoto *et al.*, 2010).

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 (Duke, 2020; Schlatter *et al.*, 2016; Zabaloy *et al.*, 2022). 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) (Mbuthia *et al.*, 2015; Morugan-Coronado *et al.*, 2022). 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 (Locke *et al.*, 2008). 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 (Kelly *et al.*, 2021).

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 (Chen *et al.*, 2022; Spinelli *et al.*, 2021). 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 (Spinelli *et al.*, 2021). 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.

3.6 CONCLUSION

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 soy-bean 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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3.8 CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

CONCLUSION GÉNÉRALE

L'objectif principal de cette thèse était de déterminer si l'utilisation de culture de couvertures pouvait apporter des bénéfices agronomiques de pratiques en semis direct dans les grandes cultures TG. Pour ce faire, trois aspects ont été spécifiquement pris en considération pour déterminer les bénéfices potentiels à la suite de l'utilisation des CC soit : sur la résilience des cultures TG dans un contexte de stress hydrique et de valeur de déficit de pression de vapeur (V_{pd}) plus élevées (Chapitre 1), sur leur potentiel à lutter efficacement contre les adventices et de réduire l'usage de HBG dans les cultures de soja et maïs TG (Chapitre 2) et sur l'augmentation de l'abondance de microorganismes dans les sols (Chapitre 3).

Dans le chapitre 1, les résultats obtenus démontrent que les CC contribuent à maintenir le potentiel d'échange gazeux du soja TG exposé à des valeurs de V_{pd} plus élevées. Les parcelles de soja dans les parcelles DSCC et DS ont été exposées à des applications de HBG similaires ($3,33 \text{ L ha}^{-1}$) ce qui peut augmenter leur sensibilité face à d'autres facteurs tels que la sécheresse et la perte élevée en eau à travers la transpiration. À des valeurs similaires de V_{pd} , la conductance stomatique sur la surface foliaire abaxiale des plantes des parcelles DSCC était significativement plus élevée que celle des plantes des parcelles DS. Cela peut s'expliquer par une plus grande tolérance aux conditions plus sèches qui peuvent provoquer une limitation de l'eau pour les plantes. Cette tolérance s'est exprimée par une nervation plus élaborée et une densité stomatique plus élevée sur les feuilles des plantes qui ont poussé dans les parcelles DSCC. Ces résultats démontrent une plasticité plus importante chez les plantes cultivées avec des CC par rapport à celles cultivées sans CC. Le développement de stomates plus petits, mais plus nombreux permet un ajustement plus rapide de l'ouverture des stomates, ce qui peut être une stratégie intéressante pour les plantes de

culture pour limiter leurs pertes d'eau lors de variations à court terme des conditions climatiques et de croissance. Cette condition n'aurait peut-être pas été possible sans le développement morphologique nécessaire pour permettre aux plantes une meilleure disposition pour la gestion en eau, représenté par une densité de nervure foliaire plus élevée également observée dans le cas actuel. Cette plasticité plus importante des plantes avec CC favoriserait donc une plus grande résilience au stress potentiel combiné de la sécheresse et de l'application d'HBG dans les champs de soja TG. Enfin, les CC semblent représenter, en partie, une solution durable pour lutter contre la sécheresse et les changements climatiques futurs. D'autres facteurs tels que le contenu en eau, la biodisponibilité en nutriment et la structure des sols, ainsi que la dose d'HBG appliquée et sa persistance dans le système de production agricole sont également à prendre en considération pour mieux comprendre la réponse des plantes TG face aux stress.

Les résultats obtenus dans le chapitre 2 ont montré que l'utilisation des CC combinée aux HBG peut représenter une alternative intéressante pour limiter les mauvaises herbes dans les cultures de soja et maïs en champ. L'approche la plus répandue par les producteurs repose sur une régie en SD sans CC avec des applications de HBG de l'ordre de $3,33 \text{ L ha}^{-1}$ ce qui était représenté ici par les parcelles DS 3.33. L'originalité de cette étude repose sur l'usage combiné de CC et de trois différentes doses d'HBG pour lutter contre les adventices. Ici, les taux de couverture des mauvaises herbes les plus faibles ont été observés dans les parcelles où les CC étaient associés à l'application de $3,33 \text{ L ha}^{-1}$ de HBG (DSCC 3.333) par rapport aux parcelles sans CC avec la même dose de HBG appliquée de $3,33 \text{ L ha}^{-1}$ (DS 3.33). Les différences entre les méthodes de gestion des cultures sont plus marquées en 2020, ce qui peut s'expliquer par une plus grande concurrence interspécifique entre les cultures et les adventices qu'en 2019. Il a été observé que les plantes les plus petites se trouvaient dans les parcelles ayant la plus faible teneur en eau. Comparativement aux valeurs obtenues pour les contenus élémentaires, pour lesquelles il n'existe aucune corrélation avec le taux de recouvrement d'adventices, une forte corrélation a été observée entre la teneur en eau et le taux de recouvrement d'adventices dans ces parcelles DSCC 0,84. De plus, cette corrélation était fortement attribuable à la présence d'adventices à feuilles larges représentées surtout par une

combinaison de laitrons des champs (*Sonchus arvensis*), de chénopode (*Chenopodium album*), de chardon des champs (*Cirsium arvense* (L.) Scop.) et de pissenlit commun (*Taraxacum officinale*). Enfin, de nombreuses similitudes ont été observées entre les parcelles DS 3,33 et les parcelles DSCC 1,87 en ce qui concerne les taux de recouvrement d'adventices, les paramètres des plantes cultivées (taille et poids sec) et le rendement en grains au cours des deux années. Les résultats suggèrent la possibilité de réduire l'utilisation de HBG jusqu'à 50 % grâce à l'utilisation de CC dans la culture du soja après la récolte et comme culture intercalaire dans la culture du maïs. Ces résultats sont encourageants pour les producteurs qui tenteraient de réduire leur usage de HBG d'autant plus que cette étude suggère que les CC n'entrent pas en compétition avec les cultures d'intérêt. Cependant, il semble plus difficile de réduire l'utilisation d'HBG en dessous des doses de 1,87 L ha⁻¹ sans avoir de répercussions négatives sur les cultures. Une approche intéressante à explorer dans le futur serait l'usage de technologies de pointe pour permettre de mieux cibler certaines interventions de contrôle des adventices et ainsi, être un complément intéressant à l'usage de CC et permettre de diminuer davantage l'usage de HBG dans les grandes cultures.

Le chapitre 3 avait pour but de voir si l'utilisation de cultures de couverture pouvait augmenter la diversité du microbiote du sol et l'abondance de certaines communautés de procaryotes et eucaryotes. L'originalité de cette étude provient du fait qu'elle a considéré l'effet combiné des CC et de trois doses d'application de HBG sur les communautés de microorganismes dans les sols de 48 parcelles expérimentales. Dans cette étude, aucune différence significative n'a été observée en matière de richesse de microorganismes des sols entre les systèmes DS et DSCC, tant en 2018 qu'en 2019, ce qui suggère que d'autres facteurs, tels que le climat ou le type de culture, peuvent jouer un rôle dominant dans la formation des communautés microbiennes du sol à court terme. Cependant, des changements spécifiques observés dans les groupes procaryotes et eucaryotes mettent en évidence les interactions complexes entre les pratiques de gestion des cultures, l'utilisation d'HBG et les communautés microbiennes du sol. Bien que les taux d'application d'HBG n'affectent pas de manière significative la richesse microbienne, certains groupes taxonomiques, en particulier *Anaeromyxobacter*, *Marmoricola* et *Enoplea*, montrent des réponses

variées selon les taux d'application et les systèmes de gestion des cultures. La présence de CC semble favoriser la croissance de certaines populations microbiennes, probablement en augmentant la disponibilité des ressources grâce à la diversité racinaire et aux exsudats associés. Bien que les taux d'application d'HBG montrent un impact subtil sur les communautés microbiennes, leurs effets pourraient devenir plus prononcés avec des doses plus élevées d'HBG ou des applications répétées. De plus, les effets combinés d'HBG et de CC sur l'abondance microbienne demeurent complexes et nécessitent des recherches supplémentaires. Des études à long terme sont nécessaires pour comprendre pleinement l'impact cumulatif du glyphosate sur la santé des sols et la dynamique microbienne. Par ailleurs, le potentiel de résistance microbienne au glyphosate doit être pris en compte, surtout en considérant l'augmentation de l'utilisation de ce type d'herbicides dans les systèmes agricoles en raison des changements climatiques. Ces résultats soulignent l'importance de prendre en compte à la fois les effets directs de l'application d'HBG et les pratiques de gestion plus larges pour maintenir la biodiversité du sol et les fonctions de l'écosystème.

Cette thèse suggère que l'utilisation de CC a puisse représenter une solution envisageable pour effectuer une transition écologique des grandes cultures TG. Toutefois, les résultats obtenus se limitent à des observations effectuées sur deux années dans un seul design expérimental. Néanmoins, les résultats prometteurs obtenus dans cette étude devront être testés dans d'autres régions du Québec, avec d'autres types de sols et en incluant d'autres cultures TG. Le choix des mélanges de CC devra être adapté aux contraintes géoclimatiques et pédologiques des nouveaux sites à l'étude.

Le fait que l'échantillonnage ait eu lieu sur les mêmes parcelles renforce également la cohérence entre les trois chapitres de thèse. Les trois sujets visés dans ces chapitres (résilience aux changements climatiques, contrôle des adventices et biodiversité des microorganismes) sont étroitement liés et dépendent de l'état de santé des sols qui devrait en principe bénéficier de la présence de CC. Lorsque les fonctions et la structure des sols sont assurées, celles-ci permettent de maintenir des rendements élevés tout en gardant des cultures durables. L'utilisation de CC pourrait

faciliter le travail des producteurs québécois à effectuer cette transition. Le gouvernement du Québec a lancé le plan d'agriculture durable (PAD) 2020-2030 qui a pour but d'appuyer le gouvernement dans ses engagements de réduction de sa dépendance à l'utilisation d'énergies fossiles (carburant pour la machinerie et production de produits de synthèse) dans le milieu agricole. L'usage de CC tel que démontré dans cette thèse, apparaît comme une solution envisageable pour atteindre plusieurs objectifs du PAD : 1) réduire le volume de pesticides de synthèse vendus et les risques associés sur la santé et l'environnement, 2) améliorer la santé et la conservation des sols agricoles, 3) diminuer l'apport de matières fertilisantes azotées et optimiser la gestion de l'eau en agriculture. Les différents objectifs du PAD sont complémentaires, ce qui fait que l'atteinte d'un objectif (ex. maintien d'un couvert végétal) aura une influence directe sur l'atteinte d'autres objectifs (ex. diminution des intrants et augmentation de la biodiversité à la surface et dans les sols). Malgré que cette transition écologique représente un défi pour certains producteurs, ces derniers peuvent espérer un appui financier en adoptant des pratiques liées à l'agriculture de conservation (ex. subventions du programme Prime-vert) ou à l'atteinte de certains objectifs du PAD. Ces incitatifs financiers faciliteront l'adoption des producteurs et démontrent l'intérêt du gouvernement à vouloir appuyer les producteurs dans leur transition écologique. La lutte contre les changements climatiques sera également une opportunité pour les producteurs pour obtenir des subventions supplémentaires qui les aideront à financer certaines interventions pour rendre leurs pratiques durables. Le gouvernement du Québec a lancé en décembre 2023 une version améliorée de son programme Prime-Vert en incluant des incitatifs pour lutter contre les changements climatiques via l'aménagement d'étangs d'irrigation et de réservoirs d'eau de pluie. Il faut souhaiter qu'à court terme, l'utilisation de CC soit considérée comme une mesure pour augmenter la résilience des cultures face aux changements climatiques et que cette mesure soit suffisamment reconnue pour l'ajouter aux types d'aménagements qui peuvent obtenir du financement du gouvernement. De fait, l'achat de semoirs spécialisés pour les cultures de couverture intercalaires fait dorénavant partie des équipements admissibles à la version améliorée du programme Prime-Vert. Il sera intéressant de suivre l'évolution de cette transition lors des prochaines années. Actuellement, les prémises de celle-ci nous permettent d'être optimistes en ce qui concerne la

motivation du Québec et de ses producteurs de grandes cultures pour améliorer l'intégrité, la santé et la résilience des sols agricoles et des cultures. Les résultats obtenus dans cette thèse appuient également l'intérêt du gouvernement du Québec à inciter les agronomes et les producteurs sur l'importance d'adopter les CC pour une agriculture québécoise durable.

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