Tillage and crop rotation shape soil-borne oomycete communities in corn, soybean and wheat cropping systems

Alison Claire Gahagan

Thesis submitted to the university of Ottawa in partial fulfilment of the requirements for Master of Science Biology Specialization in Bioinformatics

Department of Biology

Faculty of Science

University of Ottawa

© Alison Claire Gahagan, Ottawa, Canada, 2023

Abstract

Soil-borne oomycetes include plant pathogens that cause substantial losses in the agricultural sector. To better manage this important group of pathogens, it is critical to understand how they respond to common agricultural practices, such as tillage and crop rotation. Here, a long-term field experiment with a split-plot design with tillage as the main plot factor (conventional tillage [CT] vs. no till [NT], 2 levels) and rotation as the subplot factor (monocultures of soybean, corn, or wheat, and corn-soybean-wheat rotation, 4 levels) was sampled. Post-harvest oomycete communities were characterized over three consecutive years (2016-2018) by metabarcoding the Internal Transcribed Spacer 1 (ITS1) region of soil DNA extracts. The community contained 292 Amplicon Sequence Variants (ASVs) and was dominated by *Globisporangium* spp. (85.1% in abundance, 203 ASV) and *Pythium* spp. (10.4%, 51 ASV). NT decreased diversity and community compositional structure heterogeneity, while crop rotation only affected the community structure under CT. Soil and crop health represented by soybean seedling vitality was lowest in soils under CT cultivating soybean or corn, while grain yield of the three crops responded differently to tillage and crop rotation regimes. The interaction effects of tillage and rotation on most oomycetes species accentuated the complexity of managing these pathogens.

Keywords: Agronomy, amplicon sequencing, crop rotation, oomycetes, tillage

Résumé

Les oomycètes transmis par le sol sont des pathogènes dévastateurs qui causent des pertes importantes dans le secteur agricole. Pour mieux gérer ce groupe de pathogènes, il est essentiel de comprendre comment ils réagissent aux pratiques agricoles, telles que le travail du sol et la rotation des cultures. Ici, une expérience de terrain à long terme dans un plan de parcelles divisées, le travail du sol étant le facteur principal de la parcelle (travail du sol conventionnel [CT] contre non-travail du sol [NT], 2 niveaux) et la rotation étant le facteur de la sous-parcelle (monocultures de soja, de maïs ou de blé, et rotation maïs-soja-blé, 4 niveaux) a été échantillonné. Les communautés d'oomycètes du sol après la récolte ont été caractérisées sur trois années (2016-2018) par métabarcodage de la région Internal Transcribed Spacer 1 (ITS1). La communauté contenait 292 variants de séquence d'amplicon (ASV) et était dominée par *Globisporangium* spp. (85,1 % en abondance, 203 ASV) et *Pythium* spp. (10,4 %, 51 ASV). Le NT a diminué la diversité et l'hétérogénéité de la structure de composition de la communauté, et la rotation des cultures affectait la structure de la communauté seulement sous CT. Les effets d'interaction du travail du sol et de la rotation sur la plupart des espèces d'oomycètes ont accentué la complexité de la gestion de ces pathogènes. La santé du sol et des cultures, représentée par la vitalité des du soja, était la plus faible dans les sols cultivés en soja ou en maïs sous CT, tandis que le rendement en grains des trois cultures réagissait différemment aux régimes de travail du sol et de rotation des cultures.

Mots clés : Agronomie, oomycètes, rotation des cultures, séquençage d'amplicons, travail du sol.

Acknowledgements

I would like to take this opportunity to thank my supervisors and committee members, Dr. Frances Pick and Dr. Tim Xing, for their patience over the course of this project, and their unwavering support during the unforeseen delays the early 2020's have thrown at us.

I would also like to thank all the other collaborators within AAFC. Dr. Malcolm Morrison, and his team members including Thomas Hotte, Ryan Tobalt, and Sophia St. Lawrence were instrumental in the sampling and processing of soil samples, come sun, rain or snow. Dr. André Lévesque, who passed the torch onto Dr. Hai Nguyen, along with their technicians Tara Rintoul and Ekaterina Pomonovera kindly shared their lab space with me; while their students Jamal Al-Refaee and Lianne Gillen assisted in soil sampling for DNA extraction. Members of the Chen lab, particularly Dr. Yichao Shi and Dr. Devon Radford, who supported and guided me throughout the analysis and all its iterations.

I could not have completed this project without the support of everyone involved in the project, as well as my family and friends, who supported me along the way. Getting through this thesis took a village, and I could not be more grateful to the one I was able to build over the course of this project.

Table of Contents

Abstract II

Résumé III

Acknowledgements IV

List of Figures and Tables VI

Author Contributions VII

Chapter 1. Introduction 1

Chapter 2. Materials and Methods 6

2.1 Study site and experimental design 6

2.2 Soil sampling 8

2.3 DNA extraction 9

2.4 Sequencing library preparation and Illumina MiSeq sequencing 10

2.5 Metabarcoding data processing and analysis 11

2.6 Soybean seedling vitality experiment 13

2.7 Statistical analysis 14

Chapter 3. Results 17

3.1 Soil-borne oomycete community diversity and compositional structure 17

3.2 Crop yield and soybean seedling emergence 27

Chapter 4. Discussion 29

Chapter 5. Conclusions and future work 37

References 40

Appendices 45

Appendix 1: Experimental design 45

Appendix 2: Soil moisture content 46

Appendix 3: Reference database construction and taxonomic validation 47

Appendix 4: Cumulative precipitation 49

Appendix 5: Seedling vitality score 50

List of Figures and Tables

Table 1. The P values of the analysis of variance for the effects of tillage and rotation on the alpha-diversity indices. 18

Figure 1. The alpha-diversity indices of the oomycetes communities as affected by tillage (A, B) or the interaction of tillage and rotation. 19

Figure 2. The soil oomycetes community structure as affected by tillage (A) and rotation under CT (B) or under NT (C) as determined by NMDS and PERMANOVA. 19

Figure 3. The abundance of oomycetes genera and species, and how they were affected by tillage and rotation. 22

Figure 4. The impact of tillage practices on oomycetes community diversity and species abundance over the three-year study period (2016-2018). 23

Table 2. Soil oomycete species relative abundance as affected by tillage and rotation. 24-26

Table 3. The effects of tillage and rotation on corn, soybean, and wheat yields (kg ha^{-1}) in 2016-2018. 28

Figure 5. The soybean seedling vitality score (SVS) as affected by tillage and rotation. 28

Table 4. Soil nutrient concentration and pH, of soils sampled in 2018. 38

Author Contributions

A manuscript was prepared from this work that was submitted to *Plants*: "Gahagan, A.C., Shi, Y., Radford, D., Morrison, M., Gregorich, E., Aris-Brosou, S., and Chen, W. (2023). Long-term tillage and crop rotation regimes reshape soil-borne oomycete communities in soybean, corn, and wheat production systems. *Plants (*under review, preprint is available at: https://doi.org/10.20944/preprints202305.0129.v1). Author contributions are as follows. Conceptualization, C.G., M.M, W.C.; methodology, C.G. M.M., E.G.; Software, W.C., D.R; formal analysis, C.G. and Y.S; data curation, W.C, D.R; writing—original draft preparation, C.G, Y.S.; writing—review and editing, W.C., S.A-B., M.M., E.G.; visualization, C.G., Y.S.; supervision, W.C., S.A-B., M.M.; project administration, W.C.; funding acquisition, W.C..

Chapter 1. Introduction

The oomycetes (or Oomycota) contains a group of fungal-like microorganisms within the kingdom *Stramenopila*, of which 60% of the species are considered pathogenic biotrophs, hemibiotrophs, or necrotrophs (Thines, Voglmayr, & Göker, 2009), and of great concern in agriculture (Fry, 2008), aquaculture (Phillips, Anderson, Robertson, Secombes, & van West, 2008), forestry, and natural ecosystems (Cahill, Rookes, Wilson, Gibson, & McDougall, 2008). Depending on their host range, plant pathogens in the oomycetes are considered generalist or specialist species, and such information often determines the control measures for pathogens in their respective categories. Information on pathogenicity is compiled in resources such as the USDA fungal database (https://nt.ars-grin.gov/fungaldatabases/). For example, *Globisporangium ultimum* is a pathogen with a wide host range that is associated with seedling damping off disease complexes. As a generalist pathogen, *G. ultimum* is managed in plant production systems through fungicidal seed treatments specific to oomycetes (Cohen & Coffey, 1986). *Globisporangium,* which recently underwent taxonomic revision and has been reclassified from within *Pythium* to its own genus, inherited some of the most problematic plant pathogens formerly belonging to *Pythium* (*Py.*, Rossmann et al., 2021), such as the former *Pythium. sylvaticum,* and *Py. ultimum* (Uzuhashi, Kakishima, & Tojo, 2010), which are now known as *G. sylvaticum* and *G. ultimum*. Other oomycete pathogens are more limited in their host range, such as *Phytopthora (Ph.) sojae*, known only to be pathogenic on soybean (*Glycine max*) and lupins (*Lupinus*). Alongside chemical control measures*, Ph. sojae* in soybean is managed through single gene resistance pathways, or

combinations of these genes to increase pathotype resistance (Dorrance & Grünwald, 2009). The diversity of pathogen-host interactions and host specificity among soil-borne plant pathogenic oomycetes makes them a difficult group to manage. Moreover, soil-borne oomycetes are susceptible to changes in soil texture and organic matter, with a preference for cool, moist soils (Cook, Sitton, & Haglund, 1987; Cook & Haglund, 1991). Studies have shown that agronomic practices, like tillage and crop rotation, affect soil physicochemical properties and reshape the soil-borne microbiome structure (including the oomycetes community), and consequently can be disruptive to soil health and fertility. Understanding how soil-borne oomycetes communities respond to these common agronomic practices, would help clarify best-practices for regions with a high incidence of oomycetes plant disease and provide the fundamental basis for establishing effective pest management and mitigation strategies for these important phytopathogens.

The use of tillage and crop rotation in managing soil-borne oomycetes may reduce the pathogen inocula or improve the soil's natural capacity to suppress pathogenicity (Bongiorno et al., 2019; Peters, Sturz, Carter, & Sanderson, 2003; Yuan et al., 2021). Conventional tillage (CT) usually involves fall moldboard ploughing and spring cultivation, and is practiced to reduce weed establishment (Santín-Montanyá & Sombrero Sacristán, 2020) and soil compaction (Bogunovic, Pereira, Kisic, Sajko, & Sraka, 2018). Practicing CT can lead to the loss of soil tilth, increased nutrient runoff, reduced soil quality, and disruption of the soil microbiome (Abdollahi, Schjønning, Elmholt, & Munkholm, 2014). By contrast, no-till (NT) promoted beneficial fungal and bacterial taxa, compared to CT, with soils that were enriched

with plant pathogens, as reported by Srour et al (2020). The diversity of bacterial taxa was lower in topsoil, and higher in the deeper soil layers under CT, when compared to NT (Li et al., 2020). A greater microbial species diversity in soils under NT may lead to a more complex inter-species network which may reinforce the suppression effects of beneficial microorganisms against the proliferation and growth of pathogenic species (Sipilä Timo, Yrjälä, Alakukku, & Palojärvi, 2012). CT-mediated soil surface drying has been suggested as a management strategy since successful infection of the host by many oomycete species is dependent on zoospore mobility, supported by high soil water content (Liu, Gumpertz, Hu, & Ristaino, 2008).

Crop rotation, a common agricultural practice, involves planting alternative crops sequentially on the same farmland for improved soil fertility and control of weeds, pests, and diseases. It has traditionally been encouraged to manage plant pathogens by mediating the availability of host plants from year to year (Pedersen, 1992; Peralta, Sun, McDaniel, & Lennon, 2018; Peters et al., 2003). Plant disease incidence and associated yields are impacted by the selection of crops in a cropping system and how they are rotated, with monoculture having reduced yields when compared to rotations involving more than one crop (Larkin & Halloran, 2014; Lyu et al., 2020). Crop rotation enriched plant growth promoting bacterial (Li et al., 2022), and disease suppressive functional groups, such as those carrying the *prnD* gene that encodes the antifungal compound pyrrolnitrin (Peralta et al., 2018). Monoculture tomato soils were enriched in fungal genera containing potential pathogens, such as

Pseudogymnoascus, Fusarium, and *Pyrenochaeta*, compared to soils under crop rotation (Lyu et al., 2020).

Hwang et al. (2009) found that the levels of *Pythium* (*Py*.) inocula in monoculture soils, particularly pea, were greater than in rotation soils, which was reflected in disease incidence. *Pythium* and *Phytophthora* were the dominant genera recovered in a soybean-corn cropping system (Radmer et al., 2017; Rojas et al., 2017a; Zitnick-Anderson & Nelson, 2015). Oomycete pathogenicity on soybean, in particular, has been extensively studied and over 15 species of oomycetes, such as *Py. aphanidenmatum, G. ultimum, G. irregulare, G. cryptoirregulare*, have been shown to be pathogenic on soybean; although, direct inoculation of soils with these recovered oomycete pathogens does not always reflect the severity of disease symptoms observed in soybean (Radmer et al., 2017). The relationship between oomycete plant pathogens and soybean make soybean an ideal initial crop to study disease symptoms in relation to oomycete species diversity and distribution in soils of varying rotation and tillage backgrounds. The drive for maximizing yields of high-value crops can often compete with the benefits of diversifying crop rotation systems. A thorough understanding of the potential disease ramifications due to rotation selection, especially within the oomycetes where little is known about the influence of crop rotation on community structure, may help to strengthen guidelines for more productive rotations.

This study aimed to explore the shifts in the soil-borne oomycete community in response to different combinations of tillage and crop rotation practices. More specifically, the post-harvest soil oomycete communities were characterized over three consecutive years

(2016-2018) at a long-term experimental site by metabarcoding the Internal Transcribed Spacer 1 (ITS1) region. The experimental field was established using a split-plot design (two tillage levels, NT vs. CT), with four rotations arranged randomly within each tillage treatment (Supplementary Fig. S1). The rotations included monocultures of dicot soybean (*Glycine max*, SSS), monocot corn (*Zea mays*, CCC), and monocot wheat (*Triticum aestivum,* WWW), as well as corn-soybean-wheat (CSW) rotation which change the available host range for oomycetes (Rojas et al., 2017b). We hypothesized that prolonged tillage and rotation practices can 1) exert a significant influence on the diversity and abundance of microbial communities, which may lead to a consistent increase or decrease trend over the course of the three-year study period, and 2) affect crop and soil health as represented by crop yield and seedling vitality of soybean. This study aimed to provide guidelines for better agricultural management practices in managing soil-borne oomycetes.

Chapter 2. Materials and Methods

2.1 Study site and experimental design

The field site sampled was a tillage and crop rotation trial established in 1990 at the Central Experimental Farm in Ottawa, ON, Canada (45°23’13.6”N; 75°43’15.6” W). The soil was a Matilda sandy loam (Melanic Brunisol, Canadian classification) with a pH (in $CaCl_2$) of 6.8 (Morrison et al., 2017). A two-replicate, split-plot tillage–rotation experiment (see Appendix 1 for a graphical representation) was established on land that had previously grown alfalfa for 1 year and corn for several years prior to that. The main plots (89.1 m × 45.7 m each) were either managed by no-tillage (NT) or conventional tillage (CT), within which the subplots for crop rotations were 9.1 m × 45.7 m. The plots under CT were subjected to moldboard ploughing (Overum DTL 5 Furrow plow, Västervik, Sweden) in early November and cultivated in the spring with a mulch-finisher (John Deere 2310 Mulch Finisher) followed by a combination harrow (Kongskilde 2600 Triple K, Albertslund, Denmark) with rotatory baskets. Subplots were allocated to three crops (corn, soybean, or wheat) grown in monoculture (CCC, SSS, CCC) or in two 3-year rotations (corn–soybean–wheat [CSW] or corn–wheat–soybean [CWS]). The rotations were replicated so that each crop was present in the rotation every year, leading to a total of nine subplot treatments (3 monoculture, 3 CSW and 3 CWS) per replication (n = 3 for CSW, n = 3 for CWS, and n = 3 for monoculture) within each main plot. Within each main plot (tillage effect), the subplots (crop × rotation) were duplicated in complete blocks. Therefore, a total of 72 subplots were established,

including randomized and duplicated subplots representing nine rotation sequences ($n = 2 \times 9$) within each of the two duplicated main plots ($n = 2 \times 2$; Appendix 1).

Crop planting and management information were described in Morrison et al. (2017). Wheat was planted in the first two weeks of May (450 seeds m^{-2}) using a Sunflower 9312 Multifunction Drill (Beloit, KS) equipped with disc openers with a row spacing of 19 cm. Corn was seeded (7 seeds m^{-2}) in the first two weeks of May with a John Deere 6-row corn planter set to the NT option; rows were 76 cm wide. Soybean was planted (55 seeds m^{-2}) in the last two weeks of May with the Sunflower drill with 19 cm wide rows. Corn was fertilized with 224 kg N (as urea) ha^{-1} pre-plant broadcast and 40 kg ha^{-1} N-P_2O_5–K_2O (18-18-18) at seeding. Wheat plots were fertilized with 100 kg N (as urea) ha^{-1} pre-planting. Soybean received no fertilizer, as per recommendations based on soil nutrient levels and the Ontario Ministry of Agriculture, Food and Rural Affairs guidelines. Pre-plant fertilizer was applied prior to spring tillage, therefore, it was integrated into the soil layer in CT plots but remained on the surface in the NT plots. Glyphosate was used to control weeds in commercial herbicide-resistant varieties of corn and soybean. Weeds were controlled in wheat with Buctril-M at 0.2 L ha^{-1} at the seedling stage.

Crop yields for soybean and wheat were measured by harvesting a central strip of each plot (6 rows) using a plot combine (NurseryMaster, Wintersteiger, Germany). Corn yields were harvested using a John Deere combine (X9, John Deere, USA). Grain yields were adjusted to a moisture content of 13%. Corn crop residues were chopped down with a Loftness 180 flail-style chopper (Loftness, Hector MN), while soybean and wheat stubble

were left in place. All crop residue was fall incorporated in the CT plots but remained on the soil surface in the NT plots. A weather station 700 meters from the study site [45°22'57.34" N, 75°42'50.96" W] was used to collect precipitation data for the 2016, 2017, and 2018 growing seasons.

2.2 Soil sampling

In this study, only the corn-soybean-wheat rotation and monoculture plots were selected to investigate the rotation effect. Soil sampling was conducted at the end of the 2016 to 2018 growing seasons after the crop was harvested. Sampling in 2016 was considered as a proof of concept, for which only soils from the soybean and corn monoculture plots and CSW plots in soybean that year were sampled (24 plots). For the subsequent sampling years, additional rotation regimes were included: in 2017 the sampling was expanded to include the CSW rotation plots in soybean and wheat as well as the corn (CCC) and soybean (SSS) monoculture treatments (32 plots); in 2018 the wheat monoculture (WWW) was added to the sampled plots (46 plots). A total of 102 (24 [2016] + 32 [2017] + 46 [2018]) samples were collected over the three years. Due to the unbalanced sampling strategy across the years, the treatments included two tillage treatments (CT and NT) and four rotations (CCC, SSS, WWW, and CSW).

Soil sampling followed a random staggered strategy. To target the soil layer most impacted by tillage treatments, soil samples with a depth of 0-30 cm were taken using soil cores (diameter = 2 cm) in each plot using a soil core sampler (Lamotte, Chesterton, MD, USA). In 2016 and 2017, 50 cores were collected per plot which were mixed as one

composite sample, so that the extra soils could be used in the seedling vitality trials carried out in the growth cabinet (Chapter 2.6). In 2018, 15 cores were collected per plot which were homogenized as one composite sample. Before sampling in another plot, all equipment was rinsed with distilled water, then sterilized with 90% ethanol, and dried. The soil cores were pooled by plot and were transferred to a cold room (4 °C), until sampling was completed. Prior to sub-sampling the soil samples were thoroughly mixed and sieved through a 5 mm mesh to remove rocks, plant material, and insects. A 15 ml sub-sample of the homogenized soil per plot was stored at -80 °C.

Gravimetric soil moisture was measured by taking a 30 mL sample of soil at sampling for each plot, weighing it wet, and drying it at 60˚ C until the weight was stable, approximately 7 days in this case. Dried soils were weighed, and soil moisture was calculated as shown in equation 1.

$$Soil\ moisture\ content = \frac{wet\ weight\ - dry\ wieght}{wet\ weight} \quad [1]$$

2.3 DNA extraction

The DNAs were extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions with the following modifications: a 2 mL microcentrifuge tube was used during the binding step rather than a 15 mL falcon tube, and a Percellys® Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) was used instead of the MP Fast-Prep homogenizer. The soil was homogenized with silica and glass beads, then a column binding step was used to remove debris where the DNA

was bound to beads and column elution was used to remove the protein and RNA. The purified DNA was then eluted into DNAse-free water. All samples were extracted in triplicate. The extracted DNA was stored at -25˚ C.

The concentration of the DNA extract was measured using the Qubit dsDNA HS (High Sensitivity) Assay Kit on a Qubit Fluorometer (Thermo Fisher Scientific, MA, USA). The DNA extraction replicates were pooled at a concentration of 10 ng μL^{-1} in a volume of 40 µL in 96-well PCR plates, which were stored at -25 °C. The plates containing the genomic DNAs were shipped overnight on dry ice to the Génome Québec Innovation Centre (Montreal, QC, Canada) for the preparation of sequencing libraries and amplicon-based metagenomics (or metabarcoding) sequencing using the Illumina MiSeq platform target fragment length was 100-300 bp with a target output of 15 Gb.

2.4 Sequencing library preparation and Illumina MiSeq sequencing

At the Génome Québec Innovation Centre, the DNA libraries of the ITS1 region were prepared using SSU_ITS (5'-ACA CTG ACG ACA TGG TTC TAC ACG GAA GGA TCA TTA CCA CAC-3') forward primer and the OOM_LO5.8S47c (5'-TAC GGT AGC AGA GAC TTG GTC TAT TAC GTA TCG CAG TTC GCA-3') reverse primer (A. Levesque, personal communication). The initial PCR amplification was carried out in 8 µL reaction volume comprised of 7 µL of the master mix and 1 µL of sample DNA diluted to 1/50. The following thermocycling parameters were 15 minutes at 96 °C for initial melting then 33 cycles through of 30 seconds at 96 °C, 30 seconds at 52 °C and 60 seconds at 72 °C, followed by a 10-minute cool down at 72 °C. The amplicons were verified on a 2% agarose gel

quantified and were purified using the sparQ PurMag Beads (Quantabio, Beverly, MA, USA). A secondary PCR was then done to add the dual indexed barcoding adapters, for demultiplexing the sequencing data for each sample. The PCR was carried out in 7 µL reaction volume containing a 1/50 dilution of DNA to master mix. The PCR cycling parameters were: 15 minutes at 96 °C, 30 seconds at 96 °C, 30 seconds at 52 °C, 60 seconds at 72 °C and 10 minutes at 72 °C. The amplification was verified, and amplicons were purified as above. Indexation was done with 1 µL of undiluted amplicon product secondary PCR. Indexed samples were verified on a 2% agarose gel and quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, CA, USA). The sequencing library was made with an equal quantity in ng of DNA for each sample. Library DNA was cleaned with sparQ PureMag Beads. The library was quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal Kit (Kapa Biosystems, MA, USA), and average fragment size was determined using the LabChip GX (PerkinElmer, MA, USA). Before sequencing 10% of Phix control library was added to the amplicon pool for a final concentration of 8 pM, this served as a control for sequencing quality and calibration. Sequencing was performed on the Illumina MiSeq platform with the MiSeq Reagent Kit v3 with 600 cycles. Sequencing was done with LNA™ modified custom primers.

2.5 Metabarcoding data processing and analysis

The Illumina MiSeq sequencing adapters were removed from the fastq files using Cutadapt ver.4.1 (M. Martin, 2011). The paired-end raw reads were processed using DADA2 ver.1.14 (Callahan et al., 2016) implemented in QIIME2 for denoising, chimera detection, and the

amplicon sequence variants (ASVs) inference using default parameters. The raw forward and reverse reads were truncated at 200 nt based on Phred scores.

The taxonomic assignment was performed with a reference database of oomycetes ITS1 that was compiled in-house (denoted as oomycetes-ITS1-refDB) from GenBank. The oomycetes-ITS1-refDB was first downloaded in TinySeq XML format using query "Oomycetes[Organism]" AND "150:2500[slen]" AND ("internal transcribed spacer 1" OR "ITS1") NOT "sp." NOT "uncultured" NOT "clone" NOT "whole genome" NOT" metagenome" (retrieved on March 2, 2022). In-house Perl and Bash scripts were developed to parse the XML file and to retrieve National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) taxonomy for each sequence. The locations of rRNA gene regions (ITS1, 5.8S, ITS2, 28S) within each sequence were annotated by ITSx (Bengtsson-Palme et al., 2013). Only sequences including ITS1 region with sequence length between 150–500 bp were retained. The final oomycetes-ITS1-refDB database contained 26,220 oomycete ITS1 reference sequences. This database and related scripts can be downloaded from the bitbucket repository: https://bitbucket.org/wenchen_aafc/metabarcoding_oomycetes/downloads/.

The taxonomy assignments were initially classified via the q2-feature-classifer (Bokulich et al., 2018) implemented in QIIME2 against the oomycetes-ITS1-refDB database. Species-level identification of the oomycetes was improved by the Automated Oligonucleotide Design Pipeline (AODP), which identified all mutations distinguishing highly conserved DNA markers between close relatives (Chen, Radford, & Hambleton, 2021;

Zahariev & Chen, 2018). The final taxonomy was improved by comparing and validating the results of three classifiers: AODP, q2-feature-classifier, and BLASTn at each taxonomic rank, in particular, at species level. To assess the accuracy of the species-level assignments, the representative sequences of each ASV assigned to a specific species were extracted, which were combined with corresponding reference sequences of the species and its close relatives in oomycetes-ITS1-refDB. The combined sequence dataset was then aligned using MAFFT vers.7.407 (Katoh & Standley, 2013), followed by the reconstruction of an approximate maximum likelihood (ML) tree using FastTree (ver.2.1.0) with the –nt and –gtr options (Price, Dehal, & Arkin, 2010). The ML tree was visualized in FigTree (ver.1.4.4, https://github.com/rambaut/figtree/releases).

2.6 Soybean seedling vitality experiment

To evaluate the impact of tillage and rotation regimes on the potential pathogenicity of soil-borne microorganisms, including oomycetes, on soybean seedling emergence, seedling emergence tests were done under controlled growth cabinet conditions in 2016 and 2017. In this trial, 5 kg of soil were collected from selected subplots under different tillage × rotation treatments following the same procedure as the soil samples that were submitted for DNA extraction and sequencing. A total of 24 soil samples in 2016 and 32 in 2017. The soils from each subplot were placed in a plastic tray (9×12×35 cm). Thirty-two soybean seeds (variety: AC Mandor) were planted in a four column 8 row grid, 2 cm deep. The growth cabinet was set at 15 °C with a 10-hour photoperiod. Field capacity was calculated by filling a 2-inch plastic pot with field soil, saturating the soil, and weighing after 16 hours of draining. Soil

moisture was maintained by weighing the trays and adjusting the water content to 80% of field capacity twice a day with distilled water. A final seedling vitality score was used to asses plant health at harvest: a score of "1" was given to seeds that did not germinate; "2" to seeds that germinated but had broken off cotyledons and signs of rot on the stem and roots; "3" to seedlings that had emerged but had signs of necrosis on the cotyledons and delayed unifoliate emergence; "4" to seedlings with signs of necrosis on the stems and cotyledons as well as stunted and damaged unifoliates; "5" to seedlings with healthy unifoliates but signs of necrosis on the stems and cotyledons; and "6" to healthy seedlings (Appendix 5). To minimize edge effects, the seedlings of the end rows proximity to the edge of the plug tray were not evaluated. The overall seedling vitality score (SVS) for each subplot was calculated using equation 2, where i is the vitality score ranging from 1 to 6, x_i is the number of seedlings with a given vitality score, and n is the total number of seedlings.

$$\mathrm{SVS} = \left(\sum_{i=1}^{6} x_i \times i\right)/n \qquad [2]$$

2.7 Statistical analysis

All statistical analyses were performed in R (ver. 4.2.0) (Team, 2021). To avoid the risk of losing rare taxa (ASVs with low sequence counts), the ASV table was not rarefied but was Hellinger-transformed for multivariate analysis. The alpha-diversity indices are quantitative measures representing the diversity of ASVs in a sample. The Shannon Index (H), Simpson Index (D), and Chao1 index were calculated using vegan (Oksanen et al., 2022) and biodiversityR (Kindt, 2020) packages. The Shannon-based True Diversity (Shannon-TD =

exp(H)) and the Simpson-based True Diversity (Simpson-TD = 1/D) were calculated as suggested by Jost (2006).

The alpha-diversity indices, crop yield, and SVS were checked for normality using the *shapiro.test* function and were transformed as needed. Linear mixed models were used to assess the main effects of tillage and rotation, and their interaction on alpha-diversity indices, the relative abundances of species, and the SVS using the *lme* function from the nlme package (Pinheiro & Bates, 2023) at a significance level of $P \leq 0.05$. Tillage and rotation were treated as fixed effects, and year as random effect. Linear mixed models were also used to assess the effect of rotation under each tillage treatment on the relative abundances of taxa at species level, with rotation as a fixed effect, and blocks and years as random effects. Linear mixed models were used to evaluate the tillage, rotation, and their interaction on corn, soybean and wheat crop yields separately, with tillage and rotation as fixed effects, and blocks as random effect, and year as repeated measurements.

The non-metric multidimensional scaling (NMDS) was done to evaluate the overall impact of tillage and crop rotation on oomycete community heterogeneity using the *metaMDS* function in vegan (Oksanen et al., 2022). The *adonis* function from the vegan package was used to perform permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2014) for determining the main effects of tillage, rotation, and the interaction effects, and also the effect of current crop under CSW treatment on the community heterogeneity of oomycete community based on the Bray-Curtis dissimilarity. Pairwise comparisons between treatments (tillage, rotation, or their combination) were

conducted by using the *pairwise.perm.manova* function from the RVAideMemoire package (Hervé, 2022) when one factor or interaction effect is significant. The community dissimilarity over the study years under CT and NT was evaluated by using the *beta.sample* function in the betapart package (Baselga & Orme, 2012).

Chapter 3. Results

A total of 3,148,276 high-quality reads were retained in the final amplicon sequencing variants (ASV) abundance table, with 30,272 ± 5,785 (MEAN ± SD) reads per sample. In total, 292 ASVs (mean ± SD = 9 ± 3 per sample) were recovered from all of the samples.

3.1 Soil-borne oomycete community diversity and compositional structure

The alpha-diversity indices of the soil oomycete community were significantly affected by tillage practices, with higher Shannon-based True Diversity Index (Shannon-TD; $P = 0.007$) and Simpson-based True Diversity Index (Simpson-TD; $P = 0.014$) under CT than NT (Table 1, Fig. 1). Additionally, Chao1 index, an index that is more sensitive to rarer ASV, was significantly ($P = 0.001$) affected by the interaction of tillage and rotation, with higher values under CT than NT under CSW, but not under monoculture. The CSW increased Chao1 richness in comparison with CCC monoculture, but only under CT not NT. Differences in soil moisture content between the tillage and rotation treatments were not significant (Supplementary Fig. S2A). Correlation analysis also showed no significant relationship between the soil moisture content and alpha-diversity indices ($P > 0.05$, Appendix 2 Fig. S2B-D), or between the soil moisture content and the recovered oomycetes species ($P > 0.05$).

The permutational multivariate analysis of variance (PERMANOVA) results showed that soil oomycete community structure was significantly affected by tillage ($F = 4.83$, $P = 0.001$; Fig. 2B) and the interaction of tillage and rotation ($F = 1.82$, $P = 0.01$). The rotation significantly affected the oomycete community structure under CT ($F = 2.01$, $P = 0.003$, Fig

2B), but not under NT (F = 1.20, P = 0.175; Fig. 2B, C). Pairwise comparison and non-metric multidimensional scaling (NMDS) results showed that the soil oomycete community structure under CT differed significantly among the three monoculture treatments (F = 2.09, P < 0.001; Fig. 2B). However, such differences were not observed between the crops within the CSW rotation (F = 0.98, P = 0.468). PERMANOVA analysis indicated insignificant impact of soil moisture content on the beta-diversity of the soil-borne oomycetes community (P > 0.05).

Table 1. The P values of the analysis of variance for the effects of tillage and rotation on the alpha-diversity indices.

	DF[1]	Simpson-TD[2]	Shannon-TD[3]	Chao1
Tillage (T)	1	0.014	0.007	0.160
Rotation (R)	3	0.613	0.563	0.4292
T×R	3	0.217	0.041	<0.001

[1] Degrees of Freedom
[2] Simpson-based True Diversity Index
[3] Shannon-based True Diversity Index

The oomycete ITS1 ASVs were assigned to six families, seven genera, and 34 species. The predominant genera were *Globisporangium* (85.1%, 203 ASV), *Pythium* (10.4%, 51 ASV), and *Wilsoniana* (1.3%, 7 ASV). All other oomycete genera were represented by < 1% ASVs. Of the species identified, the most abundant were generalist species (Table 2) of *Globisporangium*, including *G. attrantheridium* (47.0%), *G. heterothallicum* (7.88%), *G. sylvaticum* (7.78%), *G. apiculatum* (5.92%), and *G. ultimum* (4.36%; Fig. 3A). Phylogenetic reconstruction using the representative sequences of the ASVs confirmed the accuracy of the species-level classifications (Appendix 3). Of all identified oomycetes species, 22 were

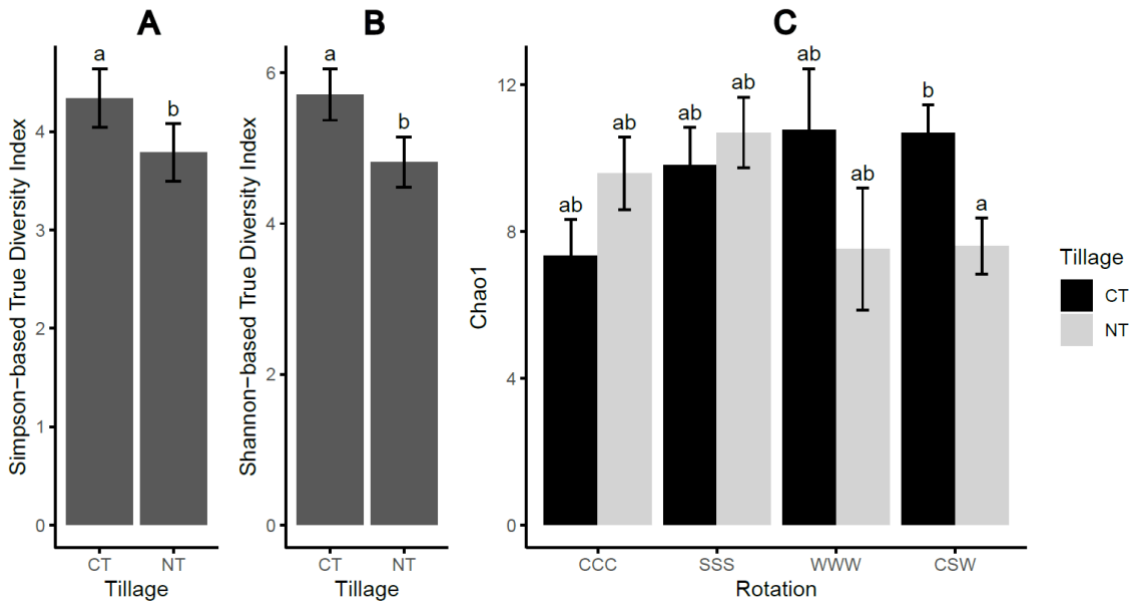


Figure 1. The alpha-diversity indices of the oomycetes communities as affected by tillage (A, B) or the interaction of tillage and rotation (C). Chao1, Chao1 richness index; CT, conventional tillage, NT, no-till; CCC, monoculture of corn, SSS, monoculture of soybean, WWW, monoculture of wheat, CSW, rotation of corn-soybean-wheat. Different letters across the treatments represent significant difference at $\alpha < 0.05$ based on Tukey's HSD test. Error bars represent one standard error.

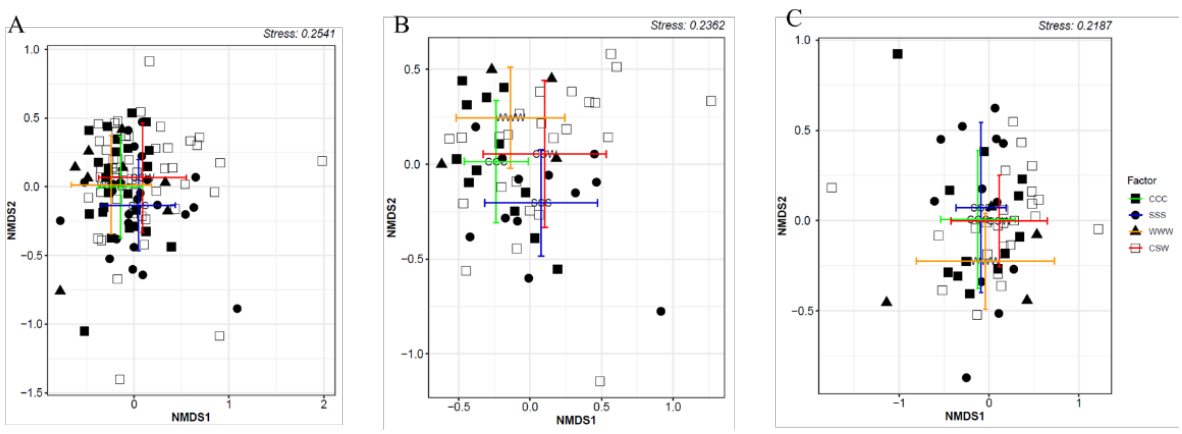


Figure 2. The soil oomycetes community structure as affected by tillage (A) and rotation under CT (B) or under NT (C) as determined by NMDS and PERMANOVA. CT, conventional tillage; NT, no-till; CCC, monoculture of corn; SSS, monoculture of soybean; WWW, monoculture of wheat; CSW, rotation of corn-soybean-wheat. The central dots represent the means of the points on the two NMDS axes for respective groups, the bars represent one standard deviation from the mean along both axes.

observed both under CT and under NT, six were only observed under CT, and six were only observed under NT (Table 2, Fig. 3B). Tillage had a significant *(P* < 0.05) effect on eight species (Table 2), with higher abundances of *G. macrosporum, G. sylvaticum, Py. arrhenomanes*, and *Wilsoniana portulacae* under NT than under CT, and higher abundances of *G. iwayamae, G. ultimum, G. apiculatum*, and *Py. volutum* under CT than under NT (Fig. 3B). Additionally, rotation had a significant effect on seven species under CT, with enriched abundances in *G. ultimum* under soybean monoculture, as well as *G. iwayamae, Pythium* sp. aff. monospermum*, Py. volutum*, and *Saprolegnia anisospore* under wheat monoculture (Table 2, Fig. 3C). *Pythium* sp. aff. monospermum had low relative abundance under CSW but a high abundance of this species (an average of 10.6%) under wheat monoculture (Table 2). *G. iwayamae* showed the same trend and was recovered in low abundance under CSW (with relative abundance of 0.2%), and in high abundance under wheat monoculture (with relative abundance of and 2.0%). By contrast, *S. anisospore* was abundant with 2.0% under wheat monoculture, but was not recovered under CSW (Table 2). Only *Py. volutum* was significantly affected by rotation under NT (Table 2).

Since tillage practices showed a stronger impact on the oomycetes community assemblage than the rotation patterns did, it was predicted that the implementation of long-term tillage regimes would lead to a consistent microbial abundance and diversity throughout the duration of the three-year study. Indeed, the Simpson-TD showed an insignificant but persistent decreasing trend over the three years under NT but not under CT (Fig. 4A), suggesting the potential of NT in reducing the number of dominant oomycetes species. The

relative abundance of *G. apiculatum, Py. volutum*, and *G. iwayamae* increased persistently under CT but not under NT, although such changes were not statistically significant (Fig. 4B-D). Under CT, the community overall dissimilarity (Sørensen dissimilarity, SOR) and the turnover component of the Sørensen dissimilarity (Simpson dissimilarity, SIM) increased over the three growing seasons, while the nestedness component of the Sørensen dissimilarity (SNE) showed an opposite trend ($P \leq 0.05$; Fig. 4E, G, I). Such a trend, however, was not observed under NT. The yearly difference in SIM and SNE components, especially between 2016 and the other two years, was more than 2-fold under CT compared to under NT (Fig. 4F, H, J). These findings suggest that continuous CT practices may lead to a decreased homogeneity of the soil oomycetes community.

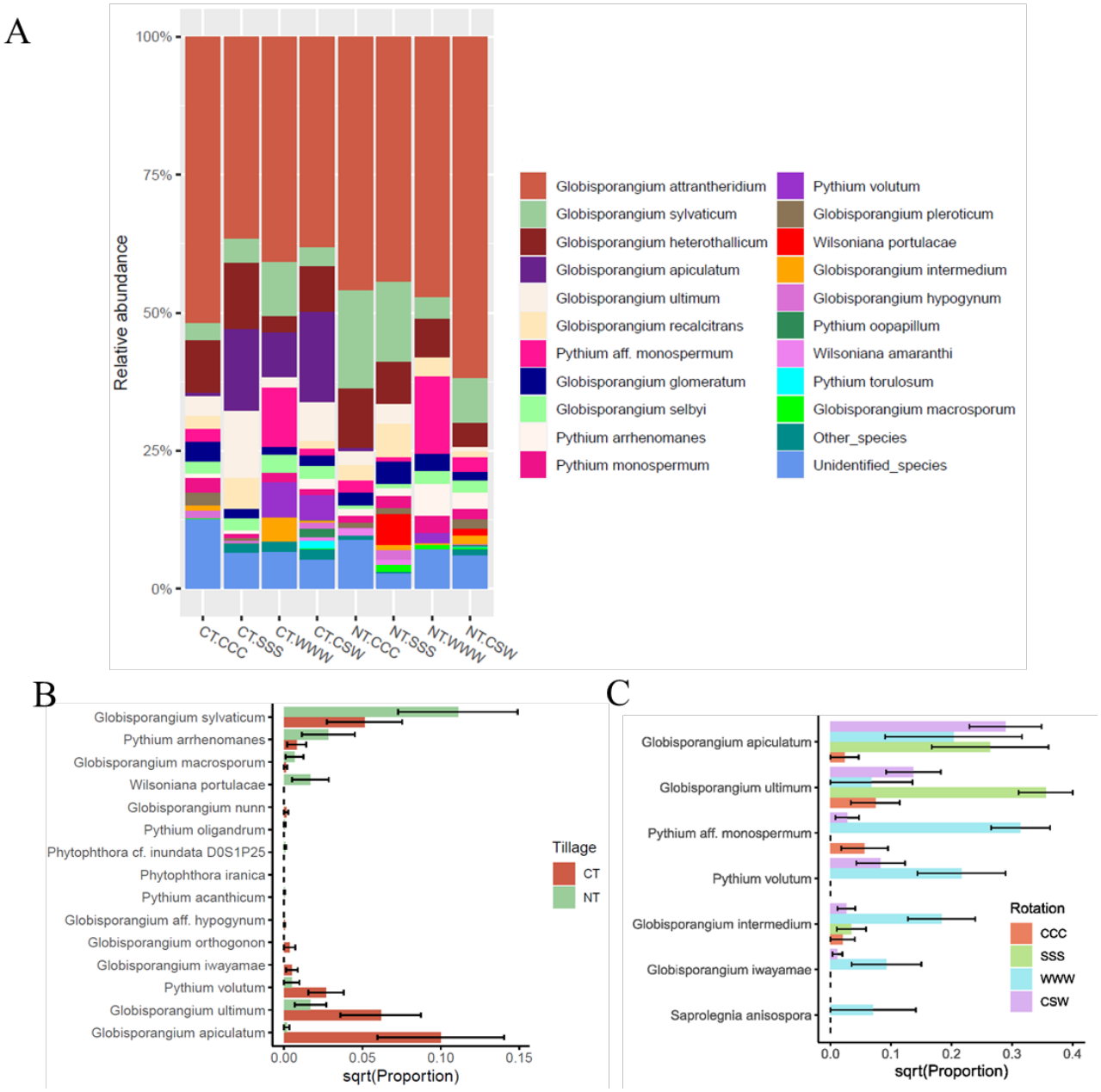


Figure 3. The abundance of oomycetes species. (A) The 20 most abundant species as affected by tillage and rotation; Other_species, the identified species which were not in the 20 most abundant; unidentified species, sequences that were not assigned to the species level. (B) The species that were significantly affected by tillage ($P < 0.05$, linear mixed effect model) and the species that were unique under CT or NT. (C) Species that were significantly affected by rotation under CT ($P < 0.05$). Error bars represent standard error. CT, conventional tillage, NT, no-till; CCC, monoculture of corn, SSS, monoculture of soybean, WWW, monoculture of wheat, CSW, rotation of corn-soybean-wheat.

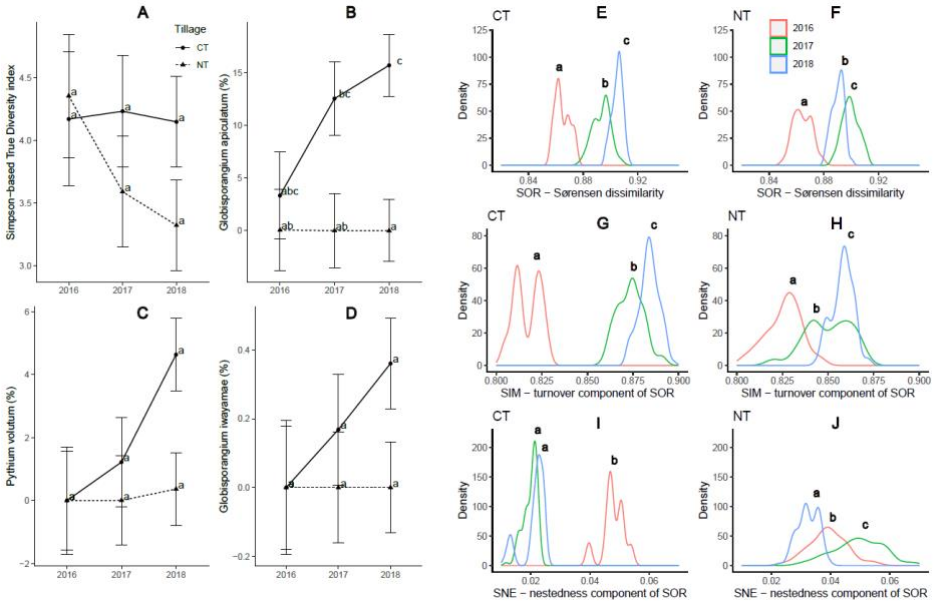


Figure 4. The impact of tillage practices on oomycetes community diversity and species relative abundance over the three-year study period (2016-2018). A) Simpson-based True Diversity Index decreased under NT but not under CT; the relative abundance of B) *Globisporangium apiculatum*, C) *Pythium volutum*, and D) *G. iwayamae* increased under CT but not under NT. E-J) The impact of tillage practices on the beta-diversity of oomycetes community: E-F) the overall community dissimilarity (Sørensen dissimilarity, SOR) and G-H) the turnover component of the Sørensen dissimilarity (Simpson dissimilarity, SIM) increased over the three growing season; I-J) the nestedness component of the Sørensen dissimilarity (SNE) decreased over the three years under CT but not under NT.

Table 2. Soil oomycete species relative abundance (%) as affected by tillage (T), either conventional tillage (CT) or no tillage (NT) and rotation (R) (including soybean monoculture (SSS), corn monoculture (CCC), wheat monoculture (WWW) and corn-soybean-wheat rotation (CSW), the associated P-values from the analysis of variance, and potential hosts and disease symptoms. Species below 0.001% relative abundance not included.

Species [a]	No. ASV	Relative abundance (%)								Analysis of variance (P value) [b]					Disease Note [c]	Known Hosts [c,d]
		CT				NT										
		CCC	SSS	WWW	CSW	CCC	SSS	WWW	CSW	T	R	T*R	R CT	R NT		
Globisporangium aff. hypogynum	1	0.000	0.000	0.000	0.153	0.000	0.000	0.000	0.000	0.320	0.78	0.772	0.782	N/A		
Globisporangium apiculatum	20	0.664	16.294	7.970	16.522	0.000	0.000	0.000	0.002	<0.001	0.036	0.031	0.041	0.761		grape
Globisporangium attrantheridium	49	57.290	33.493	40.079	41.743	38.209	46.590	47.258	59.105	0.230	0.448	0.021	0.066	0.138	Cavity spot lesions	*Daucus carota*, *Prunus*, **soybean**
Globisporangium glomeratum	11	2.273	2.341	1.381	2.454	1.770	2.658	3.176	2.208	0.96	0.833	0.936	0.988	0.734		**soybean**
Globisporangium heterothallicum	27	10.438	11.844	2.942	6.979	13.878	7.603	7.139	3.802	0.539	0.039	0.558	N/A	0.076	Damping off	Pepper**, soybean, corn,** spinach, **wheat**, lentils
Globisporangium hypogynum	3	0.000	0.930	0.000	0.000	1.115	2.277	0.000	0.728	0.055	0.075	0.902	0.061	0.381	Root rots	**soybean**
Globisporangium intermedium	3	1.000	0.000	4.290	0.388	0.000	0.995	0.281	1.700	0.755	0.074	0.001	0.004	0.407	Damping-off, rots	abutilon, antirrhinum, arabis, beet, begonia, carrot, cauliflower, chamaecyparis, cherry laurel, chrysanthemum, cotoneaster, cucumber, erica, ferns, godetia, hazel, hop, hyacinth, lettuce, leyland cypress, lupin, nasturtium, pea, pear, pelargonium, pepper, saintpaulia, strawberry, fragaria vesca, tomato, violet, yew, **soybean, corn**
Globisporangium irregulare	2	0.000	0.000	0.000	0.247	0.074	0.000	0.000	0.000	0.559	0.867	0.563	0.782	0.347	Blight, damping off, root and other rots, etc	**soybean, wheat, corn**
Globisporangium iwayamae	2	0.000	0.000	1.860	0.163	0.000	0.000	0.000	0.000	0.024	0.001	0.0007	0.002	N/A	Rots	*Poaceae*, **wheat**
Globisporangium macrosporum	2	0.000	0.000	0.000	0.181	0.055	1.220	0.797	0.546	0.029	0.658	0.738	0.782	0.682	Damping-off, root rot	iris, **soybean, corn**
Globisporangium nunn	1	0.000	0.000	0.000	0.000	1.042	0.000	0.000	0.000	0.333	0.349	0.339	N/A	0.3415		**soybean**
Globisporangium orthogonon	1	0.000	1.578	0.000	0.000	0.000	0.000	0.000	0.000	0.305	0.323	0.264	0.3061	N/A		**corn**

Globisporangium pleroticum	5	2.38	0.3683	0.000	0.193	0.952	1.170	0.000	1.760	0.7545	0.074	0.009	0.711	0.841		peas, **soybean,** lupins
Globisporangium recalcitrans	14	1.103	6.190	0.000	1.321	3.613	4.969	3.326	1.693	0.131	0.194	0.566	0.219	0.435	Root rot, damping off.	beet, hebe
Globisporangium rostratifingens	5	0.000	0.000	0.000	0.329	0.725	0.233	0.000	0.000	0.699	0.671	0.107	0.529	0.174	Root rot	pea, **soybean, corn, wheat**
Globisporangium selbyi	8	2.200	2.223	3.130	2.350	0.591	0.774	2.410	2.290	0.702	0.859	0.996	0.943	0.750	Lesions	**corn, soybean**
Globisporangium sylvaticum	25	4.970	2.344	9.556	3.796	19.620	12.984	3.839	7.282	0.006	0.330	0.086	0.334	0.063	Root disease, rots	apples, carrot, cherry laurel, cress, chrysanthemum, cucumber, garlic, lettuce, onion, pea, radish, rhododendron, spinach, strawberry, yew, **wheat**
Globisporangium ultimum	11	2.346	14.656	1.834	6.589	2.490	2.820	0.000	1.348	<0.001	0.008	0.011	0.002	0.991	Blight, damping off, root rot, etc	**soybean**, garlic, grape, hyacinth, lettuce, lily, lupin, melon, mustard, onion, parsley, pea, pear, pelargonium, pepper, poinsettia, primula, radish, rhododendron, rhubarb, spinach, strawberry, sweet pea, tomato, tulip, wallflower, yew
Phytophthora cf. inundata D0S1P25	1	0.000	0.000	0.000	0.000	0.000	0.237	0.000	0.000	0.180	0.093	0.152	N/A	0.092		
Pythium acanthicum	1	0.000	0.000	0.000	0.073	0.000	0.000	0.000	0.000	0.315	0.724	0.763	0.711	N/A	Downy mildew. Blight, damping off, rots, etc.	**soybean, corn**
Pythium aff. monospermum	3	1.956	0.000	10.579	0.949	2.768	0.774	14.025	2.828	0.263	<0.001	0.794	<0.001	0.073		grapevine
Pythium arrhenomanes	8	1.116	0.000	0.000	0.872	1.257	3.545	5.713	2.795	0.014	0.782	0.223	0.492	0.409	Blight, root rot	**corn,** rice, barley, **wheat**
Pythium monospermum	12	2.109	0.872	1.683	1.187	1.274	1.928	3.108	2.005	0.367	0.857	0.725	0.476	0.962	Downy mildew. Root necrosis, not known as a strong pathogen	cherry, juniper, spinach
Pythium oligandrum	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.338	0.763	0.772	N/A	0.762	Damping-off; root, stem, and fruit rots	**soybean, wheat**
Pythium oopapillum	2	0.000	0.158	0.000	1.536	0.000	0.000	0.000	0.404	0.35	0.48	0.931	0.678	0.761	Root rot	**soybean**
Pythium torulosum	1	0.000	0.000	0.000	1.326	0.000	0.000	0.000	0.000	0.144	0.503	0.477	0.469	N/A	damping-off, root rot	pea, **soybean, corn**
Pythium volutum	6	0.000	0.000	6.287	4.399	0.000	0.002	1.895	0.053	0.020	0.004	0.143	0.034	0.014	Root rot	melon, morning glory, **wheat,** barley, turfgrass

Saprolegnia anisospora	1	0.000	0.000	1.997	0.000	0.000	0.238	0.000	0.000	0.704	0.024	0.01	0.006	0.379		
Saprolegnia torulosa	1	0.000	0.175	0.000	0.000	0.000	0.000	0.000	1.093	0.142	0.384	0.181	0.306	0.265		
Wilsoniana amaranthi	1	0.000	0.000	0.000	0.638	1.252	0.743	0.000	0.000	0.646	0.876	0.369	0.782	0.515	White blister rust	amaranth
Wilsoniana portulacae	6	0.000	0.000	0.000	0.000	0.000	5.157	0.000	1.132	0.031	0.19	0.211	N/A	N/A	White blister rust	portulacaceae

[a] Species are in alphabetical order.

[b] *P* values are in bold for significant impact of treatments.

[c] Lifestyle and host information are acquired from https://nt.ars-grin.gov/fungaldatabases/.

[d] Hosts included in the study are in bold.

3.2 Crop yield and soybean seedling emergence

Crop yield and seedling emergence tests reflect soil and crop health under different tillage and crop rotation regimes. The growing seasons in 2016-2018 had highly variable precipitation (Appendix 4). Our results showed that corn yield was significantly affected by tillage, with ~18% higher yields in NT than in CT across 3 years (Table 3). Soybean and wheat yields were not significantly affected by tillage (Table 3). Crop rotation had significant effect on wheat yield, but not on corn and soybean yields. Wheat yields were ~78% higher under CSW than under wheat monoculture (Table 3).

When the sampled field soil was planted with soybean in growth cabinet under controlled conditions, seedling vitality (SVS) was not significantly affected by tillage or rotation. However, the interaction between tillage and rotation did significantly affect the SVS (Fig. 5). Compared with CT, NT decreased SVS by ~30% under corn monoculture, but increased SVS by 21% under soybean monoculture and by 33% under CSW. Under corn monoculture, SVS was highest under CT but lowest under NT.

Table 3. The effects[1] of tillage (T) (conventional tillage; CT vs. no-till; NT), and rotation (rotation vs. monoculture for each individual crop) on corn, soybean, and wheat yields (kg ha-1) in 2016-2018.

	Corn yield	Soybean yield	Wheat yield
Tillage			
CT	9177[b1]	2257	1854
NT	10806[a]	2652	1795
Rotation			
Monoculture	9735	2404	1298[b]
Rotation	10248	2446	2305[a]
Analysis of variance (P-values)			
Tillage (T)	0.018	0.226	0.548
Rotation (R)	0.442	0.577	<0.001
T×R	0.163	0.343	0.652

[1]Letters represent significant difference between treatments in tillage or rotation category at $P = 0.05$.

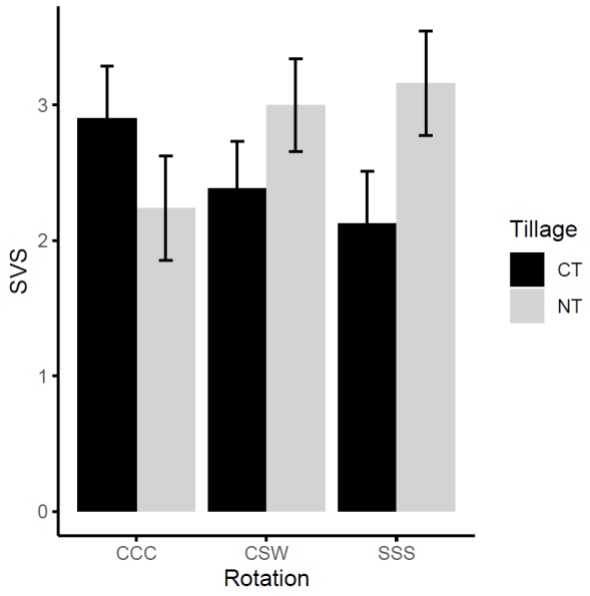


Figure 5. The soybean seedling vitality score (SVS) as affected by tillage and rotation (all pairwise comparisons $P \geq 0.05$). CT, conventional tillage, NT, no-till; CCC, corn monoculture, SSS, soybean monoculture CSW, corn-soybean-wheat rotation.

Chapter 4. Discussion

In this study, the effects of the combined long-term tillage and rotation regimes on the soil oomycete community diversity and compositional structure were evaluated, but it is important to contextualize the environment in which this study took place. To this end, the crop yield response as an indicator of soil and crop health was assessed, to better understand the long-term effects of these agricultural practices on soil and crop health. The long-term tillage and rotation had a significant effect on crop yield, which varied between crops. Soybean and wheat yields in 2016-2018 were not significantly impacted by tillage, similar to the results from a previous study on this site from 2001-2015 by Morrison et al, who reported that yields did not differ between CT and NT for either soybean or wheat (Morrison et al., 2017). However, corn yield was higher under NT than under CT, contrary to prior results at this site (Morrison et al., 2017). The differences between these two studies suggests that the beneficial effects of NT on crop yield may not be evident in the short term and need to be studied on a long-term basis, perhaps 5 years or longer (Büchi et al., 2017). In comparison with monoculture systems, crop rotation significantly increased wheat yield, especially in 2016 and 2018 where the growing seasons were drier, but it did not affect corn and soybean yields. This is in agreement with 2001-2015 data where wheat yielded 22% more when grown in rotation than in monoculture (Morrison et al., 2017), largely attributed to wheat following the nitrogen-fixing soybean in the rotation. This long-term observation supports the value of crop rotation in increasing crop productivity.

Oomycetes are vastly understudied compared to bacteria and fungi despite their importance in crop production systems, where they are responsible for severe declines in crop yields. Here we investigated the impact of different tillage and crop rotations on the soil oomycete communities in corn, soybean, and wheat cropping systems. In this study, we

identified 34 oomycetes species from 292 Amplicon Sequence Variants (ASVs). *Globisporangium* spp. and *Pythium* spp. were most abundant recovered from the study site and accounted for over 95% of the total oomycete abundance. Previous studies have also showed that *Globisporangium* and *Pythium* were dominant oomycetes taxa in other plant production systems, as demonstrated in oak rhizosphere-associated oomycetes communities (Sapp et al., 2019), where these two genera accounted for over 60% of the total community abundance. This was also the case in soils imported to Norway attached to roots of ornamental trees and shrubs, where *Pythium* (46%) was the most abundant genus, while *Globisporangium* (6%) was the second abundant (Rossmann, Lysøe, Skogen, Talgø, & Brurberg, 2021). *Globisporangium* and *Pythium* species that can cause damping-off and root rot are usually generalists that can infest a large variety of agricultural crops, as shown in Table 2 (Martin & Loper, 1999). Zitnick-Anderson and Nelson (2015) found that the *G. attrantheridium, G. heterothallicum, G. hypogynum, G. intermedium*, *and G. irregulare*, caused pre-emergence damping-off on soybean with less than 50% seedling emergence compared with 100% seedling emergence in control. *G. heterothallicum* was one of the most abundant oomycete species in the present study, especially under corn and soybean monoculture. This species has been reported as a pathogen on many crops, including corn (Gan, Chai, Lou, & Li, 2010) and soybean (Coffua, Veterano, Clipman, Mena-Ali, & Blair, 2016), and was a dominant species in North Dakota as reported by studies focusing on oomycete pathogenicity on soybean, representing 49% of the isolates (Zitnick-Anderson & Nelson, 2015). However, Radmer et al. (2017) reported that *G. heterothallicum* was less aggressive on soybean and corn, so its abundance in the population could potentially mediate the pathogenicity of more detrimental species through competition. Several species in *Pythiaceae* recovered from our study, such as *Py. arrhenomanes*, *Py. volutum*, *Py. oopapillum*

and *Py. torulosum* are reported to cause diseases on the seeds and seedlings of soybean (Broders, Lipps, Paul, & Dorrance, 2007; Rojas et al., 2017b; Zitnick-Anderson & Nelson, 2015), corn (Bakker, Moorman, Kaspar, & Manter, 2017; Reyes-Tena et al., 2018), or wheat (Chamswarng & Cook, 1985; Paulitz, 2006; Waller, 1979; Yin, McLaughlin, Paulitz, Kroese, & Hagerty, 2020).

The tillage practices, alone or in combination with rotation regimes, had a significant impact on the oomycete community's alpha- and beta-diversity. For example, NT decreased the alpha-diversity, represented by Simpson-TD and Shannon-TD. The Chao1 richness was also reduced in NT under CSW rotation, but not in the monoculture systems. The persistent decrease trend in Simpson-TD over the three years under NT but not under CT suggests a potential of NT in reducing the number of abundant oomycetes species (Fig. 4A). These observations are not in agreement with Srour et al. (2020) who found that tillage had no significant effect on Shannon's diversity index of the oomycete community at the soybean growth phase of a corn-soybean rotation regime. A meta-analysis showed that NT significantly increased bacterial community diversity but had little effect on fungal community diversity, which could be attributed to the increase of labile carbon and water holding capacities that are essential for microbial growth under NT or the moisture loss resulting in a more conducive environment for microbial growth promoted by plough tillage (Li et al., 2020). There was an overall decrease in homogeneity of the soil oomycetes community under continuous CT, making CT a less favorable practice for managing oomycetes pathogens.

Considering that oomycetes zoospores require wet soil for mobility and invasion of the plants, greater but not excessive soil moisture content can promote oomycetes growth and infection ability. Our results indicated that tillage and rotation regimes may not affect the soil moisture content (Appendix 2). This is contradictory to previous studies, which showed that

reduced tillage and crop rotation may lead to greater soil moisture and soil organic matter contents by enhancing soil aggregation, promoting biological activities, and increasing water holding capacity (Shittu, Oyedele, & Babatunde, 2017; Yang, Siddique, & Liu, 2020). The soil moisture content also did not show significant associations with the community's alpha- and beta-diversity, or the abundance of recovered oomycetes species. In summary, no connection between tillage and rotation regimes and soil moisture content was observed. The overall soil moisture was not highly variable, likely due to the fall sampling time as the soil had compacted throughout the growing season and all plots are under the same yearly precipitation, temperature, and other climate conditions.

The suppression of *Pythium* damping-off can be enhanced by the addition of compost to improve overall soil microbial activity, implying that soil organic matter plays an important role in the soil suppressiveness against oomycetes (Berendsen, Pieterse, & Bakker, 2012). Hoitink and Boehm indicated that the extent of soil-borne pathogen suppression was related to the quantity and quality of soil organic matter (Hoitink & Boehm, 1999). Bongiorno et al. (2019) found that reduced tillage can potentially increase soil suppressiveness through labile carbon and the positive effect of microbial biomass. Therefore, the increase in soil natural suppressiveness under NT as a result of increased soil organic matter could be an important reason for the decrease in the alpha-diversity of the oomycete community observed in this study.

Oomycete community compositional structure was significantly reshaped under CT compared to NT. Similar to a previous study (Srour et al., 2020), which reported that tillage was a main factor driving the soil oomycete community heterogeneity. Among the 34 identified species, six were observed under CT but absent under NT, while another eight showed the opposite trend. The impact of tillage can vary between oomycete species (Fig. 3B), likely

depending on their adaptation to changes in environmental conditions. Similar results were observed by Srour et al (2020) who recovered *G. attantheridium* only from NT plots. Furthermore, *G. sylvaticum and Py. arrhenomanes*, which have been confirmed as pathogens for corn and soybean (Coffua et al., 2016), were more abundant under NT than CT, which is consistent with previous research showing that *Pythium* spp. abundance increases under reduced tillage (Cook, Sitton, & Waldher, 1980; Pankhurst, McDonald, & Hawke, 1995). In contrast, *G. apiculatum* was in higher abundance under CT and rarely present under NT, although this species is not a confirmed pathogen (Paul, 2006). *G. ultimum* is another species that was more abundant under CT than NT, particularly under soybean monoculture (Fig. 3A), and has been reported as one of the most damaging pathogens of corn and soybean seeds and seedlings (Broders et al., 2007; Coffua et al., 2016; Jiang, Haudenshield, & Hartman, 2012; Wei et al., 2010). In addition, *Py. volutum* was highly abundant under CT but was present in low abundance under NT and was reported as being one of the most damaging pathogens in wheat (Chamswarng & Cook, 1985). Similarly, *G. apiculatum, Py. volutum,* and *G. iwayamae* were found in low abundance under NT but showed a consistent increase in abundances under CT over the three-year period, although such changes were not statistically significant (Fig. 4B-D). We hypothesize that that the soils under CT have a reduced capacity to suppress the oomycetes pathogens compared to NT. To investigate this further, the next step is to examine the bacterial and fungal communities present in the same soil samples.

The shift in soil oomycete community composition induced by tillage practices in this study is likely associated with the changes in soil physical and chemical properties induced by such disruptive practices. Previous studies have shown correlations between the abundance of some *Pythium* species and soil physicochemical properties such as pH, calcium, and magnesium

content, cation exchange capacity, and clay content (Broders et al., 2009; F. Martin & Hancock, 1986). The occurrence of some oomycete species in no-till systems may be a result of layered crop residue on the ground, which serves as an ideal habitat for primary inoculum buildup (Bockus & Shroyer, 1998; Rojas et al., 2019). It is also likely that the timing of sampling influences the oomycete community composition. Sampling was done in the fall, at a point where the majority of the plant tissue available to the soil-borne oomycete community was dead plant tissue, favoring saprophytic oomycetes such as *Pythium* and *Globisporangium*. Asad et al. (2023) found that microbiome sampling early in the growing season was most closely tied to final seed quality. It is likely that spring or summer sampling of oomycete communities may result in different trends.

Our results showed that the soil oomycete community structure differed significantly between monoculture systems. Sapkota and Nicolaisen (2018) found that crops grown prior to sampling, influenced the oomycete community composition in a field survey of arable soils (Sapkota & Nicolaisen, 2018). In this study, *G. apiculatum* and *G. ultimum* were highly abundant in soybean soils. In contrast, *Pythium* sp. aff. monospermum and *P. volutum* were highly abundant in wheat soils. This confirms the crop effect on the oomycete community, and different oomycete species prefer certain crops as hosts. The crop effect on oomycete community structure could be related to the effect of root exudates in the rhizosphere, the accumulation of crop-specific root pathogens or parasites, and also the plant-derived crop residues left over after harvest (Sapkota & Nicolaisen, 2018).

Crop rotation has been demonstrated as a good practice for reducing plant disease caused by soil-borne pathogens (Emmond & Ledingham, 1972; Frank & Murphy, 1977; Pedersen, 1992; Scholte, 1987), potentially through altering soil physical changes plus the presence of layers of

crop residue on the soil surface leading to changes in pathobiome community structure and functions. This study further showed that rotation had no significant effect on the alpha-diversity but statistically affected the beta-diversity of the oomycete community under CT. Several species, including *Py.* sp. aff. monospermum, *G. iwayamae*, and *S. anisospore*, were abundant under wheat monoculture but were in low abundance or absent in wheat soils under CSW, implying that rotation may reduce some oomycete species levels by breaking disease cycles. Such breaks in pathogen cycles were observed by Bargués-Ribera and Gokhale, where the instruction of non-host crops reduced the incidence of unspecified disease (Bargués-Ribera & Gokhale, 2020). The USDA fungal database (https://nt.ars-grin.gov/fungaldatabases/) indicates that *G. iwayamae* is present in various hosts, including wheat (Lipps & Bruehl, 1978). These two species were reported to induce plant damping-off or rot diseases.

Crop rotation had shown benefits of decreasing the abundance of oomycetes plant pathogens, possibly due to improved disease suppressive capacity of soil microbiomes in more diverse rotations (Peralta et al., 2018) and increased soil N-levels form the inclusion of soybean as a preceding crop; both eventually increased wheat yield. No previous studies reported *Pythium* sp. aff. monospermum and S. anisospore in wheat soils. *Pythium* sp. aff. monospermum has been isolated from grapevine (Spies, Mazzola, & McLeod, 2011), and *S. anisospore* is generally reported as an aquatic pathogen (Masigol et al., 2020). Rotation did not significantly affect the beta-diversity of the soil oomycete community under NT. One possible reason is that tillage and the disruption of soil structure is the major factor driving the soil oomycete community in this study. The top ten most abundant oomycete species under NT were not significantly affected by rotation. Only *Py. volutum* was significantly affected by rotation, which is not a highly abundant

species under NT and may have a negligible contribution to shifting the soil oomycete community structure.

Many oomycetes species identified are pathogens associated with soybean (Table 2) (Radmer et al., 2017; Rojas et al., 2017a), and as such, a growth chamber experiment was conducted to determine the soybean seedling vitality in the studied soils collected in 2016 and 2017, aiming to evaluate the overall health of the soils and test if there was any association with identified oomycete species. Neither tillage nor rotation showed significant impact on the seedling vitality score (SVS) independently (Table 1), however, differing trends between the rotations within the two tillage treatments highlight the effect of the interaction between tillage and rotation (Fig. 5). We did not observe significant associations between any individual oomycete species and SVS. The poorer emergence in NT-CCC, CT-SSS, and CT-CSW treatments could be associated with the presence of higher abundances of *G. heterothallicum* and *G. ultimum* (Table 2). These two species have been shown to be aggressively pathogenic on soybean (Radmer et al., 2017; Rizvi & Yang, 1996; Rojas et al., 2017a). However, without isolation and/or molecular characterization (Rojas et al., 2017b; Wei et al., 2010), no direct conclusions about oomycete contribution to low SVS can be made.

Chapter 5. Conclusions and future work

Our study shows that combined tillage and rotation regimes have pronounced impacts on soil and crop health and the oomycete communities. No-till (NT) is an effective way to suppress the overall diversity of the soil-borne oomycete community, while the selection of crop rotations is crucial under conventional tillage (CT), where the oomycete community is more responsive to changes in management practices. These findings suggest that continuous CT practices may lead to a decreased homogeneity of the soil oomycete community, while NT may be a more sustainable approach to farming that helps maintain soil health and biodiversity.

It is important to note that different oomycete species respond differently to tillage and rotation practices, which could be due to host availability and their unique adaptations to specific soil and environmental conditions. However, to gain a comprehensive understanding of the potential of tillage and rotation in mediating the pathogenicity of soil-borne oomycete, future studies should consider how soil physicochemical properties and soil depth influence the oomycetes under tillage and rotation regimes. To answer this question, the soil-borne oomycetes of the soils collected at two depths (0-15 cm and 15-30 cm) were characterized in 2018 as well as the soil physicochemical properties including pH, nitrogen, carbon, potassium and phosphorus (Table 4). Carbon and nitrogen concentrations were both significantly higher in the topsoil layer (0-15, $P = 0.001$), whereas available potassium was impacted by tillage ($P < 0.001$). The changes in soil physiochemical properties resulting from varying tillage and rotation practices at the two depths have significant implications for the habitat conditions of soil-borne oomycetes communities. As a consequence, these changes could potentially induce shifts in the diversity and compositional structure of these communities. A manuscript is in preparation where the

impacts of tillage, rotation and depth on the soil physiochemical parameters and soil-borne oomycete community structure will be further investigated.

Table 4. Soil nutrient concentration and pH, including carbon (%), nitrogen (%), pH, available phosphorus (ppm) and potassium (ppm) at two different soil depths (0-15 cm and 15-30 cm) of soils under conventional tillage (CT) or no-till (NT) management, with 3 rotation patterns, corn monoculture (CCC), soybean monoculture (SSS), wheat monoculture (WWW) and corn-soybean-wheat rotation (CSW) from 2018.

		CT					NT				
	Depth	Carbon	Nitrogen	pH	Phosphorus	Potassium	Carbon	Nitrogen	pH	Phosphorus	Potassium
CCC	0-15	1.51	0.12	7	36.5	77.5	2.06	0.15	6.94	56.25	117.5
	15-30	1.34	0.11	7.05			1.55	0.12	7.11		
CSW	0-16	1.93	0.15	6.99	39.5	70	2.01	0.16	7.18	47.5	96.67
	15-31	1.77	0.13	6.99			1.31	0.11	7.31		
SSS	0-17	2.32	0.17	7.1	45.75	77.5	1.97	0.16	7.07	48.5	105
	15-32	2.04	0.15	7.16			1.31	0.11	7.24		
WWW	0-18	2.31	0.16	7.14	45.25	70	2.14	0.16	7.12	47	117.5
	15-33	1.9	0.14	7.11			1.27	0.1	7.39		

Finally, the interrelationships between oomycetes, bacterial, and fungal taxa in soil samples and how these relationships may be influenced by tillage and rotation practices will be studied. This investigation involves analyzing the bacterial and fungal communities alongside the oomycete community structure. To elucidate these relationships, co-occurrence ecological networks will be reconstructed, to provide insights into potential biocontrol agents capable of antagonizing the growth and pathogenicity of soil-borne oomycetes. Future research should employ culture-dependent and molecular diagnostic assays to validate the sequencing results and ascertain the pathogenic potential of soil-borne pathogens within the communities. This validation is particularly crucial for the seedling vitality trial, as confirming the role of oomycetes as causal agents for decreased seedling vitality will bolster the interpretation of these findings.

Overall, this research represents a significant advancement in the comprehension of oomycete community diversity and structure and their response to tillage and crop rotation practices. These aspects have received relatively less attention compared to other microbial groups, such as fungi and bacteria. Thus, this study fills an important knowledge gap and contributes to a more comprehensive understanding of soil microbial dynamics in agricultural systems.

References

Abdollahi, L., Schjønning, P., Elmholt, S., & Munkholm, L. J. (2014). The Effects of Organic Matter Application and Intensive Tillage and Traffic on Soil Structure Formation and Stability. *Soil and Tillage Research, 136*, 28-37. doi:https://doi.org/10.1016/j.still.2013.09.011

Anderson, M. J. (2014). Permutational Multivariate Analysis of Variance (PERMANOVA). In *Wiley StatsRef: Statistics Reference Online* (pp. 1-15).

Asad, N. I., Wang, X.-B., Dozois, J., Azarbad, H., Constant, P., & Yergeau, E. (2023). Early Season Soil Microbiome Best Predicts Wheat Grain Quality. *FEMS Microbiology Ecology, 99*(1), fiac144. doi:10.1093/femsec/fiac144

Bakker, M. G., Moorman, T. B., Kaspar, T. C., & Manter, D. K. (2017). Isolation of Cultivation-Resistant Oomycetes, First Detected as Amplicon Sequences, from Roots of Herbicide-Terminated Winter Rye. *Phytobiomes, 1*(1), 24-35. doi:10.1094/pbiomes-10-16-0011-r

Bargués-Ribera, M., & Gokhale, C. S. (2020). Eco-evolutionary agriculture: Host-pathogen Dynamics in Crop Rotations. *PLoS Comput Biol, 16*(1), e1007546. doi:10.1371/journal.pcbi.1007546

Baselga, A., & Orme, C. D. L. (2012). betapart: an R Package for the Study of Beta Diversity. *Methods in Ecology and Evolution, 3*(5), 808-812. doi:https://doi.org/10.1111/j.2041-210X.2012.00224.x

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The Rhizosphere Microbiome and Plant Health. *Trends in Plant Science, 17*(8), 478-486. doi:https://doi.org/10.1016/j.tplants.2012.04.001

Bockus, W., & Shroyer, J. (1998). The Impact of Reduced Tillage on Soilborne Plant Pathogens. *Annual review of phytopathology, 36*(1), 485-500.

Bogunovic, I., Pereira, P., Kisic, I., Sajko, K., & Sraka, M. (2018). Tillage Management impacts on Soil Compaction, Erosion and Crop Yield in Stagnosols (Croatia). *CATENA, 160*, 376-384. doi:https://doi.org/10.1016/j.catena.2017.10.009

Bokulich, N. A., Kaehler2, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., . . . Caporaso, J. G. (2018). Optimizing Taxonomic Classification of Marker-gene Amplicon Sequences with QIIME 2's q2-feature-classifier Plugin. *Microbiome, 6*(90).

Bongiorno, G., Postma, J., Bünemann, E. K., Brussaard, L., de Goede, R. G. M., Mäder, P., . . . Thuerig, B. (2019). Soil Suppressiveness to *Pythium ultimum* in Ten European Long-term Field Experiments and Its Relation with Soil Parameters. *Soil Biology and Biochemistry, 133*, 174-187. doi:https://doi.org/10.1016/j.soilbio.2019.03.012

Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Characterization of *Pythium* spp. Associated with Corn and Soybean Seed and Seedling Disease in Ohio. *Plant Disease, 91*(6), 727-735. doi:10.1094/PDIS-91-6-0727

Broders, K. D., Wallhead, M. W., Austin, G. D., Lipps, P. E., Paul, P. A., Mullen, R. W., & Dorrance, A. E. (2009). Association of Soil Chemical and Physical Properties with *Pythium* Species Diversity, Community Composition, and Disease Incidence. *Phytopathology, 99*(8), 957-967. doi:10.1094/phyto-99-8-0957

Büchi, L., Wendling, M., Amossé, C., Jeangros, B., Sinaj, S., & Charles, R. (2017). Long and Short Term Changes in Crop Yield and Soil Properties Induced by the Reduction of Soil Tillage in a Long Term Experiment in Switzerland. *Soil and Tillage Research, 174*, 201-129.

Cahill, D. M., Rookes, J. E., Wilson, B. A., Gibson, L., & McDougall, K. L. (2008). *Phytophthora cinnamomi* and Australia's Biodiversity: Impacts, Predictions and Progress Towards Control. *Australian Journal of Botany, 56*(279-310).

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-resolution Sample Inference From Illumina Amplicon Data. *Nat Methods, 13*(7), 581-583. doi:10.1038/nmeth.3869

Chamswarng, C., & Cook, R. (1985). Identification and Comparative Pathogenicity of *Pythium* Species from Wheat Roots and Wheat-field Soils in the Pacific Northwest. *Phytopathology, 75*, 821-827.

Chen, W., Radford, D., & Hambleton, S. (2021). Towards Improved Detection and Identification of Rust Fungal Pathogens in Environmental Samples Using a Metabarcoding Approach. *Phytopathology, 112*(3), 535-548. doi:10.1094/PHYTO-01-21-0020-R

Coffua, L. S., Veterano, S. T., Clipman, S. J., Mena-Ali, J. I., & Blair, J. E. (2016). Characterization of *Pythium* spp. Associated with Asymptomatic Soybean in Southeastern Pennsylvania. *Plant Disease, 100*(9), 1870-1879. doi:10.1094/pdis-11-15-1355-re

Cohen, Y., & Coffey, M. D. (1986). Systemic Fungicides and the Control of Oomycetes. *Annual Reviews in Phytopathology, 24*, 311-338.

Cook, R., Sitton, J., & Haglund, W. (1987). Influence of Soil Treatments on Growth and Yield of Wheat and Implications for Control of *Pythium* root rot. *Phytopathology, 77*(8), 1192-1198.

Cook, R., Sitton, J., & Waldher, J. (1980). Evidence for *Pythium* as a Pathogen of Direct-drilled Wheat in the Pacific Northwest. *Plant Disease, 64*(1), 61-61.

Cook, R. J., & Haglund, W. A. (1991). Wheat Yield Depression Associated with Conservation Tillage Caused by Root Pathogens in the Soil Not Phytotoxins From the Straw. *Soil Biology and Biochemistry, 23*(12), 1125-1132. doi:https://doi.org/10.1016/0038-0717(91)90024-E

Dorrance, A., & Grünwald, N. J. (2009). *Phytophthora sojae*: Diversity Among and Within Populations. In *Oomycete Genetics and Genomics* (pp. 197-212).

Emmond, G. S., & Ledingham, R. J. (1972). Effects of Crop Rotation on Some Soil-borne Pathogens of Potato. *Canadian Journal of Plant Science, 52*(4), 605-611. doi:10.4141/cjps72-093

Frank, J. A., & Murphy, H. (1977). The Effect of Crop Rotations on *Rhizoctonia* Disease of Potatoes. *American Potato Journal, 54*, 315-322.

Fry, W. (2008). *Phytophthora infestans*: the Plant (and R gene) Destroyer. *Molecular Plant Pathology, 9*, 385-402.

Gan, H., Chai, Z., Lou, B., & Li, J. (2010). Pythium heterothallicum new to China and its pathogenicity. *Mycosystem, 29*(4), 494-501.

Hervé, M. (2022). RVAideMemoire: Testing and Plotting Procedures for Biostatistics. *R package version 0.9-81-2*, https://CRAN.R-project.org/package=RVAideMemoire. doi:https://CRAN.R-project.org/package=RVAideMemoire

Hoitink, H., & Boehm, M. (1999). Biocontrol Within the Context of Soil Microbial Communities: A Substrate-dependent Phenomenon. *Annual review of phytopathology, 37*, 427.

Hwang, S., Ahmed, H., Gossen, B., Kutcher, H., Brandt, S., Strelkov, S., . . . Turnbull, G. (2009). Effect of Crop Rotation on the Soil Pathogen Population Dynamics and Canola Seedling Establishment. *Plant Pathology Journal, 8*(3), 106-112.

Jiang, Y. N., Haudenshield, J. S., & Hartman, G. L. (2012). Characterization of *Pythium* spp. From Soil Samples in Illinois. *Canadian Journal of Plant Pathology, 34*(3), 448-454. doi:10.1080/07060661.2012.705326

Jost, L. (2006). Entropy and diversity. *Oikos, 113*(2), 363-375. doi:doi:10.1111/j.2006.0030-1299.14714.x

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol Biol Evol, 30*(4), 772-780. doi:10.1093/molbev/mst010

Kindt, R. (2020). BiodiversityR: Package for Community Ecology Suitability Analysis. *https://github.com/cran/BiodiversityR*.

Larkin, R. P., & Halloran, J. M. (2014). Management Effects of Disease-Suppressive Rotation Crops on Potato Yield and Soilborne Disease and Their Economic Implications in Potato Production. *American Journal of Potato Research, 91*(5), 429-439. doi:10.1007/s12230-014-9366-z

Li, Y., Li, L., Sui, P., Liu, X., Zhang, T., & Li, X. (2022). Crop Rotation to Diversify the Soil Microbiome in the Semi-arid Area of Inner Mongolia, China. *Archives of Agronomy and Soil Science, 1-16*.

Li, Y., Song, D., Liang, S., Dang, P., Qin, X., Liao, Y., & Siddique, K. H. M. (2020). Effect of No-tillage on Soil Bacterial and Fungal Community Diversity: A Meta-analysis. *Soil and Tillage Research, 204*, 104721. doi:https://doi.org/10.1016/j.still.2020.104721

Lipps, P., & Bruehl, G. (1978). Snow Rot of Winter Wheat in Washington. *Phytopathology, 68*(1), 120-121.

Liu, B., Gumpertz, M. L., Hu, S., & Ristaino, J. B. (2008). Effect of Prior Tillage and Soil Fertility Amendments on Dispersal of *Phytophthora capsici* and Infection of Pepper. *European Journal of Plant Pathology, 120*(3), 273-287. doi:10.1007/s10658-007-9216-7

Lyu, J., Jin, L., Jin, N., Xie, J., Xiao, X., Hu, L., . . . Yu, J. (2020). Effects of Different Vegetable Rotations on Fungal Community Structure in Continuous Tomato Cropping Matrix in Greenhouse. *Frontiers in Microbiology, 11*. Retrieved from https://www.frontiersin.org/articles/10.3389/fmicb.2020.00829

Martin, F., & Hancock, J. (1986). Association of Chemical and Biological Factors in Soils Suppressive to *Pythium ultimum*. *Phytopathology, 76*(11), 1221-1231.

Martin, F. N., & Loper, J. E. (1999). Soilborne Plant Diseases Caused by *Pythium* spp.: Ecology, Epidemiology, and Prospects for Biological Control. *Critical Reviews in Plant Sciences, 18*(2), 111-181. doi:10.1080/07352689991309216

Martin, M. (2011). Cutadapt Removes Adapter Sequences from High-throughput Sequencing Reads. *EBMnet.journal, 17*(1), 10-12.

Masigol, H., Khodaparast, S. A., Mostowfizadeh-Ghalamfarsa, R., Rojas-Jimenez, K., Woodhouse, J. N., Neubauer, D., & Grossart, H.-P. (2020). Taxonomical and Functional Diversity of *Saprolegniales* in Anzali Lagoon, Iran. *Aquatic Ecology, 54*(1), 323-336. doi:10.1007/s10452-019-09745-w

Morrison, M. J., Cober, E. R., Gregorich, E., Voldeng, H. D., Ma, B., & Topp, G. C. (2017). Tillage and Crop Rotation Effects on the Yield of Corn, Soybean and Wheat in Eastern Canada. *Canadian Journal of Plant Science, 98*, 183–191. doi:10.1139/cjps-2016-0407

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R., . . . Wagner, H. (2022). vegan: Community Ecology Package. *R package version 2.6-2. https://CRAN.R-project.org/package=vegan, 2*(9).

Pankhurst, C. E., McDonald, H. J., & Hawke, B. G. (1995). Influence of Tillage and Crop Rotation on the Epidemiology of *Pythium* Infections of Wheat in a Red-brown Warth of South Australia. *Soil Biology and Biochemistry, 27*(8), 1065-1073. doi:https://doi.org/10.1016/0038-0717(95)00009-4

Paul, B. (2006). A New Species of *Pythium* Isolated From a Vineyard in France. *FEMS Microbiology Letters, 263*(2), 194-199. doi:10.1111/j.1574-6968.2006.00422.x %J FEMS Microbiology Letters

Paulitz, T. C. (2006). Low Input No-till Cereal Production in the Pacific Northwest of the U.S.: The Challenges of Root Diseases. *European Journal of Plant Pathology, 115*(3), 271-281. doi:10.1007/s10658-006-9023-6

Pedersen, E. A. (1992). The Effect of Crop Rotation on Development of the *Septoria* Disease Complex on Spring Wheat in Saskatchewan. *Canadian Journal of Plant Pathology, 14*(2), 152-158. doi:10.1080/07060669209500892

Peralta, A. L., Sun, Y., McDaniel, M. D., & Lennon, J. T. (2018). Crop Rotational Diversity Increases Disease Suppressive Capacity of Soil Microbiomes. *9*(5), e02235. doi:https://doi.org/10.1002/ecs2.2235

Peters, R. D., Sturz, A. V., Carter, M. R., & Sanderson, J. B. (2003). Developing Disease-suppressive Soils Through Crop Rotation and Tillage Management Practices. *Soil and Tillage Research, 72*(2), 181-192. doi:https://doi.org/10.1016/S0167-1987(03)00087-4

Phillips, A. J., Anderson, V. L., Robertson, E. J., Secombes, C. J., & van West, P. (2008). New Insights Into Animal Pathogenic oomycetes. *Trends in Microbiology, 16*(1), 13-19. doi:https://doi.org/10.1016/j.tim.2007.10.013

Pinheiro, J., & Bates, D. (2023). nlme: Linear and Nonlinear Mixed Effects Models. https://CRAN.R-project.org/package=nlme.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2--Approximately Maximum-likelihood Trees for Large Alignments. *PLoS One, 5*(3), e9490. doi:10.1371/journal.pone.0009490

Radmer, L., Anderson, G., Malvick, D. M., Kurle, J. E., Rendahl, A., & Mallik, A. (2017). *Pythium, Phytophthora,* and *Phytopythium* spp. Associated with Soybean in Minnesota, Their Relative

Aggressiveness on Soybean and Corn, and Their Sensitivity to Seed Treatment Fungicides. *Plant Disease, 101*(1), 62-72. doi:10.1094/pdis-02-16-0196-re

Reyes-Tena, A., Vallejo-González, R., Santillán-Mendoza, R., Rodríguez-Alvarado, G., Larsen, J., & Fernández-Pavía, S. P. (2018). *Pythium arrhenomanes* Causal Agent of Root Rot on Yellow Maize in Mexico. *Australasian Plant Disease Notes, 13*(1), 6. doi:10.1007/s13314-018-0291-8

Rizvi, S., & Yang, X. (1996). Fungi Associated with Soybean Seedling Disease in Iowa. *Plant Disease, 80*(1), 57-60.

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., . . . Chilvers, M. I. (2017a). Oomycete Species Associated with Soybean Seedlings in North America—Part I: Identification and Pathogenicity Characterization. *Phytopathology, 107*(3), 280-292. doi:10.1094/phyto-04-16-0177-r

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., . . . Chilvers, M. I. (2017b). Oomycete Species Associated with Soybean Seedlings in North America—Part II: Diversity and Ecology in Relation to Environmental and Edaphic Factors. *Phytopathology, 107*(3), 293-304. doi:10.1094/phyto-04-16-0176-r

Rojas, J. A., Witte, A., Noel, Z. A., Jacobs, J. L., & Chilvers, M. I. (2019). Diversity and Characterization of Oomycetes Associated with Corn Seedlings in Michigan. *Phytobiomes, 3*(3), 224-234. doi:10.1094/pbiomes-12-18-0059-r

Rossmann, S., Lysøe, E., Skogen, M., Talgø, V., & Brurberg, M. B. (2021). DNA Metabarcoding Reveals Broad Presence of Plant Pathogenic Oomycetes in Soil From Internationally Traded Plants. *Frontiers in Microbiology, 12*. doi:10.3389/fmicb.2021.637068

Santín-Montanyá, M. I., & Sombrero Sacristán, A (2020). The Effects of Soil Tillage Techniques on Weed Flora in High Input Barley Systems in Northern Spain. *Canadian Journal of Plant Science, 100*(3), 245-252. doi:10.1139/cjps-2019-0178

Sapkota, R., & Nicolaisen, M. (2018). Cropping History Shapes Fungal, Oomycete and Nematode Communities in Arable Soils and Affects Cavity Spot in Carrot. *Agriculture, Ecosystems & Environment, 257*, 120-131. doi:https://doi.org/10.1016/j.agee.2018.01.032

Sapp, M., Tyborski, N., Linstädter, A., López Sánchez, A., Mansfeldt, T., Waldhoff, G., . . . Rose, L. E. (2019). Site-specific Distribution of Oak Rhizosphere-associated Oomycetes Tevealed by Cytochrome c Oxidase Subunit II Metabarcoding. *Ecology and Evolution, 9*(18), 10567-10581. doi:https://doi.org/10.1002/ece3.5577

Scholte, K. H. (1987). The Effect of Crop Rotation and Granular Nematicides on the Incidence of *Rhizoctonia solani* in Potato. *J Potato Research, 30*, 187-199.

Shittu, K. A., Oyedele, D. J., & Babatunde, K. M. (2017). The Effects of Moisture Content at Tillage on Soil Strength in Maize Production. *Egyptian Journal of Basic and Applied Sciences, 4*(2), 139-142. doi:doi: 10.1016/j.ejbas.2017.04.001.

Sipilä Timo, P., Yrjälä, K., Alakukku, L., & Palojärvi, A. (2012). Cross-Site Soil Microbial Communities under Tillage Regimes: Fungistasis and Microbial Biomarkers. *Applied and Environmental Microbiology, 78*(23), 8191-8201. doi:10.1128/AEM.02005-12

Spies, C. F. J., Mazzola, M., & McLeod, A. (2011). Characterisation and Detection of *Pythium* and *Phytophthora* Species Associated with Grapevines in South Africa. *European Journal of Plant Pathology, 131*(1), 103-119.

Srour, A. Y., Ammar, H. A., Subedi, A., Pimentel, M., Cook, R. L., Bond, J., & Fakhoury, A. M. (2020). Microbial Communities Associated With Long-Term Tillage and Fertility Treatments in a Corn-Soybean Cropping System. *Front Microbiol, 11*, 1363. doi:10.3389/fmicb.2020.01363

Team, R. C. (2021). R: A Language and Environment for Statistical Computing. In. Vienna, Austria: R Foundation for Statistical Computing.

Thines, M., Voglmayr, H., & Göker, M. (2009). Taxonomy and Phylogeny of the Downy Mildews (*Peronosporaceae*). In *Oomycete Genetics and Genomics* (pp. 47-75).

Uzuhashi, S., Kakishima, M., & Tojo, M. (2010). Phylogeny of the Genus *Pythium* and Description of New Genera. *Mycoscience, 51*(5), 337-365. doi:https://doi.org/10.1007/S10267-010-0046-7

Waller, J. M. (1979). Observations on *Pythium* Root Rot of Wheat and Barley. *Plant Pathology, 28*(1), 17-24. doi:https://doi.org/10.1111/j.1365-3059.1979.tb02612.x

Wei, L., Xue, A., Cober, E., Babcock, C., Zhang, J., Zhang, S., . . . Liu, L. (2010). Pathogenicity of *Pythium* Ppecies Causing Seed Rot and Damping-off in Soybean Under Controlled Conditions. *Phytoprotection, 91*, 3-10. doi:https://doi.org/10.7202/1008539ar

Yang, T., Siddique, K. H. M., & Liu, K. (2020). Cropping Systems in Agriculture and Their Impact on Soil Health-A Review. . *Global Ecology and Conservation, 23*. doi:doi: https://doi.org/10.1016/j.gecco.2020.e01118

Yin, C., McLaughlin, K., Paulitz, T. C., Kroese, D. R., & Hagerty, C. H. (2020). Population Dynamics of Wheat Root Pathogens Under Different Tillage Systems in Northeast Oregon. *Plant Disease, 104*(10), 2649-2657. doi:10.1094/pdis-03-19-0621-re

Yuan, X., Wang, B., Hong, S., Xiong, W., Shen, Z., Ruan, Y., . . . Dini-Andreote, F. (2021). Promoting Soil Microbial-mediated Suppressiveness Against *Fusarium* Wilt Disease by the Enrichment of Specific Fungal Taxa via Crop Rotation. *Biology and Fertility of Soils, 57*(8), 1137-1153. doi:10.1007/s00374-021-01594-w

Zahariev, M. A.-O., & Chen, W. (2018). Cluster Oligonucleotide Signatures for Rapid Identification by Sequencing. *BMC Bioinformatics, 19*(1471-2105 (Electronic)). doi:doi: 10.1186/s12859-018-2363-3

Zitnick-Anderson, K. K., & Nelson, B. D. (2015). Identification and Pathogenicity of Pythium on Soybean in North Dakota. *Plant Disease, 99*(1), 31-38. doi:10.1094/pdis-02-14-0161-re

Appendices

Appendix 1: Experimental design

The sampled field was planted designed in a split-plot design with two blocks of tillage at two levels (CT and NT), with rotation as the subplot factor with nine levels. The rotation levels included corn, soybean and wheat in monoculture, and two rotation schemes (corn-soybean-wheat and wheat-soybean-corn) these schemes were present in each stage for a total of three treatments per rotation scheme. Within each tillage block there were two replications for a total of four replications per treatment and 72 total plots. Sampling expanded from 2016 to 2018, so in this appendix includes figure S1, where sampled plots are highlighted in yellow.

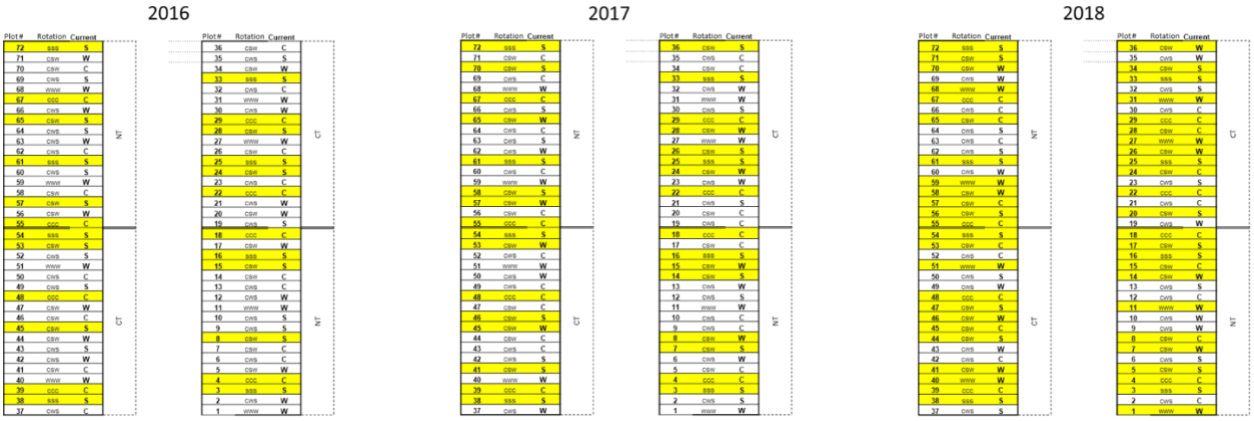


Fig. S1. Field maps of the split-plot experimental design in each field sampling year (2016-2018), tillage levels (CT: conventional tillage, NT: no-till), rotation patterns (CCC: corn monoculture, SSS: soybean monoculture, WWW: wheat monoculture, CSW: corn-soybean-wheat rotation, CWS: corn-wheat-soybean rotation), as well as current crop (C: corn, S: soybean, W: wheat) are indicated for each field plot.

Appendix 2: Soil moisture content

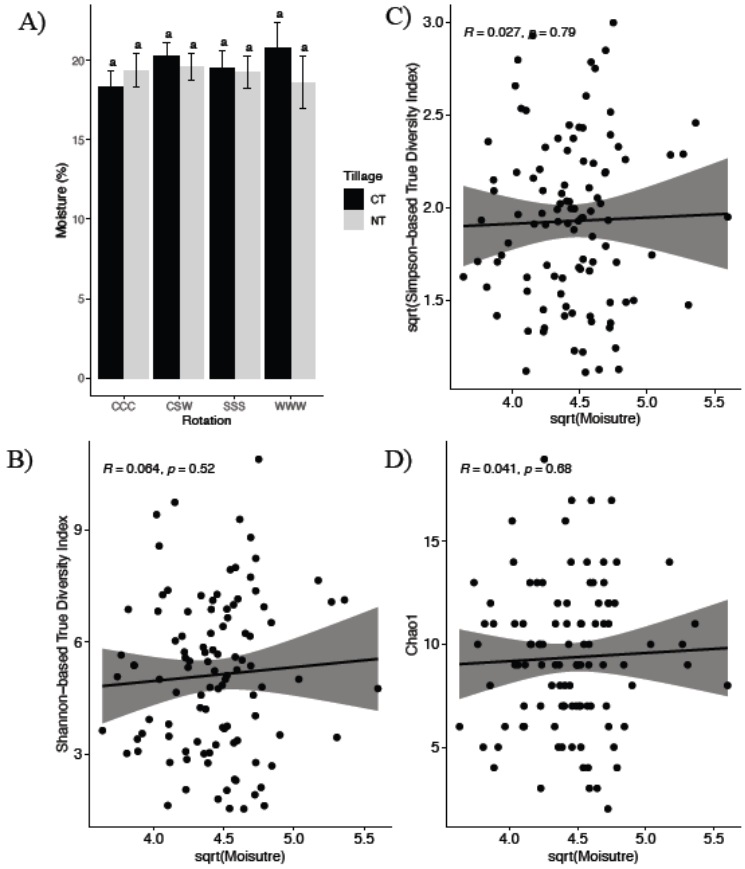


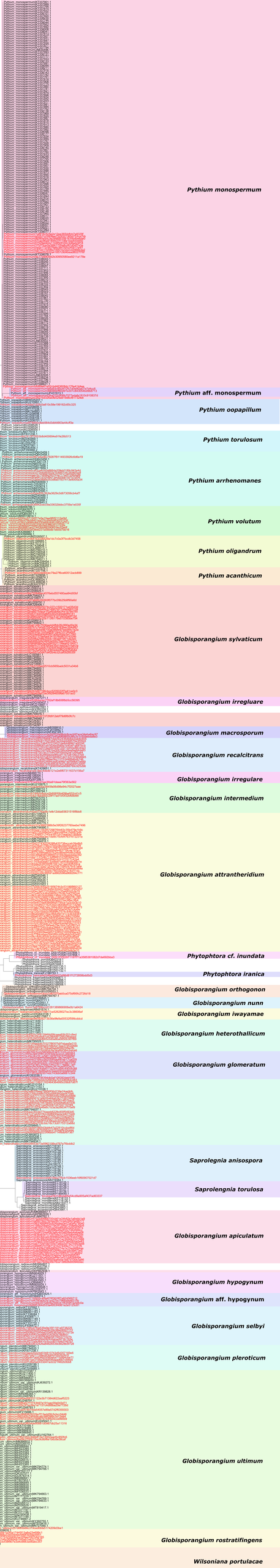
Fig. S2. A) The impact of tillage and rotation regimes on the soil moisture content, as measured by weighing soils at sampling, drying down the soils at 60˚C and calculating gravimetric soil moisture content (%). The correlation relationships between the soil moisture content and the B) Shannon-based true diversity index, C) Simpson-based true diversity index, and D) the Chao1 index of the soil-borne oomycetes community.

Appendix 3: Reference database construction and taxonomic validation

The identification of the oomycetes species within the ASV recovered from the community relied on the reference database. The oomycetes ITS1 reference sequence database was constructed from GenBank 26,200 sequences. These sequences were curated manually to remove less reliable reference sequences. The ASV were identified using a comparative approach with AODP, q2-feature-classifier, and BLASTn.

These classifications were assessed at each taxonomic level. Genus level classifications were taken from the q2-feature-classifier when the confidence of the assignment was over 90%. At the species level, when the AODP confidence was above 97% the species level classification was kept. When the confidence was between 97% and 80%, the genus level classification was used. From 80% to 60% only the family level classification was kept. Any reference sequence below 60% was left unclassified.

After initial classification an additional validation step was taken for the representative sequences that were classified to the species level. The representative sequences identified to the species level, as well as the representative sequences used assign species, and some manually selected outgroup reference sequences were aligned using MAFFT. A phylogenetic tree was constructed from this alignment in FastTree, using the -nt and -gtr options. This tree was then visualized using the FigTree. Identifications that conflicted with the overall structure of the phylogeny were manually assessed, and in some cases the taxonomic assignment at the species level was removed. This was done progressively until species level assignments were as reliable as possible as demonstrated in Figure S3.



Supplementary Fig. S3. Validation of the classification accuracy of oomycetes ASVs at the species level using phylogenetic tree reconstruction. The 230 oomycetes ASVs (highlighted in red) was combined with reference sequences downloaded from the Genbank for multiple sequence alignment and Approximate Maximum Likelihood tree reconstruction.

Appendix 4: Cumulative precipitation

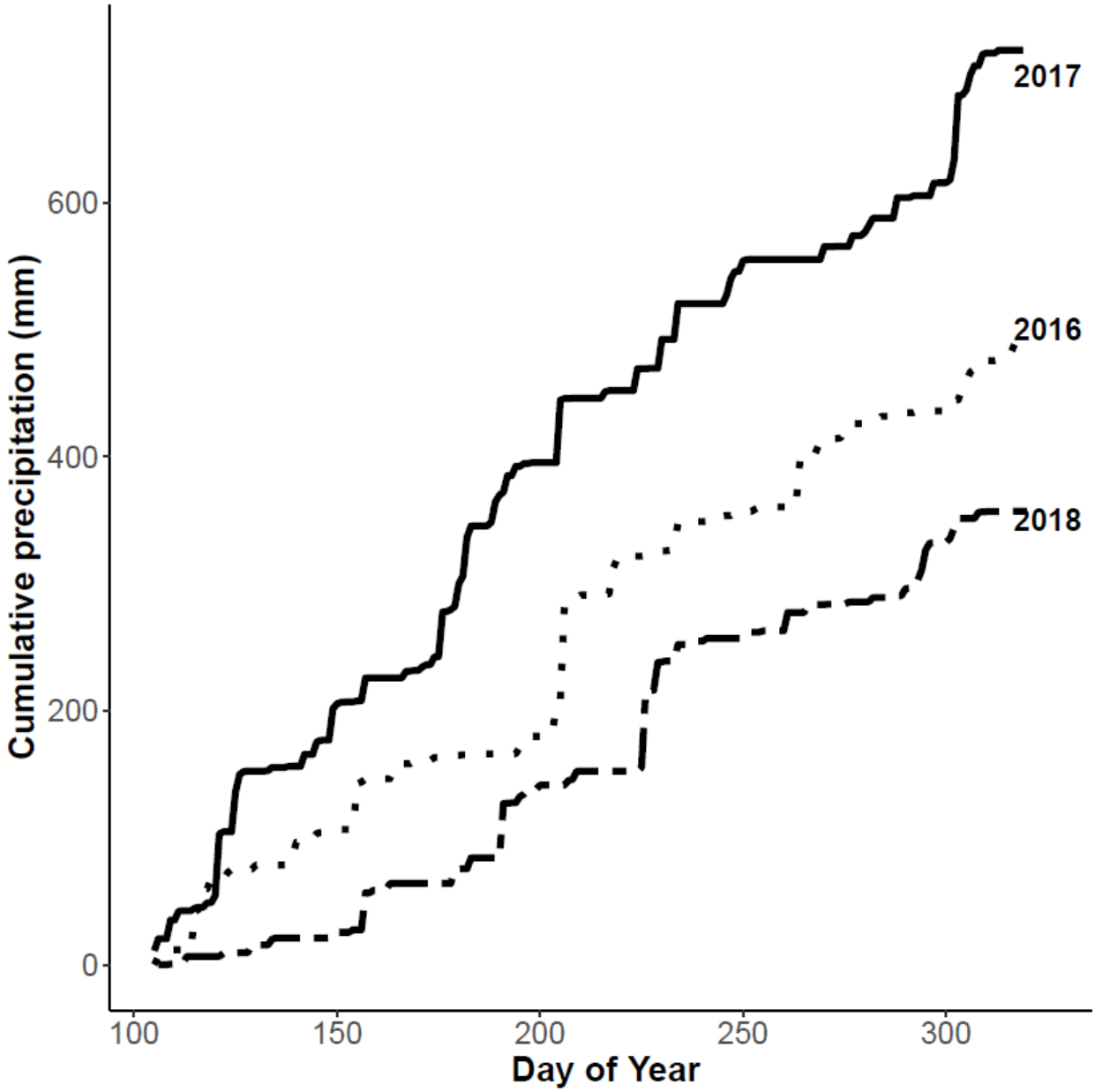


Fig. S4. Cumulative precipitation at the field site over the study years (2016-2018).

Appendix 5: Seedling vitality score

Supplementary Fig. S5. Examples of soybean plants from each vitality score (1-6) rating where: 1, no germination; 2, broken-off cotyledons and signs of necrosis on roots; 3, seedlings with signs of necrosis on the cotyledons and stunted unifoliate emergence; 4, seedlings with signs of necrosis on the cotyledons and minimal stunting of the unifoliates; 5 seedlings with minimal cotyledon necrosis and healthy unifoliate development; 6, healthy seedlings with no signs of disease. Soybean plants were harvested after 21 days of growth in field soil at 15 ˚C constant temperature at 80% of saturated pot capacity. Scale bar = 1 cm.