**Variation in soybean rhizosphere oomycete communities from Michigan fields with contrasting disease pressures.** Zachary A. Noel, Hao-Xun Chang, Martin I. Chilvers

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**Abstract**

Noel Z.A., Chang, HX., Chilvers M.I., **Variation in soybean rhizosphere oomycete communities from Michigan fields with contrasting disease pressures.**

Many oomycete species can contribute to significant losses in soybean plant density and root mass. In this study, soybean oomycete rhizosphere communities were characterized from a location with and without historical disease pressure to examine the effect of location, soybean genotype, and seed treatment on oomycete communities. Soybean oomycete rhizosphere communities were dominated by *Pythium* and *Pythium ultimum* var. *ultimum* was the most abundant oomycete OTU making up on average > 30 % relative abundance in high disease pressure and low disease pressure sites, indicating that historical disease pressure was not due to the presence or absence of highly pathogenic species, but perhaps an imbalance of species. High-disease pressure sites contained more species and were less even in 2015. There was no evidence of seed treatment or genotype on oomycete community despite the improvement in plant density and root biomass in high-disease pressure sites. However, interestingly seed treatment increased with the addition of insecticides, potentially pointing to intertrophic interactions with oomycetes and seedling death. Overall, this study represents an extensive survey of oomycete communities in soybean rhizosphere soils from Michigan.

**Introduction**

Soybean (*Glycine max* (L.) Merr) is regarded as a key crop for global food security (Singh et al. 2007) with a worldwide harvest of 223 million tons and it is ranked the fourth most important crop worldwide (Hartman, et al. 2011). Successful seed germination and emergence are important for soybean establishment in fields, but a number of pathogens can kill soybean seeds before emergence or can reduce root biomass of soybean seedings leading to stunting and eventually yield loss. One of the most destructive pathogens are oomycetes such as the genera *Pythium* and *Phytophthora* that can infect host in both seed or seedling stages. Symptoms of seed rot can range from necrotic lesions on a germinating seed to a totally dead seed colonized by oomycetes, and symptoms of seeding rot can include root mass reduction and water-soaked lesions along the hypocotyl and stem, which may result in seedling stunting, reduced seedling vigor, or death. Moreover, seedling rot can negatively influence yield even when the symptoms are not severe to cause plant death (Martin, 2009; LéVesque 2011). Some growers favor minimum or no-till, which increases the crop debris left over from the previous season, and/or favor planting soybean earlier for increasing growing days and yield (Vossenkemper et al. 2015). Both agricultural practices can increase exposure of seed and developing seedlings to conditions favoring rotting diseases due to slow soil warming and increased organic debris act as reservoirs that can harbor inoculum (Pankhurst 1995; Larkin 2015).

In previous studies, over 80 oomycetes species belonging to genera *Phytophthora*, *Pythium*, *Phytopythium*, and *Aphanomyces* have been identified to be associated with soybean (Rojas et al. 2017ab; Broders et al. 2009; Zitnick-Anderson and Nelson 2015). While some oomycetes such as *Phytophthora sojae* or *Pythium ultimum* are well known to be pathogenic, others are mycoparasitic or entomopathogenic (Martin and Loper 1999, Paul et. al. 1999, Su et al., 2001; Sholte et al. 2004, Ribeiro and Butler, 1995) suggesting potential multitrophic and complicated interactions among plant, oomycetes community, and other organisms such as agricultural pests. Under this circumstance, disease occurrence should be understood from a community point of view, and disease management need to be precise for the oomycetes that are involved in pathogenesis in this agroecosystem. Therefore, studying the oomycete community and its association with the disease severity or agricultural practices will provide advanced information for oomicide efficacy screening and resistance breeding to achieve precision agriculture.

While traditional culture-based surveys have been used to survey oomycetes communities, a major disadvantage of this methodology is the labor needed for pathogen isolation, characterization, and maintenance (Rojas et al. 2017a, Coffua et al., 2016). Culture-based survey may also have biases by the isolation protocol or the culture medium used, and it is also known that some oomycete species are fastidious or hard to culture (Bakker et al, 2017). An alternative methodology is the culture-independent approach using high-throughput amplicon sequencing or metabarcoding, which has been widely used technique to provide a more comprehensive glimpse of the composition of a microbial community (REF). Metabarcoding studies of bacteria or fungi have been applied in profiling microbial community to understand the association between microbial community and traits of interest (REF), but metabarcoding studies of oomycetes are less common despite their importance in plant disease, ecosystem functioning, and community assembly (Agler et al. 2016). With a curated oomycete ITS database (Robideau et al. 2011) and improved strategies to preferentially amplify oomycetes ITS sequences from environmental samples (Sapkota and Nicolaisen 2015, Riit et al. 2016, Taheri et al. 2017), there is an increasing interest and ability to characterize oomycete communities using metabarcoding (Rojas et al., 2019; Agler et al. 2016; Coince et al. 2013; Vannini et al. 2013; Sapkota and Nicolaisen 2015; Singer et al. 2016; Bakker et al. 2017; Riit et al, 2016; Duran et al. 2018; Coffua et al., 2016). Applying metabarcoding strategy to profile oomycetes in field samples would be a fast approach to provide in-depth understanding of the structure of oomycetes community for rotting diseases of soybean.

There are several factors affecting the structure of oomycetes community, and it has been recognized that location and edaphic factors drive oomycete community predominantly (Rojas et al. 2017ab; Broders et al. 2009; Zitnick-Anderson and Nelson 2015, Taheri et al. 2017). However other agronomic factors have not been examined in detail. For example, soybean genotypes have been shown to recruit different beneficial microbial taxa (Mendes et al., 2014), and there are inter- and intraspecific variation in sensitivity to anti-oomycete chemicals (oomicides) used within soybean seed treatments (Broders et al. 2007; Matthiesen et. al. 2016; Noel et al. *in press*; Radmer et al. 2016; Weiland et al. 2014) suggesting the possibility of a specific oomycete lineages being selected or counter-selected in the soybean rhizosphere in the presence in different oomicide-genotype combinations. Moreover, because soybean seed treatments usually contain oomicides along with a number of other active ingredients such as fungicides, nematicides, or insecticides, the likelihood of these chemicals on influencing seedling rot diseases or shaping the structure of oomycete communities is considerable. For example, soybean seed treatments have been observed to be more effective and consistent in field sites in Allegan county of Michigan, where heavy oomycete damage has been observed (Rossman et al. 2018). Herein, this study aimed to provide a profile of the oomycete community present in soybean rhizosphere soils and compare the structure of oomycetes communities between high disease pressure fields in Allegan county and low disease pressure field sites in Ingham county of Michigan. This study characterized oomycete communities between these two counties in two years, investigated the association between oomycetes community and disease severity as well as seed treatments, and identified the taxonomic difference of the oomycete that link to the disease pressures between these sites.

**Materials and Methods**

**Experimental design and field setup.** Field experiments were set up in two locations, Allegan county (with high disease stress) and Ingham county (with low disease stress) of Michigan, in two years (2015 and 2016). In each location-year combination, a complete randomized factorialdesign with four soybean genotypes, four treatments, and six replicates was set up in plots (6.10 m by 3.05 m), resulted in a total of 96 plots in each of four location-year combinations. Full seed treatment formulations and application rates were described in Rossman et al (2018). In brief, seed treatments used in this study were generalized based on the target pests, herein abbreviated as non-treated control (NTC), fungicides (F), fungicides plus insecticides (FI), and fungicides plus insecticides plus a biological control nematode protectant (FIN) (Supplemental table 1). Soybeanseedswere planted 3.8 cm below ground, in six rows with 38 cm row spacing, and at a seeding rate of 395,000 seeds ha-1. In all locations, the crop planted in the previous growing season was corn. The coordinates, plating dates, and plant sampling dates for each location-year combination along with bulk soil texture and nutrient levels for each location-year as characterized by the MSU Soil and Plant Nutrient Laboratory were documented (Table 1)

**Sample collection.** For each location-year combination, three measurements were taken for representing disease stresses, including plant density, root biomass, and yield. The four middle rows in each six-row plot were harvested for yield quantification in the end of season, but the plant density was measured by counting the number of emerged soybeans in two of the four harvested rows of each plot at the V1 growth stage. Meanwhile, rhizosphere samples were collected from two side rows (non-harvested rows), and ten random emerged plants (excluding plants in 2.74 meters from either end of a row) were collected in each non-harvested row. Loosely adhering soil was shaken from the roots, and these twenty plants were pooled to represent a plot and stored together on ice to be transported to the lab for processing in the following day. Root tissue was determined based on the soil line, and ten random roots were cut, washed with tap water, and dried before measuring the root biomass. The remaining ten roots were used for rhizosphere soil collection. Rhizosphere soil was washed from roots by vortexing for 15 seconds in a 50ml tube with 35ml 10mM NaCl solution (Shakya et al., 2013). Roots were removed from the tube and the remaining NaCl solution was centrifuged for 10 minutes at 3500 rpm to pellet rhizosphere soil. The supernatant was decanted, and the rhizosphere pellet was frozen at -20ºC then lyophilized and stored in sterile coin envelopes with desiccants prior to DNA extraction.

**Oomycetes ITS1 amplification and sequencing.** For rhizosphere soil samples, total DNA was extracted from 0.35 g of lyophilized rhizosphere soils using the Qiagen PowerMag® Soil DNA Isolation Kit (Toronto, ON, Canada) following the manufacturer's recommendations. A water control and artificial oomycetes community (Rojas et al. 2019) containing 15 species mixed equivalently and adjusted to a final concentration of 0.05 ng/µL were included in polymerase chain reaction (PCR) amplification for internal transcribed spacer 1 (ITS1) of oomycetes using a three-step PCR program modified based on the protocol from Lundberg et al. (2013).

In the PCR step one, samples were amplified using primers ITS6 (5’-GAAGGTGAAGTCGTAACAAGG-3’) and ITS7 (5’-AGCGTTCTTCATCGATGT-3’) (Cooke et al. 2000) with an annealing temperature of 59ºC, which preferentially amplifies oomycetes ITS1 while minimizes fungal ITS contamination (Sapkota and Nicolaisen 2015). In the PCR step two and step three, ITS1 amplicons were amplified by frameshift primers and then by a ten bp barcode plus Illumina adapters, respectfully. All PCR steps contained a final concentration of 1X buffer, 0.2mM dNTP, 0.8mg ml-1 bovine serum albumin (BSA), 0.2 μM primers and 1 U DreamTaq Polymerase (ThermoFisher Scientific, USA) and 2 μl DNA template for the PCR step one and step two. The PCR step three contained 4 μl of aliquots from PCR step two. Thermal cycling conditions for PCR step one were as followed: 95ºC for 5 min followed by 15 cycles of 95ºC for 15 seconds, 59ºC for 30 seconds and 72ºC for 30 seconds followed by a final extension at 72ºC for 7 min. Thermal cycling conditions for PCR step two were as followed: 95ºC for 5 min followed by 10 cycles of 95ºC for 20 seconds, 57ºC for 30 seconds and 72ºC for 35 seconds followed by a final extension at 72ºC for 7 min. Thermal cycling conditions for PCR step three were as followed: 95ºC for 5 min followed by 10 cycles of 95ºC for 20 seconds, 63ºC for 50 seconds and 72ºC for 1 minute 20 seconds followed by a final extension at 72ºC for 7 min. PCR products were normalized using SequalPrepTM Normalization Plate Kit (ThermoFisher Scientific, USA), pooled then concentrated 20:1 with Amicon® Ultra 0.5 mL filters (EMDmillipore, Germany). The amplicon library was purified, and size selected with Agencourt AMPure XP magnetic beads at 0.6X sample to bead volume (Beckman Coulter, USA) and subsequently paired-end sequenced in 250 bp on an Illumina MiSeq using the v2 500 cycles kit (Illumina, USA).

**Data processing.** ITS1 paired-end reads were quality evaluated with FastQC and then demultiplexed according sample barcodes in QIIME 1.9.1 (Caporaso et al., 2010), and primers were removed from reads with Cutadapt 1.8.1 (Martin 2011), and then quality filtered using USEARCH 9.1.13 (Edgar 2010) based on read quality and expected error threshold obtained from VSEARCH stats 2.3.2 (Rognes et al. 2016). Qualified reads were then trimmed to equal length and singletons were removed using USEARCH 9.1.13 (Edger 2010). De novo OTU clustering was performed based on 97% similarity using the UPARSE algorithm, which includes a chimera detection step (Edger 2013). An OTU table was generated using a custom python script and taxonomy was assigned to each OTU using CONSTAX with a confidence threshold of 80 % (Gdanetz et al. 2017), which is a robust algorithm that generates a consensus taxonomy from the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al. 2007), UTAX (Edgar 2013), and SINTAX (Edgar 2016). The reference database used for taxonomy assignment included the curated oomycete ITS sequences from Robideau et al. (2011) and the UNITE version 7.2 1.12.2017 fungal database (UNITE community, 2017). OTUs that were identified as fungal were removed from further analysis. OTU sequences identified in the phylum Oomycota were BLAST search against the NCBI nucleotide database (accessed January 2019) to corroborate taxonomy assignments. If CONSTAX assigned an OTU to a species or if the top BLAST matched an OTU sequence to a species with over 90 % identity and a bitscore ≥ 300, the OTU was grouped to oomycete clades according to Robideau et al. (2011) and LéVesque and De Cock (2004). Samples with less than 1000 reads were dropped from analysis due to low sequencing coverage.

**Statistical Analysis.** Data were imported into R 3.2.2 (R core team 2016) and analyzed using the packages ‘phyloseq’ 1.24.2 (McMurdie and Holmes, 2013) and ‘vegan’ 2.5.3 (Oksanen et al. 2018). All samples were rarefied to an even depth before analysis. Alpha-diversity was estimated for each sample and only OTUs observed more than once were used before estimating α-diversity. OTU richness, evenness, and Shannon diversity was used as α-diversity metrics. To examine differences in beta-diversity between samples, non-metric multidimensional scaling (NMDS) (k = 2) was performed on Bray-Curtis distances (Bray and Curtis, 1957). A permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis distances, was used to test for differences in community centroids using the ‘adonis2’ function in the package ‘vegan’. Differences in community multivariate dispersion were tested with the ‘betadisp’ function in the R package ‘vegan’. Stepwise model building was used to select a constrained model for input into distance-based redundancy analysis (db-RDA) to examine the variation in Bray-Curtis distances due to plant density, root biomass, and yield. A Monte-Carlo permutation test was used to test for significance of constrained factors within db-RDA. Indicator species analyses was performed using the package ‘indicspecies’ 1.7.6 to identify OTUs significantly associated with covariates.

**Data availability.** OTU table, metadata, and taxonomy files along with code are available on (<https://github.com/noelzach/XXXXXX>). Raw sequenced data was deposited in the Sequence Read Archive (SRA) SRA-ID.

**Results**

**Overview of experimental design and factors.** A two-year field study in two locations where one with high disease stress (Allegan county of Michigan) and another with low disease stress (Ingham county of Michigan) was established to understand the association among genotypes, seed treatments, and oomycete rhizosphere communities to the severity of soybean root and seed rotting diseases. Among three disease severity measurements, plant density was the most consistent indicator as it was lower in Allegan than Ingham in both years, especially for 2015 where Allegan had on average 17.62 plants m-2 compared to Ingham which had on average 31.72 plants m-2 (Figure 1A). Root biomass and yield reflected this tendency, but the reduction of root biomass and yield in Allegan was more evident in 2015 than 2016 (Figure 1B and 1C).

When the seed treatments were applied, plant density and root biomass in Allegan was significantly higher for the FIN treatment compared to the NTC for all genotypes tested for both years. However, no significant improvement rescue in either plant density or root biomass was observed when only F were applied in Allegan, which indicated the influence of FI or FIN interaction was more important in determining the outcome of plant density and root biomass, rather than just fungicide or fungicide-insecticide. There was no significant improvement in plant density, root weight, or yield due to seed treatment in Ingham regardless of soybean genotype (Supplemental Table 2)

**Oomycete community composition in soybean rhizospheres.** In respect to the importance of oomycetes in the disease severity difference between Allegan and Ingham counties (Rossman et al., 2018),an ITS-amplicon sequencing strategy was applied to illuminate the structure of oomycetes community between these two locations. A total of 2,628,469 quality filtered reads were obtained, and after data processing, reads were clustered into 621 OTUs. Among these OTUs, 230 OTUs were assigned to the kingdom Stramenopila; and 219 OTUs were classified into phylum Oomycota using CONSTAX. In summary, 219 oomycete OTUs were identified from a total of 361 rhizosphere samples from Allegan and Ingham.

The most abundant genus was *Pythium* at 86.3 % across the rhizosphere samples. *Phytophthora* comprised of 3.2 % and the genera *Lagenidium*, *Apodachlya*, *Albugo*, *Plasmopara*, *Phytopythium*, *Peronospora*, *Hyaloperonospora*, *Brevilegnia*, *Plectospira*, and *Achlya* together comprised of 1.8 % across rhizosphere samples, and 8.7 % of the OTUs were not confidently assigned to an oomycete genus (Fig. 2A). Pythium clade I was the most abundant clade in Ingham 2015, Allegan 2015, and Allegan 2016 making up 66.3 %, 55.0 %, and 44.4 % across the rhizosphere samples. In Ingham 2016, Pythium clade F (including notable pathogen species like *Pythium irregulare* and *Pythium sylvaticum*) was most abundant making up 41.1 % of the reads (Fig. 2C). The most abundant OTU was identified as *Pythium ultimum* var*. ultimum* (OTU1 in Pythium clade I) and was found in Ingham 2015, Allegan 2015, and Allegan 2016 (Fig. 2D), while an unknown Pythium species (OTU2 in Pythium clade F) was the most abundant in Ingham 2016 (Fig. 2D). Other frequently observed OTUs were identified as *Pythium heterothallicum* (OTU 3 and 7 in Pythium clade I), which was present in all location-year combinations.

**α-diversity** **analysis for Allegan and Ingham.** In order to understand the oomycete communities, α-diversity were estimated for each year-location combination. There was no significant difference in Shannon index (H’) between locations (Fig. 3A); however, when the diversity was breaking down into Plieou’s evenness (J) and richness (S), the richness was significantly higher in Allegan than Ingham in 2015 (*P*  < 0.0001) (Fig. 3B) whereas the evenness was significant lower in Allegan than Ingham in 2015 (*P* < 0.01) (Figure 3C). In addition, there were no significant differences in α-diversity metrics due to variety or seed treatment within any location-year combination, suggesting the α-diversity of oomycetes community may be relatively similar and not being affected by soybean variety or seed treatment.

**β-diversity analysis and identification of unique oomycete communities in Allegan.** Rhizosphere communities were highly clustered based on location and year and the interaction contained significantly different centroids (*P* < 0.001) and multivariate dispersion (*P* < 0.001) (Fig. 4A). Similar to α-diversity, neither soybean genotypes nor seed treatment influenced β-diversity. Most oomycetes OTUs were associated with multiple year-location combinations, however, there were 21 OTUs uniquely associated with Allegan 2015 (Fig. 4B; Table 2), 9 OTUs in Allegan 2016, and 5 OTUs in Allegan both 2015 and 2016 but not in Ingham (Supplemental Table 3). These 21 OTUs, unique to Allegan 2015 included OTUs identified as *Pythium ultimum* var. *ultimum*, *Pythium heterothallicum*, *Pythium irregulare*, and *Laganidium giganteum*, and *Pythiaceae* sp. which added up to 5.61% relative abundance (Table 2).

Focusing on Allegan 2015, a model selection in the distance-based redundancy analysis (db-RDA) pointed out a significant association between oomycetes community and plant density (*P <* 0.001) and root biomass (*P <* 0.005) but not yield using Monte-Carlo permutation test. However, only 3.89 % of the total variation in oomycete communities could be attributed to plant density and root biomass. Rhizosphere samples from plots with increased plant density and root biomass were associated with positive db-RDA1 scores. Rhizosphere samples from plots with increased root biomass were more associated with positive db-RDA2 scores whereas samples with increased plant density was more associated with negative db-RDA2 scores (Fig. 5A).

Among plots in Allegan 2015, OTU18 *Pythium* sp. nov (Clade B) was significantly associated with higher plant density and OTU41 *Pythium ultimum* var. *ultimum* (Clade I) was significantly associated with higher root biomass. On the other hand, OTU135 *Saprolegniaceae* sp. was significantly associated with lower plant density and OTU71 Oomycete sp. was significantly associated with lower root biomass (Table 3). Among these OTUs identified from indicator species analysis, OTU41 and OTU71 were also found to be unique to Allegan 2015 (Table 2), which indicating their potential importance in the association between oomycetes communities and disease severity at Allegan.

**Discussion**

This study was motivated from the observation of increased and consistent seedling disease in Allegan field sites compared with Ingham. Therefore, a two-year field study was conducted to profile oomycete communities from over 300 soybean rhizosphere soils. Consistent with previous observations (Rossman et al. 2018) disease pressure was greater in Allegan than in Ingham, especially in 2015 where plant density and root biomass was significantly reduced compared to Ingham (Fig 1). Rhizosphere soils were dominated by *Pythium* and community profiles were different depending on location and year (Fig. 4A). Disease symptoms most consistent with oomycete pressure were most prominent in Allegan 2015 and oomycete communities were associated with variation in plant density and root biomass in Allegan 2015, and unique OTUs associated with high disease pressure were identified.

In all location-year combinations, oomycete communities in soybean rhizosphere samples were dominated by *Pythium*. Notably, this included notable pathogenic species like *Pythium ultimum* var. *ultimum* and *Pythium heterothallicum* and putatively beneficial oomycetes (i.e. mycoparasites). For example, OTU4 was identified as a *Pythium* sp. in clade D with a 100 % match to *Pythium oligandrum* and had 3.62 and 3.51 % mean relative abundance in Allegan 2015 and Ingham 2016. *Pythium oligandrum*, *Pythium acanthicum*, and *Pythium periplocum* are well-known soil dwelling antagonists of fungi and oomycetes in clade D (Martin and Loper 1999, Paul et. al. 1999, Ribeiro and Butler, 1995).

The observation of *Pythium* dominance corroborates observations of other culture-based and culture independent metabarcoding studies where *Pythium* was dominate in agricultural soils (Rojas et al. 2017ab; Broders et al. 2009; Taheri et al. 2017; Coince et al. 2013; Vannini et al. 2013; Sapkota and Nicolaisen 2015; Singer et al. 2016; Bakker et al. 2017; Riit et al, 2016; Duran et al. 2018; Coffua et al., 2016; Sapp et al., 2018; Schlatter et al. 2017). Historically, soybean breeding efforts have primarily focused on *Phytophthora sojae* because of soybean’s gene-for-gene interaction (Dorrance and Grunwald 2009). There have been studies focused on *Pythium* resistance breeding (Rosso et al. 2008; Rupe et al. 2011; Kirkpatrick et al 2006; Lin et al., 2018; Sasko et al 2016; Ellis et al. 2013) but genetic resistance not known to be or is not intentionally applied in the field like for *Phytophthora sojae*.

Despite the lack of observed disease pressure in Ingham, it was not because pathogenic species were absent. The most abundant OTUs in Ingham 2015 was identified as *Pythium ultimum var. ultimum*, and *Pythium heterothallicum*, yet little disease was observed. Allegan in 2015 was on average less even than Ingham 2015 indicating that although Allegan 2015 contained more OTUs than Ingham 2015, rhizosphere samples were dominated by a few taxa. The most abundant OTU in Allegan 2015 was identified as *Pythium ultimum var. ultimum*. This species is a well-known opportunistic plant pathogen notorious for infecting plants at early developmental stages and under stress. According to PRISM Climate Group database (Prism Climate Group, Oregon State University, 2016) Allegan county Michigan in 2015 experienced 26.86 mm rain two days after planting compared to no rain in Ingham 2015 two days following planting. This may have increased favorable conditions for oomycete growth and stressed germinating seeds*.*

There were 21 OTUs found to be unique to Allegan 2015 significantly associated with oomycetes community based on indicator species analysis. Notably, OTU41, also identified as *Pythium ultimum* var. *ultimum* was significantly associated with higher than average plant density in Allegan 2015. This may indicate that the increased resource availability provided by increased plant density and root mass is attractive to fast growing oomycetes. Interestingly, OTU1 was also identified as *Pythium ultimum var. ultimum* and was the most abundant in Allegan 2015 but was associated with both high and low plant density. Based on these data it could be hypothesized that with increased niche space provided by increased plant density allows for specific *Pythium ultimum* genotypes to coexist.

The results of the db-RDA indicated that although small, some variation in plant density and root biomass could be attributed to oomycete community composition in Allegan 2015. Other edaphic factors such as, soil pH and soil temperature could also explain why disease was not observed in Ingham field sites as these factors can influence pathogenicity (Martin and Loper 1999; Rojas et al. 2017a). It is possible that disease stress is not simply resulted from the present or absence of some pathogens, instead, it depends on the abundance of pathogens along with possibly a multitrophic or synergistic interaction between these unique oomycetes to other organisms. Another observation to support this statement is that when the seed treatments were applied, plant density and root biomass in Allegan was significantly higher for the (FIN) treatment compared to the non-treatment control (NTC) but not for the F or FI seed treatments (Supplemental Table 2). On the other hand, there was no significant improvement in plant density, root weight, or yield due to seed treatment in Ingham regardless of soybean genotype (Supplemental Table 2). These results indicated the possibility of soil pests (insects or nematodes) feeding on roots may provide higher risk of oomycetes infection and disease stress. Interestingly, two OTUs identified as *Lagnidium giganteum* were unique to Allegan 2015 (Table 4). Members of the Lagnidium genus are known pathogens of animal hosts and the presence of these isolates along with the observation of increased plant stand with insecticides is intriguing. Facilitation of plant death by pathogenic oomycetes may be influenced by the presence of insects or nematode damage allowing easier entry into plant tissue (Graham and McNeill, 1972; Willsey et. al., 2017). Neonicotinoid insecticides that do not have activity against oomycetes directly may indirectly influence oomycete communities. Furthermore, neonicotinoid insecticides can induce systemic acquired resistance (SAR) and prime plant defenses (Ford et al. 2010). Insect larval root feeding injury, presumably from seedcorn maggot (*Delia platura*) has been observed in Allegan field sites, but an extensive insect surveys were not conducted (Rossman et al. 2018). Additional study using metagenome sequencing may reveal other pests or organisms in Allegan, and analyses on multiple organisms together with oomycetes may improve the explanation of variance.

Oomycetes are important drivers of community assembly but are often overlooked and an understudied portion of the microbiome (Agler et al., 2016). This study represents a survey of oomycete communities from a location previously observed to have high disease pressure compared to one that did not. Communities were different based on location, however field sites without historical disease pressure were not devoid of pathogenic oomycetes. Therefore, we hypothesize that oomycete interactions with edaphic factors, weather conditions at planting, and possible interactions with other soil dwelling organisms are responsible for the disease pressure observed in Allegan. It will be important for future studies to document differences in these communities. Nonetheless, this study improves our understanding of oomycete diversity in soybean rhizosphere which will aid in recommendations for plant breeders and oomicide recommendations.

**Acknowledgements**

This project was supported by Agriculture and Food Research Initiative competitive grant number 2011-68004-30104 from the USDA National Institute of Food and Agriculture, Michigan Soybean Promotion Committee, United Soybean Board, North Central Soybean Research Program. We would also like to acknowledge Steven Gower from Asgrow, Phil Schneider and Kerrek Griffes from Gratiot Agricultural Professional Services, and Karen Zuver from Pioneer for supplying the seed used in this study. We would like to thank John Boyse and Randy Laurenz for management of the field trial plots. We would also like to thank X and X for critical review of this manuscript.

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**Tables and Figures**

Figure 1. The effect of location tested within year on plant density (A), root biomass (B) and yield (C) of plants from non-treated seed across all genotypes. T-test P value is shown within each figure.

Figure 2. (A) Genus-level relative abundance of oomycete communities for each year-location combination. (B) Relative abundance of putative life history categories. (C) Clade-level relative abundance of oomycete OTUs in the genera *Pythium*, *Phytophthora*, and *Phytopythium*. (D) Mean relative abundance of OTUs where the OTU was observed greater than 2 % mean relative abundance at least one site.

Figure 3. Influence of location on oomycete alpha diversity within year as estimated by (A) Shannon diversity index (*H*’) and (B) richness (S) and (C) Plieou’s evenness (J). T-test P value is shown within each figure.

Figure 4. Between sample diversity of oomycete rhizosphere communities. (A) Non-metric multidimensional scaling ordination of soybean rhizosphere oomycete communities based on log-transformed and Wisconsin double standardized Bray-Curtis distances. Point shapes represent year (2015 or 2016) sampled and color represents location (Ingham or Allegan). Ellipses represent 95 % confidence interval of a multivariate normal distribution for each year-location combination. (B) Venn-diagram or the number of OTUs significantly associated with each year-location combination.

Figure 5. Association of oomycete communities with plant density and root biomass in Allegan 2015. (A) Distance based redundancy analysis (db-RDA) of rhizosphere oomycete communities based on log-transformed and Wisconsin double standardized Bray-Curtis distances in Allegan 2015. Variation in root biomass and plant density were significantly associated with oomycete community composition based on a Monte Carlo permutation test. Arrows represent direction of increasing root biomass and plant density. Points represent plots sampled are scaled to the mean root biomass per plant and colored by mean plant density.

Table 1. Field location and soil properties description

Table 2. OTUs significantly associated uniquely with Allegan 2015

Table 3. OTUs significantly associated with high or lower than average plant density or root biomass

Supplemental Table 1. Seed treatment and variety descriptions

Supplemental table 2. Effect of seed treatment on plant density, root biomass, and yield for each location, year, genotype combination

Supplemental table 3. Indicator OTUs associated with each location-year combination