Mid-Year Report 2021
Pennsylvania Soybean On-Farm Network

Paul Esker, Delbert Voight, Andrew Frankenfield, Heidi Reed, Liz Bosak, Alyssa Collins, and Terry Bell, Co-PIs

In 2021, our on-farm network is focused on no-till deep ripping for soybeans, expanding cover crop options after soybeans, Ilevo seed treatment trials, good inoculation practices, and slug monitoring. We also tried to establish a series of new trials on the use of microbial compounds in soybean. Unfortunately, agreements could not be made between Penn State and the company.

As a team, we continued to deal with COVID-19 restrictions, which while less than in 2020, still impacted our ability to establish some trials as planned. In other ways though, we were able to increase our workloads in the laboratory, which is helping to process samples more efficiently this year.

Slug Monitoring Project: Since 2018, Penn State Extension Educators in eighteen counties have monitored slug populations in over thirty field sites. Problem slug fields were identified by the cooperating farmer. Slug traps were placed in each field to monitor juvenile and adult slug species each week before planting. After the crop emerged, crop damage was monitored.

For all four seasons, none of the monitored fields required replanting due to excessive damage by slugs. Crop damage was assessed by looking at each individual plant in ten row feet and scoring the damage at 0, 25%, 50%, or 75% leaf area removed. The average crop damage for all four years never exceeded 25%. Slug populations remained low for most fields in 2021. However, there were higher numbers of gray garden slugs reported compared to the previous three years. Unfortunately, using prior history does not necessarily predict a problem slug year in subsequent years for all fields. Some fields with a prior history continue to have higher slug numbers but this is not always the case. This fall, fields will be monitored again after harvest for slug populations and any feeding damage on cover crops.

Weekly reports from 2021 can be accessed at https://extension.psu.edu/2021-pennsylvania-slug-monitoring-project .

Good Inoculation Practices: There are two main locations one in Rock Springs and the other is at the Southeast AG Center. These two locations are under full control of the researchers and have 6 replications of each treatment. In addition to the two main locations there is one replicated study at the Daren Grumbine farm. Despite best intentions the two other on farm locations were not planted and will not be included in fall harvest results. At this point planting occurred on time with excellent stands resulting. Assessment data has been collected by the intern Derek Metcalf. Assessment data included pop up and mid-season population, pop up and

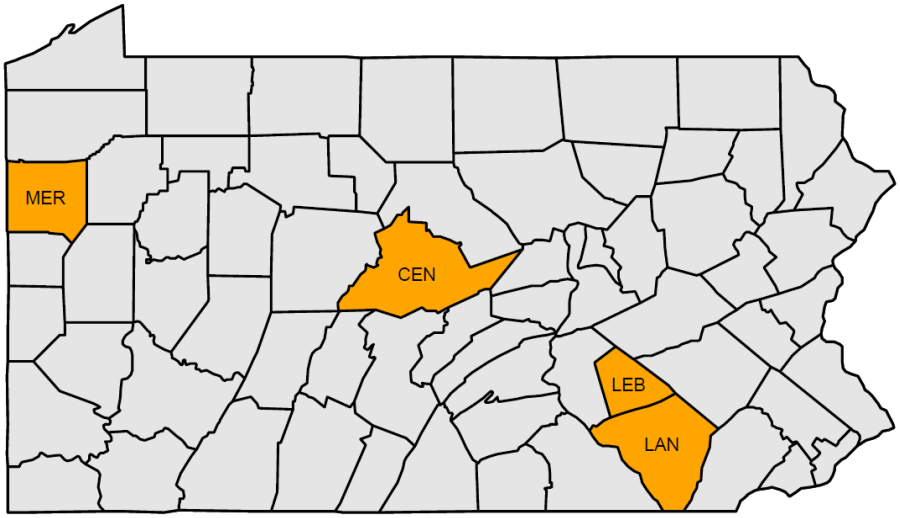
mid-season height, pop up and mid-season nodulation counts as well as mid-season chlorophyl and NDVI measurements. All that remains in the harvest activity to begin observing any results.

No-till Deep Ripping for Soybeans:

Expanding cover crop options after soybeans: So far for the 2021-2022 growing season, the research farms have been flagged and are ready for broadcast as soon as they hit R6. We have at least one cooperator signed up in Lancaster, who plans to use a drone to seed. We have approximately three additional leads for cooperators in other counties but are hoping to finalize agreements. The aim is to do broadcasting at the end of August - mid September, compared to late September-October last year. A simplified summary of 2020-2021 was in FCN a few weeks ago.

Ilevo seed treatment trials: Trials in 2021 were established in: (1) Centre County, (2) Lebanon County, (3) Lancaster County, (4), Mercer County 1, and (5) Mercer County 2. Bulk soil samples were collected from each site prior to planting and were used for determining soilborne fungal pathogen density, plant parasitic nematode density and soil nutrient profile. Sampling of root ball samples for microbiome work was carried at VE, VC and V1 stages using a selected block. Green seeker readings a were recorded at R2 growth stage to evaluate normalized difference vegetation index (NDVI) to determine crop health. At R2 growth stage, the initial plantstand of each Ilevo treated and control plot was recorded. Fifteen plants per plot were collected for destructive measurements and were evaluated in the lab for disease incidence. At harvest, yield from each plot will be collected separately.

We are currently receiving data from educators for non-destructive measurements and microbiome samples are also being processed. Data will be analyzed soon after we have a complete data set.



Additional trials:

*Saved Seed Trial***:** There is one main location at the Southeast Research Center to assess the viability of using saved soybean seed and resulting cost savings. In addition to the main locations there are 4 locations where growers are comparing their own soybean varieties to the saved seed delivered to them. The locations are in Montgomery, Schuylkill, and two in the Lebanon area. Data collected is the same as the GIP trial for the southeast center and with 6 replications. However, on farm locations will only have yield data to determine relative ROI. The saved seed costs about $13.00 per bag and additional $3.50 for cleaning charge as well as a $6.00 per bag cost to treat with Apron Max and Cruiser. The grand total of 22.50 per bag is realized compared to the 60 plus for a unit of seed from industry there would need to be about a 3 bu difference between competitive varieties to the saved seed. The fit for Pa is for the double crop market where the risk is much higher in planting later after wheat and or barley and may prove economical for soybean growers to consider particularly as seed costs escalate. The only observed aspect is that the seed germ for the saved seed was nearly 98% vs the 85% reported on the purchased soybean seed for this season.

Uniform fungicide trials: We have established two uniform fungicide trials at our primary research farms. These are looking at a combination of foliar fungicides applied at the early pod growth stage, along with one in-furrow treatment. We are currently taking field notes, but disease intensity to date is low. Currently weather conditions though are favorable for an increase in foliar disease.

Publications and Presentations (provided at end of report):

Bandara, A., Weerasooriya, D., Bell, T., and Esker, P. 2021. Collective impact of agronomic practices on the diversity and abundance of pathogenic and beneficial fungal genera in soil. American Phytopathological Society Plant Health 21 Online.

Bandara, A., Weerasooriya, D., Bell, T., and Esker, P. 2021. Differential effect of agronomic practices on the diversity and abundance of beneficial bacterial genera in soil. American Phytopathological Society Plant Health 21 Online.

Bandara, A., Weerasooriya, D., Bell, T., and Esker, P. 2021. Soil nutrients and texture affect the diversity and abundance of pathogenic and beneficial fungal genera in soil. American Phytopathological Society Plant Health 21 Online.

Bandara, A., Weerasooriya, D., Bell, T., and Esker, P. 2021. Soil texture and nutrients influence the diversity and abundance of beneficial bacterial genera in agricultural fields. American Phytopathological Society Plant Health 21 Online.

Weerasooriya, D.K., Bandara, A.Y., and Esker, P.D. 2021. Species composition and genetic diversity of soilborne *Fusarium* species. American Phytopathological Society Plant Health 21 Online.

Weerasooriya, D.K., Bandara, A.Y., and Esker, P.D. 2021. Pathogenicity of soilborne *Fusarium* spp. from Pennsylvania. American Phytopathological Society Plant Health 21 Online.

Weerasooriya, D.K., Bandara, A.Y., Maggio, J., Mowery, I., and Esker, P.D. 2021. Effectiveness of seed-applied fungicides for managing soybean seedling diseases in Pennsylvania. American Phytopathological Society Plant Health 21 Online.

Collective impact of agronomic practices on the diversity and abundance of pathogenic and beneficial fungal genera in soil

Ananda Bandara, Dilooshi Weerasooriya, Terrence Bell, Paul Esker

Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University

INTRODUCTION

- Agronomic practices such as cover cropping, reduced tillage, and crop rotation are important management practices that soybean growers use to improve soil health and reduce erosion.
- Application of manure is a common soil fertility management practice among soybean growers. Manure also improves ~~the~~ soil organic matter content.
- These practices have a strong influence on soil health, which also considers plant health, through both changes of physicochemical characteristics and influence on soil microbial communities.
- Agronomic practices are used to improve soil health, and can, in some cases, increase soil disease suppression.
- The impact of various agronomic practices on soil microbial community have been explored under experimentally manipulated conditions. However, understanding their combinatorial effects under natural agroecosystems can provide new insights on sustainable microbiome management.

OBJECTIVE

To unravel the impact of agronomic management practices on the fungal communities in bulk soil collected from agricultural fields in Pennsylvania.

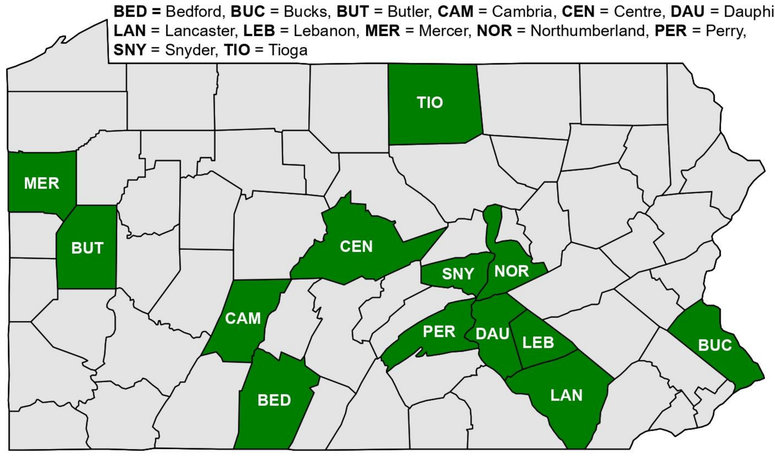
MATERIALS AND METHODS

Bulk soil samples were collected (n = 20) from 14 farms in Pennsylvania with histories of different cultural practices. Samples from each farm were composited (n = 4) and DNA was extracted from each composite sample.

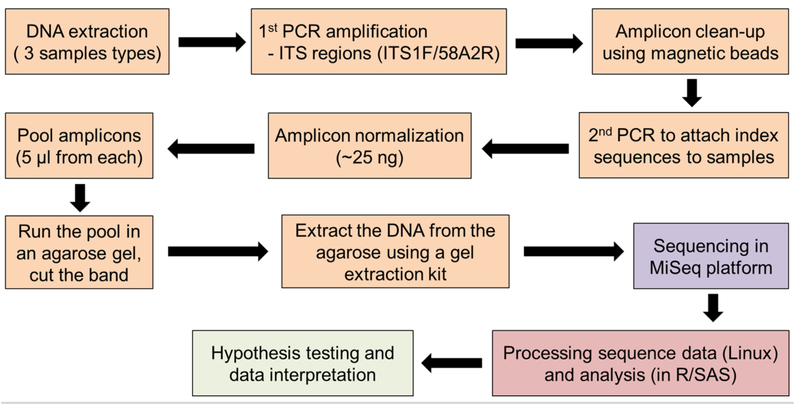
Management practices related to each focal farm

Location	Cover crop	Manure	Tillage	Seed treatment	Crop rotation	Maturity group
Bedford	Yes	No	No	No	OTHER	Early three
Bucks	Yes	No	Yes	Yes	OTHER	Early three
Butler	No	Yes	No	No	OTHER	Two
Cambria	No	No	No	No	CSC	Two
Centre	Yes	No	No	No	CSC	Two
Dauphin	No	Yes	No	Yes	CSC	Late three
Lancaster	Yes	Yes	No	Yes	OTHER	Early three
Lebanon1	Yes	Yes	Yes	Yes	OTHER	Late three
Lebanon2	No	Yes	Yes	Yes	OTHER	Late three
Mercer	Yes	No	No	Yes	CSC	Two
Northumberland	Yes	Yes	No	Yes	CSC	Two
Perry	Yes	Yes	No	No	OTHER	Early three
Snyder	Yes	Yes	No	Yes	CSC	Late three
Tioga	No	Yes	No	Yes	OTHER	Early three

BED = Bedford, **BUC** = Bucks, **BUT** = Butler, **CAM** = Cambria, **CEN** = Centre, **DAU** = Dauphin, **LAN** = Lancaster, **LEB** = Lebanon, **MER** = Mercer, **NOR** = Northumberland, **PER** = Perry, **SNY** = Snyder, **TIO** = Tioga



Pennsylvania state map depicting the focal counties of the study.

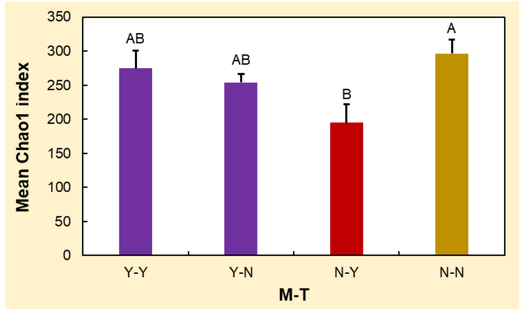


Major steps of the metagenomics pipeline.

RESULTS

Impact of agronomic practices on fungal α-diversity (= within sample diversity using Chao1 index)

Practice	ANOVA	
	F	*P*
CC	0.19	0.6655
M	0.64	0.4272
T	0.74	0.3955
CR	1.14	0.2924
CC_M	0.73	0.5403
CC_T	1.62	0.1994
CC_CR	1.12	0.3534
M_T	**2.88**	**0.0470**
M_CR	1.10	0.3602
T_CR	0.66	0.5201
CC_M_T	2.00	0.0875
CC_M_CR	1.04	0.4133
CC_T_CR	1.17	0.3403
M_T_CR	1.74	0.1457
CC_M_T_CR	1.39	0.2242



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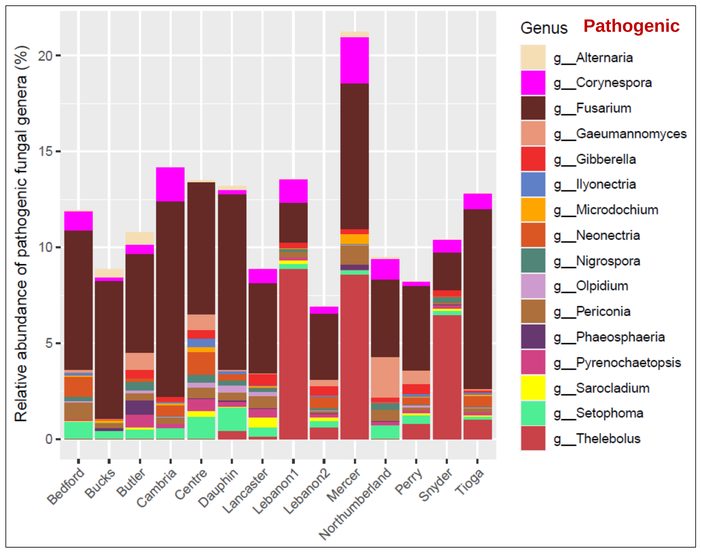
M = Manure (Yes, No)
T = Tillage (Yes, No)
CR = Crop Rotation (corn/soybean/corn, Non corn/soybean/corn)

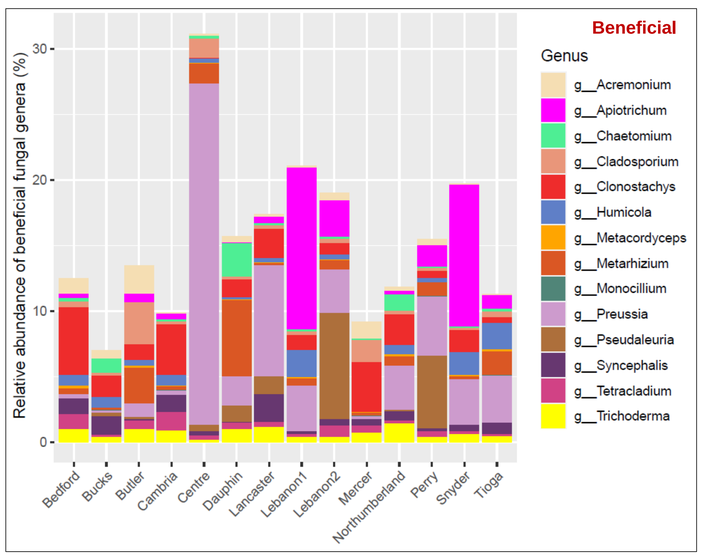
Impact of agronomic practices on fungal β-diversity (= diversity between samples)

Agronomic Practice	Pathogenic genera-based (n = 16)				Beneficial genera-based (n = 14)				Entire genera-based (n = 372)			
	PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION	
	R^2	*P*	F	*P*	R^2	*P*	F	*P*	R^2	*P*	F	*P*
CC	0.042	0.034	0.874	0.348	0.041	0.007	1.422	0.248	0.032	0.016	0.247	0.621
M	0.070	0.003	4.086	0.050	0.072	0.001	0.711	0.415	**0.062**	**0.001**	0.247	**0.628**
T	0.041	0.047	1.166	0.297	0.026	0.137	0.001	0.971	0.032	0.012	0.659	0.433
CR	0.045	0.025	4.361	0.047	0.026	0.125	0.057	0.807	0.033	0.009	0.317	0.561
CC_M	0.140	0.002	5.011	0.003	0.159	0.001	9.336	0.001	0.132	0.001	8.952	0.001
CC_T	0.136	0.001	1.703	0.166	0.117	0.001	3.497	0.020	0.101	0.001	3.172	0.041
CC_CR	0.138	0.004	4.719	0.010	0.102	0.002	2.152	0.107	**0.095**	**0.001**	1.224	**0.294**
M_T	0.135	0.001	1.841	0.157	0.135	0.001	2.389	0.071	0.132	0.001	7.489	0.001
M_CR	0.133	0.003	2.112	0.116	0.142	0.001	0.450	0.722	0.132	0.001	3.467	0.020
T_CR	0.070	0.031	1.694	0.198	0.052	0.073	0.402	0.672	**0.062**	**0.006**	0.937	**0.403**
CC_M_T	0.273	0.001	2.147	0.063	0.268	0.001	2.938	0.014	0.246	0.001	5.692	0.001
CC_M_CR	0.237	0.002	2.877	0.016	0.272	0.001	6.826	0.001	0.247	0.001	4.181	0.002
CC_T_CR	0.264	0.001	3.232	0.016	0.178	0.001	2.736	0.026	0.174	0.001	3.042	0.024
M_T_CR	0.189	0.002	1.956	0.110	0.203	0.001	1.756	0.139	0.197	0.001	7.995	0.001
CC_M_T_CR	**0.401**	**0.001**	1.319	**0.262**	**0.384**	**0.001**	1.635	**0.112**	0.361	0.001	3.007	0.005

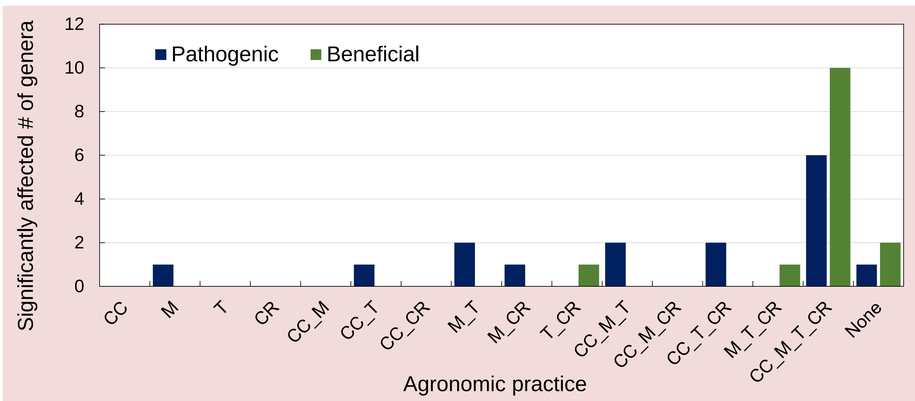
CC = Cover Crops (Yes, No); **M** = Manure (Yes, No); **T** = Tillage (Yes, No); **CR** = Crop Rotation (Corn/Soybean/Corn, Other)

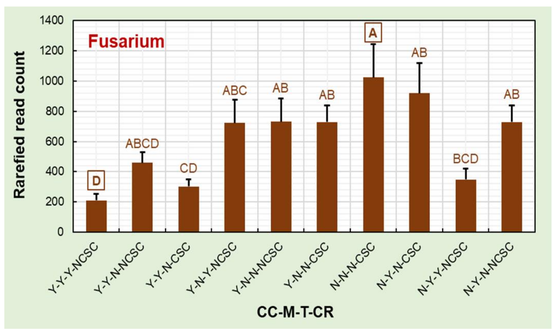
Spatial variation of the relative abundance of major pathogenic and beneficial fungal genera found in Pennsylvania agricultural fields

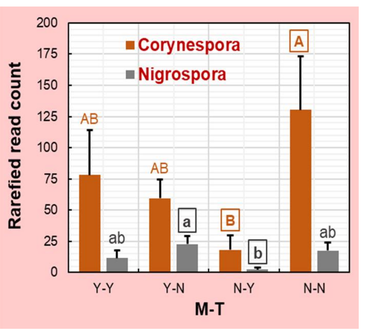


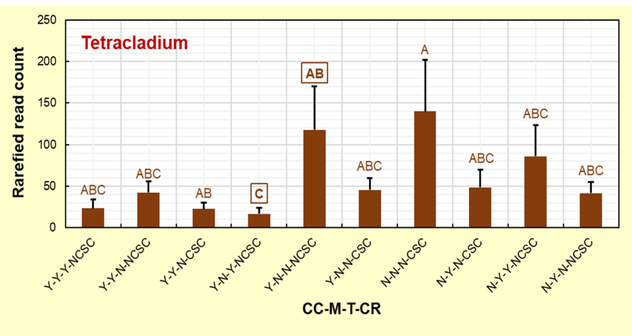


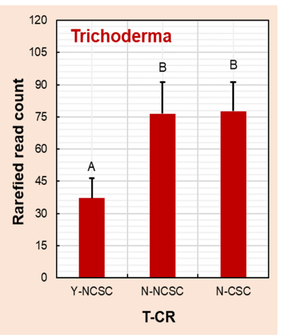
Impact of agronomic practices on the abundance (read counts) of pathogenic and beneficial fungal genera











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CONCLUSIONS

- Manure and tillage together affected fungal alpha diversity
- A significant proportion of the pathogenic and beneficial fungal genera-based beta diversity is explained by four agronomic practices in combination
- Agronomic practices have differential impact on the abundance of pathogenic/beneficial fungal genera
- Rational use of agronomic practices can promote beneficial fungal taxa and demote pathogenic fungal taxa in agricultural fields

ACKNOWLEDGEMENTS

Support for this project was from the Pennsylvania Soybean Board. This project was also supported by the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474. We thank our farmer cooperators and the following extension educators who contributed to site identification and sample collection from the Pennsylvania Soybean On-Farm Network: Adriana Murillo-Williams, Andrew Frankenfield, Anna Busch, Casey Guindon, Claire Coombs, Del Voight, Elizabeth Bosak, Jeff Graybrill, Justin Brackenrich, Nicole Santangelo, Rachel Milliron, and Zachary Larson.

Differential effect of agronomic practices on the diversity and abundance of beneficial bacterial genera in soil

Ananda Bandara, Dilooshi Weerasooriya, Terrence Bell, Paul Esker

Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University

INTRODUCTION

- Soybean farmers adopt various agronomic practices to improve the profitability of their crop.
- Certain alternative agronomic practices are becoming increasingly common in row crop agriculture in order to manage resource inputs and soil health. For example, the use of no-till and reduced tillage strategies have increased in row crops since the early 2000's in the United States.
- Additionally, cover crops, manure application, and crop rotation are major agronomic practices employed by soybean farmers.
- Although various benefits have been demonstrated for different agronomic practices, their effects on soybean-associated bacterial communities are not well-understood.
- Understanding the impact of different agronomic practices and crop management decisions on beneficial and pathogenic bacterial taxa is important to make informed decisions on their appropriate use.

OBJECTIVE

To unravel the impact of agronomic management practices on the bacterial communities in bulk soil collected from agricultural fields in Pennsylvania.

MATERIALS AND METHODS

Bulk soil samples were collected (n = 20) from 14 farms in Pennsylvania with different histories of cultural production practices. Samples from each farm were composited (n = 4) and DNA was extracted from each composite sample.

Management practices related to each focal farm

Location	Cover crop	Manure	Tillage	Seed treatment	Crop rotation	Maturity group
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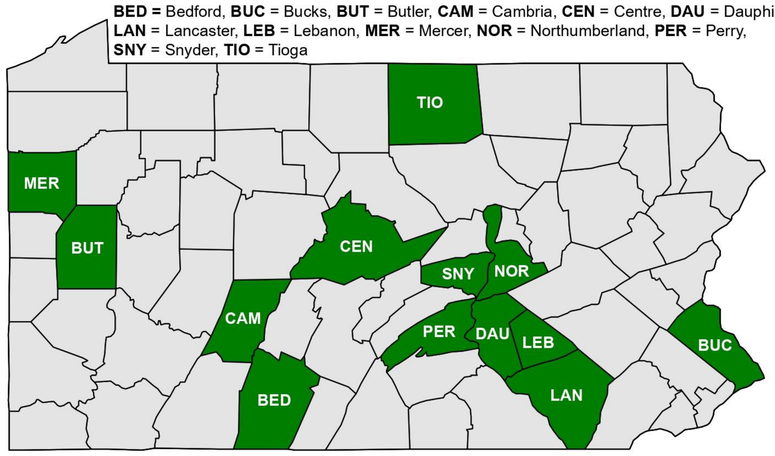
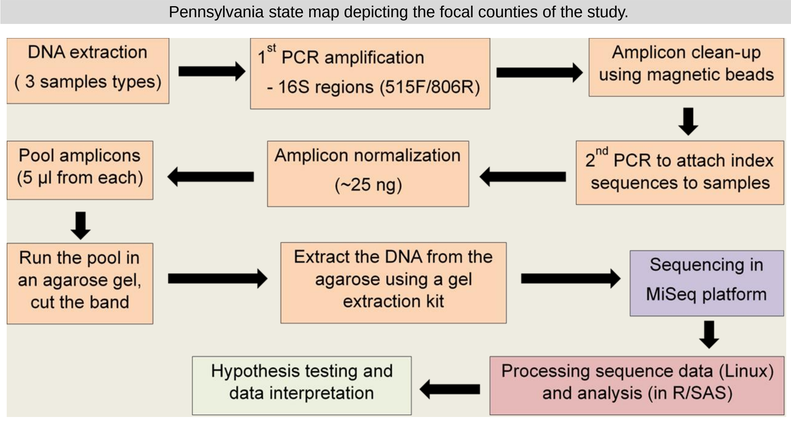


Pennsylvania state map depicting the focal counties of the study.

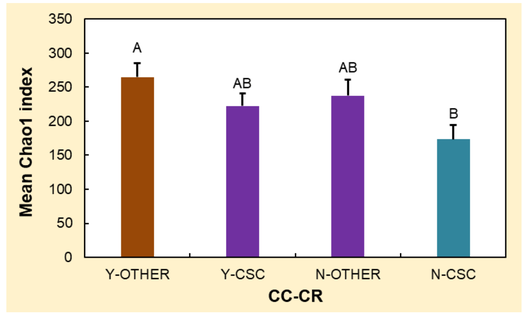


Major steps of the metagenomics pipeline.

RESULTS

Impact of agronomic practices on bacterial α-diversity (= within sample diversity using Chao1 index)

Practice	ANOVA	
	F	*P*
CC	1.69	0.2003
M	0.07	0.7995
T	0.11	0.7460
CR	4.28	0.0447
CC_M	1.04	0.3849
CC_T	1.39	0.2595
CC_CR	**3.01**	**0.0407**
M_T	0.06	0.9792
M_CR	1.46	0.2380
T_CR	2.43	0.1007
CC_M_T	0.92	0.4916
CC_M_CR	1.53	0.1907
CC_T_CR	2.45	0.0591
M_T_CR	1.03	0.4153
CC_M_T_CR	1.41	0.2152



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T = Tillage (Yes, No)
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Impact of agronomic practices on bacterial β-diversity (= diversity between samples)

Agronomic Practice	Entire genera based (n = 383)				Beneficial genera based (n = 23)			
	PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION	
	R^2	*P*	F	*P*	R^2	*P*	F	*P*
CC	0.025	0.020	0.1404	0.713	0.026	0.056	0.218	0.643
M	0.030	0.001	3.4234	0.074	0.027	0.035	3.598	0.066
T	0.025	0.015	1.2245	0.283	0.028	0.026	0.144	0.701
CR	0.026	0.007	0.6344	0.423	0.036	0.002	0.842	0.353
CC_M	0.081	0.001	5.8693	0.006	0.085	0.001	5.042	0.006
CC_T	0.087	0.001	7.4891	0.002	0.097	0.001	2.521	0.068
CC_CR	0.073	0.002	0.5655	0.643	0.084	0.001	0.925	0.427
M_T	0.092	0.001	5.0282	0.006	0.105	0.001	2.322	0.084
M_CR	0.083	0.001	1.2883	0.299	0.089	0.001	1.410	0.240
T_CR	0.051	0.001	0.0791	0.923	0.060	0.003	0.026	0.972
CC_M_T	0.170	0.001	4.814	0.002	0.187	0.001	2.505	0.042
CC_M_CR	0.160	0.001	3.2177	0.008	0.177	0.001	4.039	0.004
CC_T_CR	0.144	0.001	2.1621	0.074	0.160	0.001	0.892	0.495
M_T_CR	0.146	0.001	6.8127	0.001	0.162	0.001	3.109	0.021
CC_M_T_CR	**0.258**	**0.001**	**1.9672**	**0.061**	**0.281**	**0.001**	**1.691**	**0.122**

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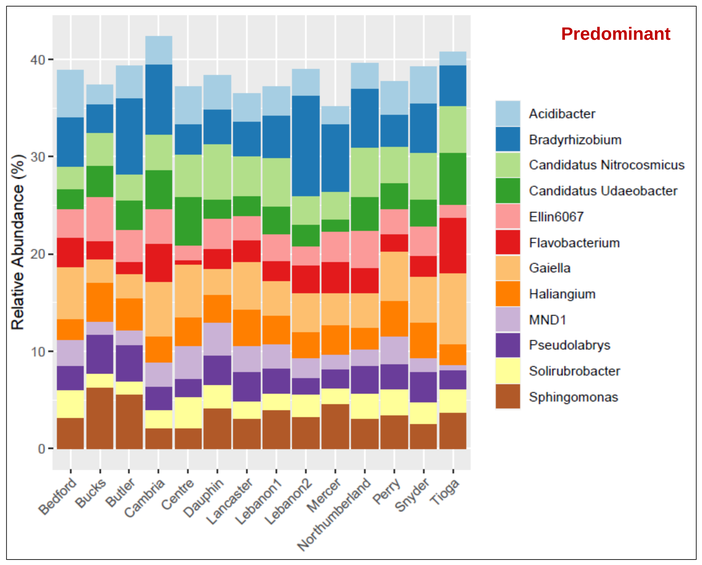
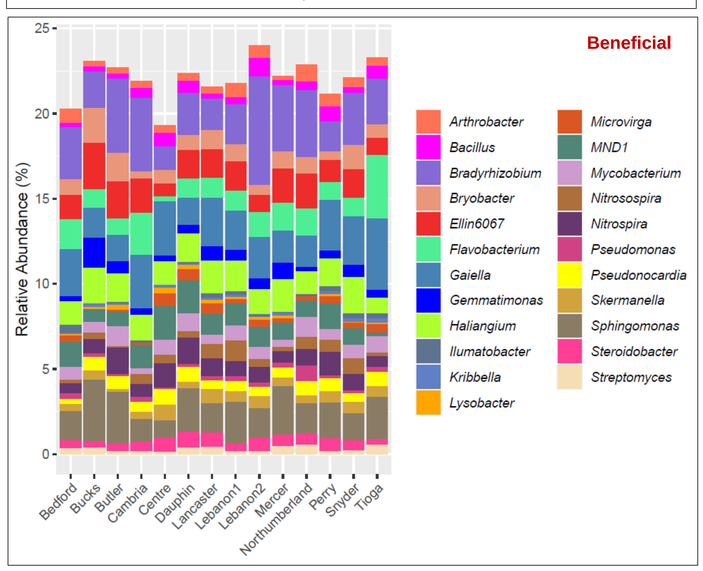
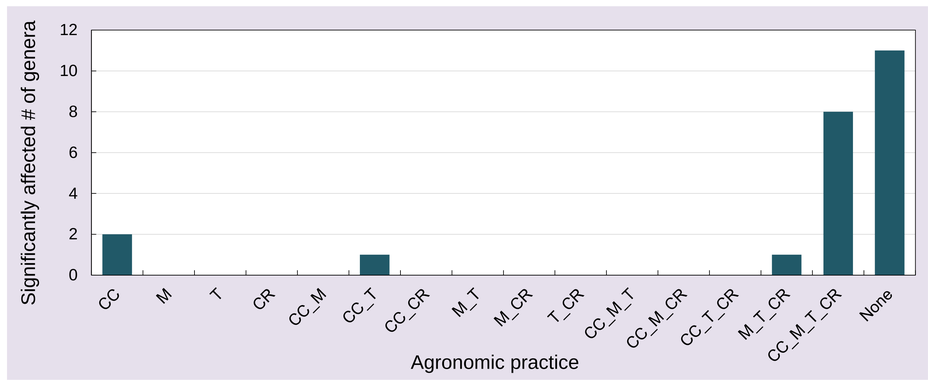
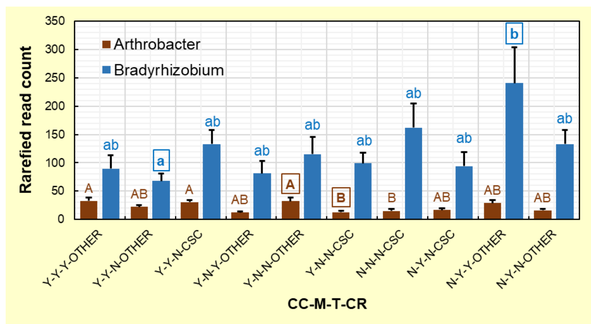
Spatial variation of the relative abundance of the predominant bacterial genera and major beneficial bacterial genera found in Pennsylvania agricultural fields

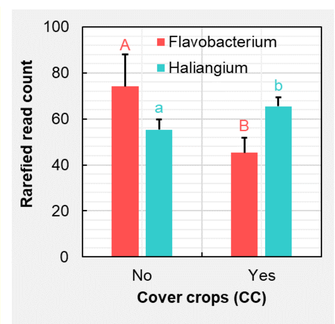


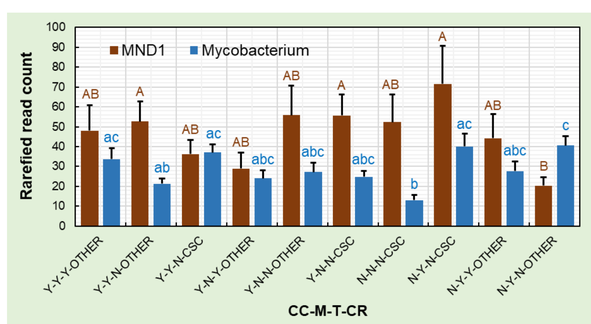


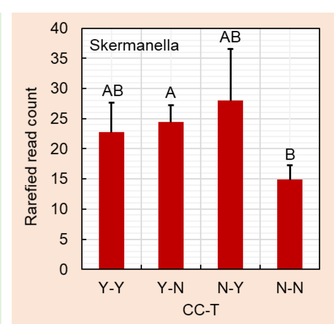
Impact of agronomic practices on the abundance (read counts) of beneficial bacterial genera











CC = Cover Crops (Yes, No); **M** = Manure (Yes, No); **T** = Tillage (Yes, No); **CR** = Crop Rotation (Corn/Soybean/Corn, Other)

CONCLUSIONS

- Major known pathogenic bacterial genera were not abundant in Pennsylvania agricultural fields
- Bacterial alpha diversity (Chao richness) is greater in fields where diversified crop rotations are practiced along with the use of cover crops
- A significant proportion of the entire and beneficial bacterial genera-based beta diversity is explained by four agronomic practices in combination
- Agronomic practices have differential impact on the abundance of beneficial bacterial genera. Their rational use can increase density of certain beneficial bacterial genera in agricultural fields

ACKNOWLEDGEMENTS

Support for this project was from the Pennsylvania Soybean Board. This project was also supported by the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474. We thank our farmer cooperators and the following extension educators who contributed to site identification and sample collection from the Pennsylvania Soybean On-Farm Network: Adriana Murillo-Williams, Andrew Frankenfield, Anna Busch, Casey Guindon, Claire Coombs, Del Voight, Elizabeth Bosak, Jeff Graybrill, Justin Brackenrich, Nicole Santangelo, Rachel Milliron, and Zachary Larson.

Soil nutrients and texture affect the diversity and abundance of pathogenic and beneficial fungal genera in soil

Ananda Bandara, Dilooshi Weerasooriya, Terrence Bell, Paul Esker

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INTRODUCTION

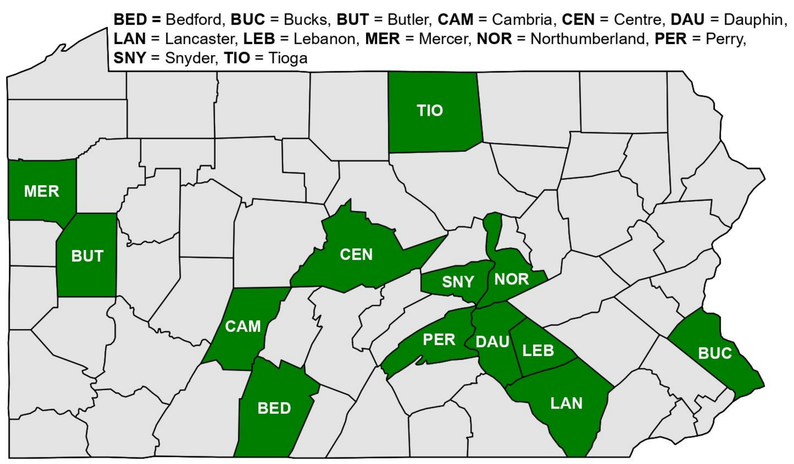
- Soil nutrients and soil texture are directly related to plant health.
- They can also affect the soil microbiome.
- For instance, nitrogen addition was shown to negatively affect soil bacterial and fungal diversity (Wang et al. 2018).
- Further, the decrease in soil microbial diversity under N addition was shown to be associated with the decrease in microbial biomass (Treseder, 2008; Liu and Greaver, 2010; Zhou et al. 2017; Wang et al. 2018).
- A study by Xue et al (2018) showed that soil electrical conductivity (EC), clay content and pH explain most of the variations in soil microbial structure.
- Understanding the impact of soil nutrients on pathogenic and beneficial microbial taxa can be useful when deciding optimum soil fertility levels for crop production.

OBJECTIVE

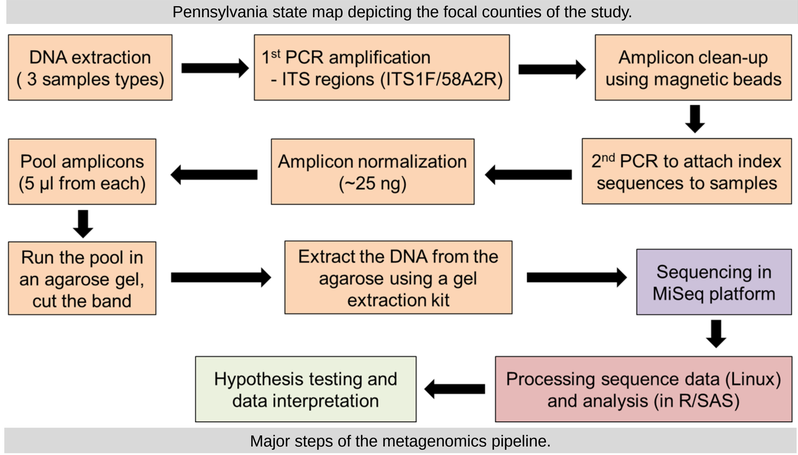
To unravel the impact of soil attributes such as soil nutrients and soil texture on the fungal communities in bulk soil collected from agricultural fields in Pennsylvania.

MATERIALS AND METHODS

- Bulk soil samples were collected (n = 20) from 14 farms in Pennsylvania.
- Samples from each farm were composited (n = 4).
- Part of each composite sample was used to assess the soil attributes (P, K, Mg, Ca, S, Zn, Cu), pH, soil cation exchange (CEC), organic matter (OM), and texture (sand/silt/clay)..
- Based on the observed values for each attribute, samples were classified into three groups: high, medium, and low (ex: high/medium/low P sample).
- Another part from individual composite soil samples were used to extract DNA.
- Extracted DNA was used for PCR targeting fungal ITS region (ITS1F/58A2R primers). Libraries were prepared and sequenced in MiSeq platform.
- Initial sequence processing was performed using DADA2 pipeline.
- Subsequent analyses were carried out in R using appropriate packages.



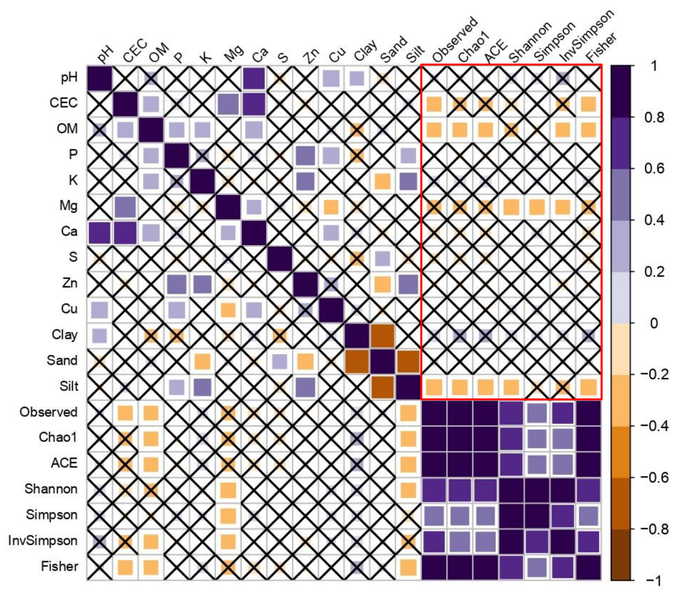
Pennsylvania state map depicting the focal counties of the study.



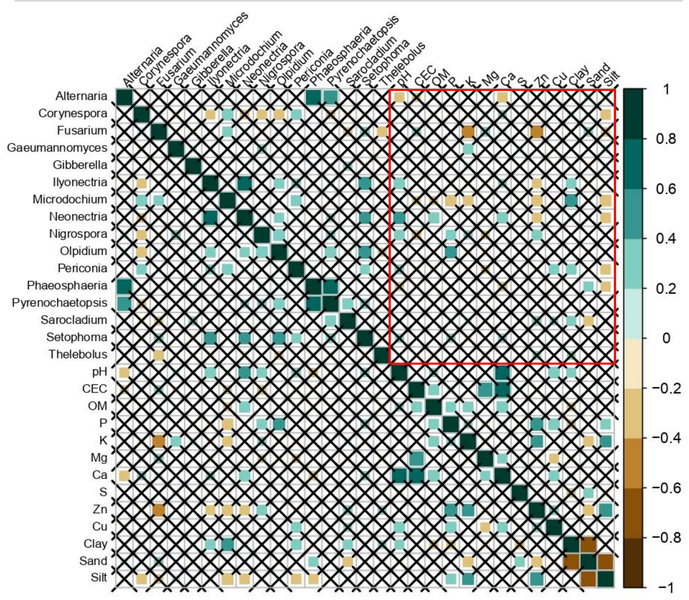
Major steps of the metagenomics pipeline.

RESULTS

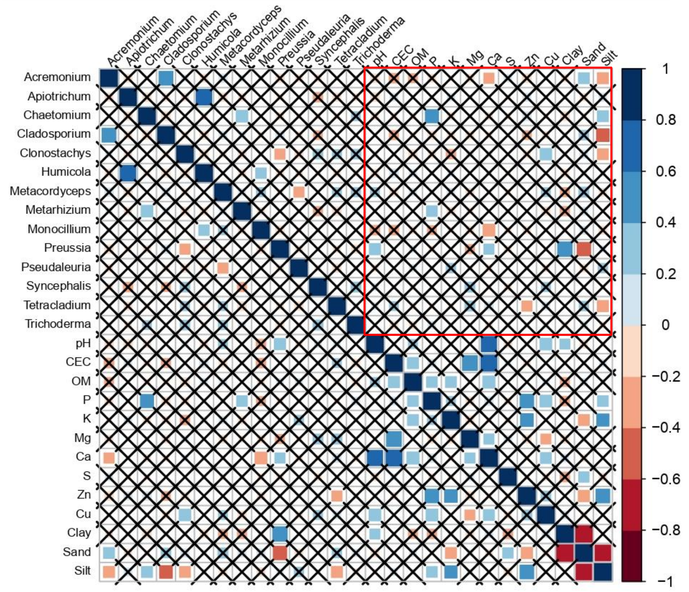
Correlation between soil attributes and alpha diversity indicators (non-significant correlations at α = 0.05 are shown by cross marks)



Correlation between soil attributes and read counts of major pathogenic genera (non-significant correlations at α = 0.05 are shown by cross marks)



Correlation between soil attributes and read counts of major beneficial genera (non-significant correlations at α = 0.05 are shown by cross marks)

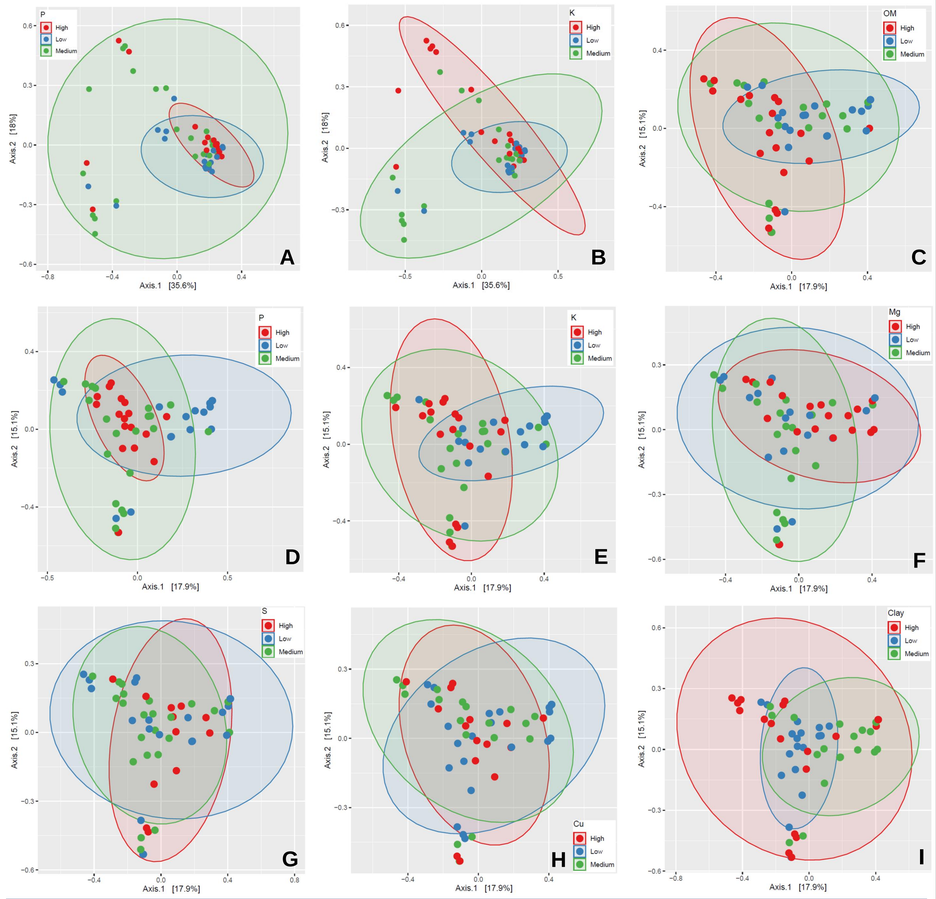


Impact of different soil attributes on fungal β-diversity (= diversity between samples)

Soil attribute	Pathogenic genera-based (n = 16)				Beneficial genera-based (n = 14)				Entire genera-based (n = 372)			
	PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION	
	R^2	*P*	F	*P*	R^2	*P*	F	*P*	R^2	*P*	F	*P*
pH	0.049	0.060	1.076	0.367	0.048	0.008	0.773	0.472	0.041	0.285	1.625	0.218
CEC	0.035	0.191	0.824	0.443	0.034	0.086	0.108	0.912	0.034	0.599	0.863	0.435
OM	0.042	0.112	0.047	0.946	0.061	0.001	0.301	0.753	0.054	0.014	0.510	0.609
P	0.067	0.013	1.212	0.293	0.068	0.001	0.267	0.737	0.064	0.004	1.835	0.184
K	0.052	0.048	1.053	0.341	0.040	0.026	1.075	0.328	0.068	0.002	1.217	0.295
Mg	0.024	0.523	0.148	0.863	0.043	0.010	0.442	0.658	0.039	0.323	0.152	0.865
Ca	0.038	0.169	0.548	0.605	0.032	0.112	0.932	0.397	0.049	0.054	0.240	0.783
S	0.034	0.227	0.402	0.654	0.047	0.009	0.139	0.853	0.046	0.097	1.028	0.371
Zn	0.035	0.209	0.402	0.654	0.045	0.005	5.961	0.007	0.077	0.001	7.236	0.005
Cu	0.025	0.503	0.341	0.705	0.044	0.009	0.657	0.511	0.048	0.072	0.728	0.515
Clay	0.028	0.396	0.454	0.645	0.072	0.001	0.644	0.510	0.078	0.001	2.738	0.072
Sand	0.048	0.072	0.212	0.787	0.059	0.001	0.280	0.740	0.071	0.001	0.725	0.462
Silt	0.130	0.001	4.134	0.027	0.057	0.001	0.291	0.743	0.093	0.001	1.320	0.309

CC = Cover Crops (Yes, No); **M** = Manure (Yes, No); **T** = Tillage (Yes, No); **CR** = Crop Rotation (Corn/Soybean/Corn, Other)

PCoA plots showing the clustering patterns of samples based on selected soil attributes. Ellipses show the 95% confidence region. A, B: Pathogenic fungi; C, D, E, F, G, H, I: beneficial fungi



CONCLUSIONS

- Soil organic matter, CEC, Mg, and silt content negatively correlated with alpha diversity.
- Bray Curtis dissimilarity-based principal coordinate plots did not reveal clear separation of samples based on soil attributes.
- PERMANOVA however, revealed significant effects (α = 0.05) of P and K on pathogenic genera-based β-diversity.
- All soil attributes except pH, CEC, Ca and Zn had significant effects on beneficial genera-based β-diversity.
- Negative correlations were observed between several soil attributes and pathogenic fungal genera (ex: K and *Fusarium*, *Microdochium*; silt and Corynespora, Microdochium, Neonectria, Periconia).
- Positive correlations were observed between certain soil attributes and beneficial fungal genera (ex: P and Metarhizium, Chaetomium; clay and Preussia).
- When taken together, results indicated how fungi-based soil health can be improved through soil nutrient management.

ACKNOWLEDGEMENTS

Support for this project was from the Pennsylvania Soybean Board. This project was also supported by the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474. We thank our farmer cooperators and the following extension educators who contributed to site identification and sample collection from the Pennsylvania Soybean On-Farm Network: Adriana Murillo-Williams, Andrew Frankenfield, Anna Busch, Casey Guindon, Claire Coombs, Del Voight, Elizabeth Bosak, Jeff Graybrill, Justin Brackenrich, Nicole Santangelo, Rachel Milliron, and Zachary Larson.

Soil texture and nutrients influence the diversity and abundance of beneficial bacterial genera in agricultural fields

Ananda Bandara, Dilooshi Weerasooriya, Terrence Bell, Paul Esker

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INTRODUCTION

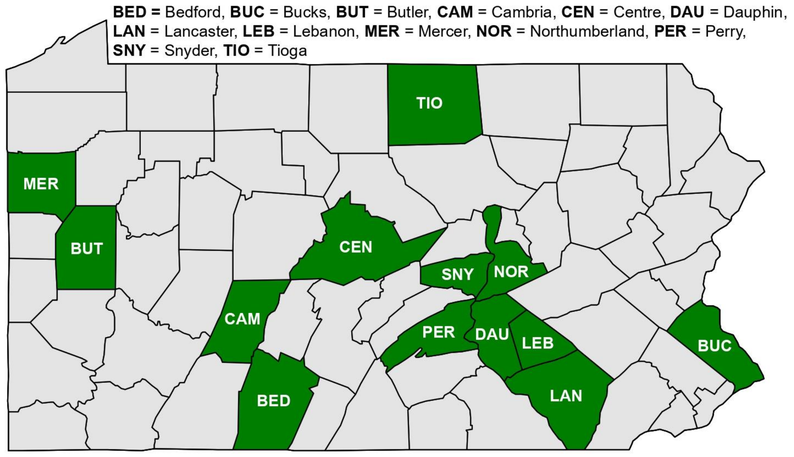
- Soil nutrients and soil texture are important determinants of crop productivity and can have profound impacts on soil bacterial community structure and function.
- Nutrient stoichiometry has been shown to be a strong predictor of bacterial diversity and composition at a regional scale (Baquerizo et al. 2016)
- Nitrogen addition was shown to negatively affect soil bacterial and fungal diversity (Wang et al. 2018).
- Further, the decrease in soil microbial diversity under N addition was shown to be associated with the decrease in microbial biomass (Treseder, 2008; Liu and Greaver, 2010; Zhou et al. 2017; Wang et al. 2018).
- Understanding the impact of soil nutrients on pathogenic and beneficial microbial taxa can be useful when deciding optimum soil fertility levels for crop production.

OBJECTIVE

Investigate the impact of soil attributes such as soil nutrients and soil texture on the bacterial communities in bulk soil collected from agricultural fields in Pennsylvania.

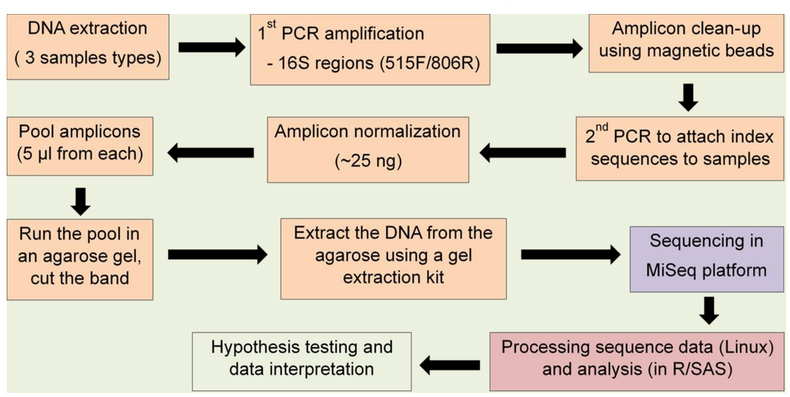
MATERIALS AND METHODS

- Bulk soil samples were collected (n = 20) from 14 farms in Pennsylvania.



Pennsylvania state map depicting the focal counties of the study.

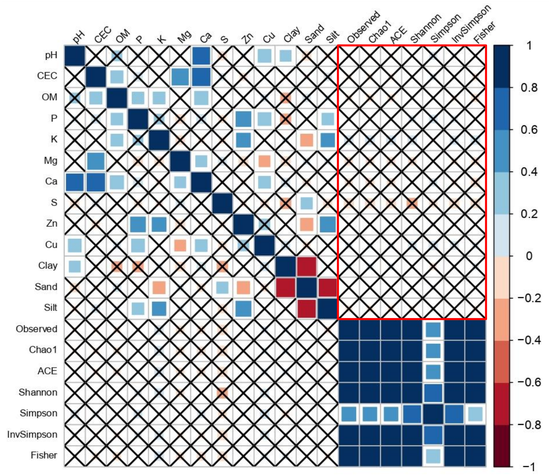
- Samples from each farm were composited (n = 4).
- Part of each composite sample was used to assess the soil attributes (P, K, Mg, Ca, S, Zn, Cu), pH, cation exchange capacity (CEC), organic matter (OM), and texture (sand/silt/clay).
- Based on the observed values for each attribute, samples were classified into three groups: high, medium, and low (ex: high/medium/low P sample).
- Another part from individual composite soil samples were used to extract DNA.
- PCR was performed targeting bacterial 16S rRNA genes (515F/806R primers). Libraries were prepared and sequenced in MiSeq platform.
- Initial sequence processing was performed using DADA2 pipeline.
- Subsequent analyses were carried out in R using appropriate packages.



Major steps of the metagenomics pipeline.

RESULTS

Correlation between soil attributes and alpha diversity indicators (non-significant correlations at α = 0.05 are shown by cross marks)

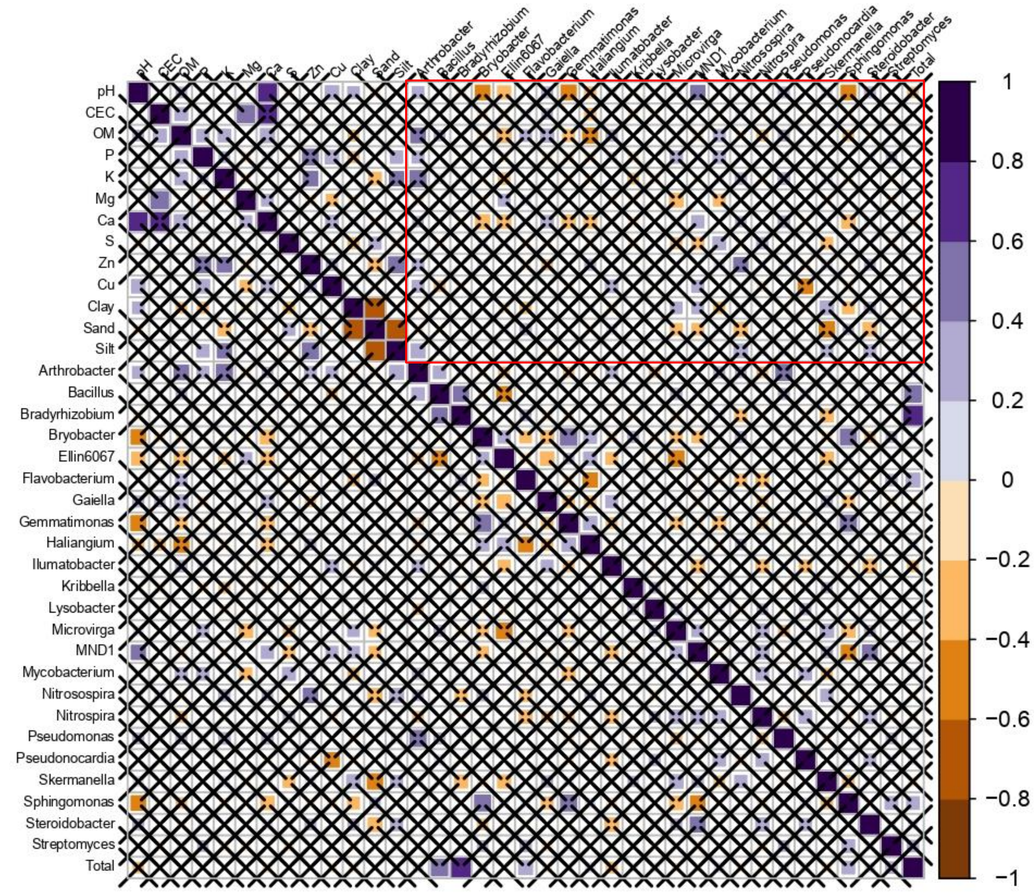


Impact of different soil attributes on fungal β-diversity (= diversity between samples)

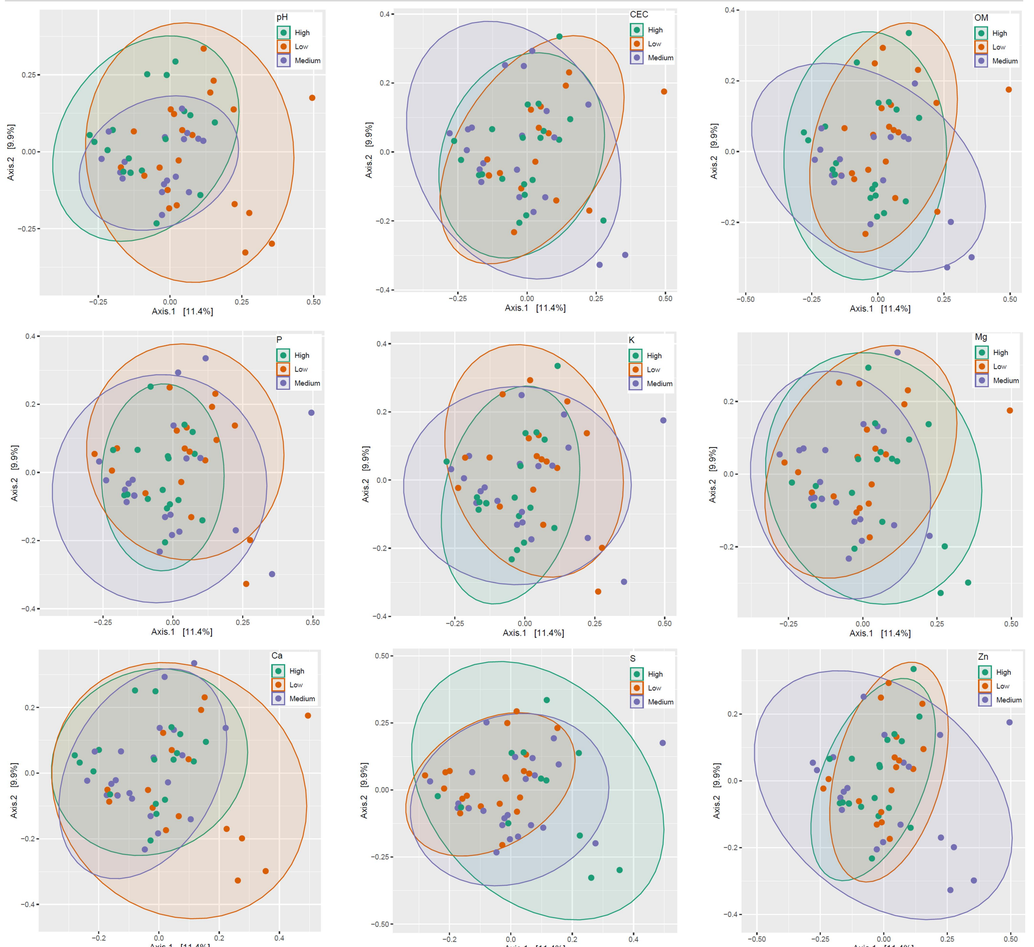
Soil attribute	Entire genera based (n = 383)				Beneficial genera based (n = 23)			
	PERMANOVA		β DISPERSION		PERMANOVA		β DISPERSION	
	R^2	*P*	F	*P*	R^2	*P*	F	*P*
pH	0.047	0.003	2.440	0.120	0.061	0.002	2.529	0.086
CEC	0.032	0.462	0.042	0.960	0.030	0.599	0.058	0.948
OM	0.045	0.001	0.388	0.673	0.041	0.072	0.143	0.868
P	0.040	0.032	4.132	0.027	0.038	0.158	2.845	0.076
K	0.036	0.141	3.734	0.030	0.033	0.387	2.105	0.129
Mg	0.037	0.106	1.790	0.179	0.045	0.021	1.277	0.280
Ca	0.043	0.005	1.029	0.372	0.033	0.381	0.832	0.449
S	0.049	0.001	0.851	0.435	0.046	0.022	1.471	0.239
Zn	0.045	0.002	6.489	0.004	0.048	0.008	2.600	0.076
Cu	0.036	0.096	2.935	0.042	0.042	0.045	2.780	0.068
Clay	0.039	0.018	1.658	0.176	0.036	0.212	1.113	0.311
Sand	0.040	0.025	3.106	0.049	0.043	0.042	1.184	0.305
Silt	0.043	0.006	1.213	0.304	0.039	0.105	1.220	0.324

CC = Cover Crops (Yes, No); **M** = Manure (Yes, No); **T** = Tillage (Yes, No); **CR** = Crop Rotation (Corn/Soybean/Corn, Other)

Correlation between soil attributes and read counts of major beneficial genera (non-significant correlations at α = 0.05 are shown by cross marks)



PCoA plots showing the clustering patterns of samples (based on beneficial genera-based beta diversity) as a function of selected soil attributes. Ellipses show the 95% confidence region.



CONCLUSIONS

- Pathogenic bacterial genera were not common in Pennsylvania agricultural fields.
- None of the soil attributes were significantly correlated with alpha diversity measures.
- Bray Curtis dissimilarity-based principal coordinate plots did not reveal clear separation of samples based on soil attributes.
- PERMANOVA however, revealed significant effects (α = 0.05) of pH, Mg, S, Zn, Cu, and Sand on beneficial genera-based β-diversity.
- Positive correlations were observed between certain soil attributes and some genera (ex: OM and Arthrobacter, Flavobacterium, Gaiella, Mycobacterium).
- Some attributes were negatively correlated with certain genera (Ca and Bryobacter, Ellin6067, Gemmatimonas, Haliangium, Sphingomonas).
- Overall, results indicated the potential use of nutrient management to enhance the abundance of beneficial bacterial genera in soil.

ACKNOWLEDGEMENTS

Support for this project was from the Pennsylvania Soybean Board. This project was also supported by the USDA National Institute of Food and Federal Appropriations under Project PEN04660 and Accession number 1016474. We thank our farmer cooperators and the following extension educators who contributed to site identification and sample collection from the Pennsylvania Soybean On-Farm Network: Adriana Murillo-Williams, Andrew Frankenfield, Anna Busch, Casey Guindon, Claire Coombs, Del Voight, Elizabeth Bosak, Jeff Graybrill, Justin Brackenrich, Nicole Santangelo, Rachel Milliron, and Zachary Larson.

Species composition and genetic diversity of soilborne *Fusarium* species from 17 counties in Pennsylvania

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INTRODUCTION

- Species of the genus Fusarium are well known as pathogens causing important diseases such as Fusarium wilt, damping-off and Fusarium root rot in soybean (Zang et al., 2010).
- Key soilborne pathogen profiles in Pennsylvania soybean farmer fields that also includes *Fusarium* spp. have shown substantial location scale variability over the years (Esker at al, 2019) while the knowledge on *Fusarium* species diversity and composition is scarce.
- The current study examined 313 *Fusarium* isolates subjected to morphological and molecular identification at the species level targeting partial sequences of TEF-1α and RPB2 genes.
- Outcomes from this study will reveal important information on genetic diversity of soilborne *Fusarium* species and their distribution in Pennsylvania
- This study also helps to provide important new knowledge towards strategizing future experiments to establish best management methods to control *Fusarium* diseases in PA crop fields.

OBJECTIVE

- To determine species composition and genetic diversity of 313 soilborne *Fusarium* isolates acquired from 22 farmer fields across Pennsylvania.

MATERIALS AND METHODS

- During summer 2018 and 2019, soil samples were collected from 22 different farmer fields in Pennsylvania.

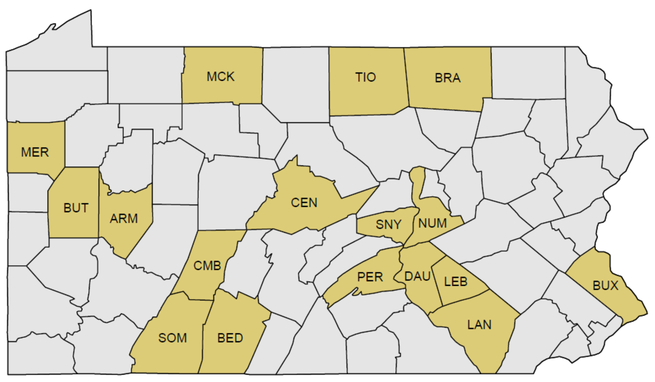

Figure 1. Seventeen Counties in Pennsylvania from which putative *Fusarium* isolates were obtained from soil samples: McKean (MCK), Tioga (TIO), Bradford (BRA), Mercer (MER), Butler (BUT), Armstrong (ARM), Centre (CEN), Snyder (SNY), Northumberland (NUM), Cambria (CMB), Perry (PER), Dauphin (DAU), Lebanon (LEB), Somerset (SOM), Bedford (BED), Lancaster (LAN), Bucks (BUX).

- Soil samples were plated on Nash and Snyder medium (Nash and Snyder, 1962). Single-spore cultures from isolates were obtained and used for DNA extraction using Lucigen MasterPure™ Yeast DNA Purification Kit.
- For species confirmation, single-spore isolates were morphologically characterized on PDA and subjected to PCR targeting partial sequences of translation elongation factor 1α (TEF-1α) and the RNA polymerase II second largest subunit (RPB2) gene. PCR amplicons were sequenced, and homology was explored using Fusarium MLST and NCBI databases.
- Multiple sequence alignment of the sequence data was performed using ClustalW. The molecular phylogenetic tree was constructed with MEGA 7 software (Kumar, Stecher, and Tamura 2015) using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993).

RESULTS

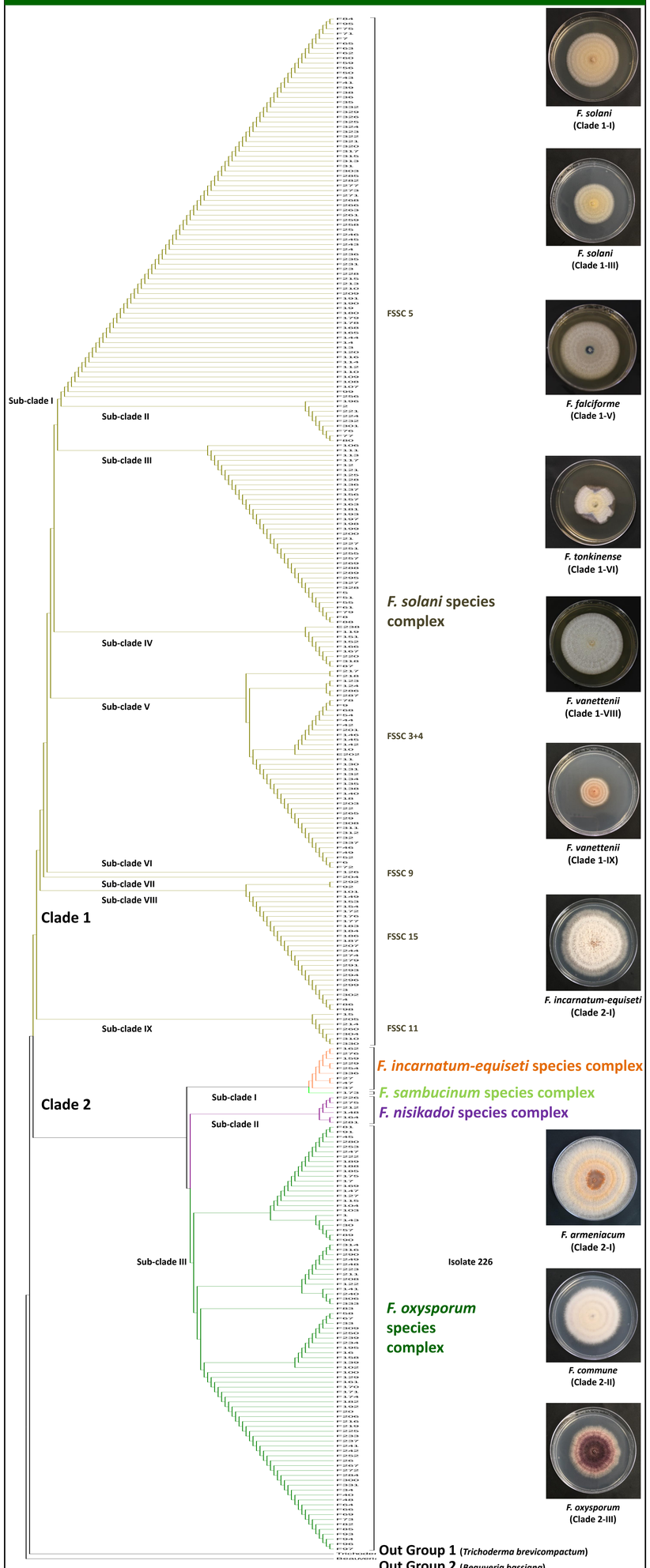

Figure 2. Molecular phylogenetic tree including 313 *Fusarium* isolates and two out groups developed using the Maximum Likelihood method. The tree with the highest log likelihood (-2468.13) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Images of representative isolates from some of the clades are shown on right.

Table 1. Species information and morphological characters of selected *Fusarium* isolates representing each of the major and sub-clades based on phylogenetic analysis.

Isolate ID	Clade	Molecular and morphological identification at species level	County	Spore length (µm)	Spore width (µm)	Number of septa	Aggressiveness (Growth rate in mm/day)
F71	1-I	*F. solani*	Bucks	35.6(±4.9)	5.6(±0.5)	3	69.9(±3.3)
F301	1-II	*F. solani*	Somerset	49.6(±4.6)	4.4(±0.7)	3 to 5	66.5(±3.3)
F251	1-III	*F. solani*	Bradford	48.2(±4.3)	4.6(±0.6)	4 to 5	62.1(±3.3)
F238	1-IV	*F. solani*	Lebanon	58.5(±4.1)	4.6(±0.6)	3 to 5	64.7(±3.3)
F10	1-V	*F. falciforme*	Mercer	44.5(±2.9)	4.8(±0.5)	3 to 4	71.6(±3.3)
F126	1-VI	*F. tonkinense*	Armstrong	49(±2.7)	4.7(±0.7)	4 to 5	53.9(±3.3)
F204	1-VII	*F. falciforme*	Dauphin	52(±3.4)	5.4(±0.4)	3 to 4	45(±3.3)
F3	1-VIII	*F. vanettenii*	Lancaster	50.1(±2.5)	4.8(±0.6)	5 to 6	52(±3.3)
F260	1-IX	*F. vanettenii*	Butler	55.8(±5.9)	4(±0.9)	5	44.5(±3.3)
F173	2-I	*F. armeniacum*	Snyder	49.6(±6.2)	3.2(±0.5)	5 to 7	87.1(±3.3)
F159	2-I	*F. incarnatum-equiseti*	Cambria	55.9(±5.0)	3.5(±0.6)	4 to 5	84(±3.3)
F226	2-II	*F. commune*	Tioga	31.6(±2.8)	2.5(±0.2)	3	65.9(±3.3)
F33	2-III	*F. oxysporum*	Northumberland	33(±2.5)	3.4(±0.4)	2 to 3	70(±3.3)

DISCUSSION

- Analysis revealed that the majority (67.1%) of isolates belonged to the *Fusarium solani* species complex (FSSC 3+4, FSSC 5, FSSC 9, FSSC 11 and FSSC 15), while the rest were categorized into *Fusarium oxysporum* (FOSC) (27.8%), *Fusarium incarnatum-equiseti* (FIESC) *(2.9%), Fusarium nisikadoi* (FNSC) (1.9%) and *Fusarium sambucinum* (FSAMSC) *(0.3%) species* complexes (Figure 2).
- Agreeing with previous findings, molecular phylogeny analysis resolved all isolates from FSSC into one monophyletic clade (Clade 1) and the rest of the isolates into a separate monophyletic clade (Clade 2) (O'Donnell et al., 2018). FOSC isolates were grouped separately from FNSC into subclade 2-II and 2-III (Baayen et al., 2001), while isolates from FSAMSC and FIESC were grouped together in subclade 2-I (Villani et al., 2019) (Figure 2). Isolates also showed a wide variability in terms of colony and spore morphological characters (Table 1).
- Overall, *Fusarium* species diversity in Pennsylvania was found to be relatively low. This can help to narrow management strategies. However, before a concrete conclusion could be made on control methods, relevant pathogenicity, aggressiveness and fungicide sensitivity assays on a selected set of isolates from each clade would be essential.

CONCLUSIONS

- Results revealed important information on species composition and genetic diversity of *Fusarium* species found in 17 counties in Pennsylvania.
- Findings provide guidance for subsequent aggressiveness and fungicide sensitivity assays with commonly used fungicides on the isolates and should help improve recommendations for managing pathogenic *Fusarium* spp. in PA field crops.

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Pathogenicity of soilborne *Fusarium* spp. from Pennsylvania

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INTRODUCTION

- *Fusarium* root rot is a common disease caused by key soilborne pathogens of the genus *Fusarium.* Multiple *Fusarium* species have been found to be in association with *Fusarium* root rot in soybean (*Glycine max*) in the United States (Arias et al., 2011) creating large losses in soybean production worldwide.
- Different *Fusarium* species have shown varying degrees of pathogenicity based on their genetic profile and the environments where they were isolated from (Chang et al., 2018; Naeem et al., 2019).
- Isolates used for this pathogenicity study were selected based on a prior molecular phylogenetic analysis based on the homology analysis of partial sequences of the translation elongation factor 1-α (EF1- α) and RNA polymerase II second largest subunit (RPB2) genes on Fusarium MLST and NCBI database.
- Pathogenicity assays on new *Fusarium* isolates from diverse locations included in this study should therefore improve our understanding of their impact on soybean seedling disease incidence.
- Moreover, findings of this study will assist selecting important phylogenetic clades for further investigations on aggressiveness, and fungicide sensitivity.

OBJECTIVE

- To investigate pathogenicity of 20 *Fusarium* isolates representing phylogenetically distinct clades based on previous molecular phylogeny analysis.

MATERIALS AND METHODS

- One to three isolates from each clade of the phylogenetic tree were used for the pathogenicity assay. Selected isolates belonged to *Fusarium solani*, *Fusarium oxysporum*, *Fusarium incarnatum-equiseti*, *Fusarium nisikadoi* and *Fusarium sambucinum* species complexes.

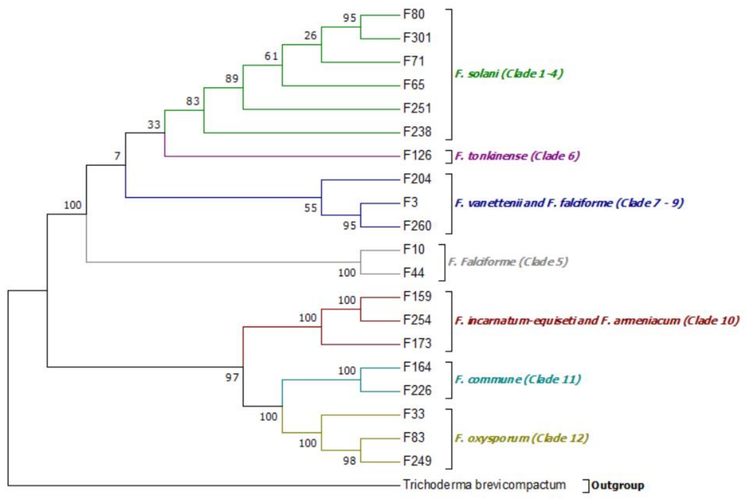

Figure 1. Phylogenetic tree showing tested isolates and 12 clades they were selected from based on their representative clades as resulted from prior molecular phylogeny analysis.

- Each single-spore isolate were plated on PDA plates and incubated at 25°C with 12h light/12h dark for seven days.
- Pathogenicity of isolates were tested following a rolled-towel assay using soybean variety SC9277R arranged in randomized complete block design with three replicates. Each replicate comprised 20 soybean seeds inoculated with a suspension of 2.5 x 105 conidia/mL or sterile water as the control. The experiment was repeated two times.
- After seven days, seeds were assessed for percent mycelial coverage, seed weight, root length and germination rate.
- Disease severity was determined using a scale from 0 to 4 where, 0 = healthy seed germination, 1 = delayed growth with negligible or no discoloration, 2 = germination with isolated lesions, 3 = developed with the merged lesion, and 4 = colonized seeds with no germination (Figure 2). Disease severity index (DSI) was calculated using the following formula.

$$\text{DSI} = \frac{\Sigma(\text{severity rating X seeds per rating})}{(\text{total seeds X highest severity rating})} \text{ X } 100$$

Figure 2. Scoring system used for calculating disease severity index. 0=healthy seed germination, 1=delayed growth with negligible/no discoloration, 2=germination with isolated lesions, 3=developed with the merged lesion, and 4=colonized seeds with no germination.

RESULTS

Table 1. Mean separation results for disease occurrence and growth parameters of soybean after inoculation with the representative *Fusarium* isolates.

Isolate IDz	Molecular Identification	PMC (%)	Seed Weight (g)	Root Length (cm)	Germination (%)	Disease Severity Index (DSI)
Control	-	0(±5.7)^{e}	0.64(±0.03)^{ab}	3.18(±0.26)^{a}	95(±5.8)^{a}	0(±0.75)^{b}
F173	*F. armeniacum*	45.8(±5.7)^{cd}	0.56(±0.03)^{abc}	1.41(±0.26)^{b}	61.7(±5.8)^{b}	6(±0.75)^{a}
F164	*F. commune*	84.2(±5.7)^{a}	0.51(±0.03)^{abc}	1.73(±0.26)^{b}	58.3(±5.8)^{b}	6.8(±0.75)^{a}
F226		75(±5.7)^{abc}	0.49(±0.03)^{abc}	1.71(±0.26)^{b}	47.9(±5.8)^{b}	8.7(±0.75)^{a}
F10	*F. falciforme*	49.2(±5.7)^{bcd}	0.54(±0.03)^{abc}	1.91(±0.26)^{ab}	62.5(±5.8)^{b}	6.4(±0.75)^{a}
F44		62.5(±5.7)^{abcd}	0.54(±0.03)^{abc}	1.81(±0.26)^{b}	58.3(±5.8)^{b}	6.5(±0.75)^{a}
F204		67.5(±5.7)^{abcd}	0.48(±0.03)^{bc}	1.54(±0.26)^{b}	60.8(±5.8)^{b}	6.3(±0.75)^{a}
F159	*F. incarnatum-equiseti*	58.3(±5.7)^{abcd}	0.58(±0.03)^{abc}	1.8(±0.26)^{b}	59.6(±5.8)^{b}	7.3(±0.75)^{a}
F254		42.5(±5.7)^{d}	0.53(±0.03)^{abc}	1.84(±0.26)^{b}	55(±5.8)^{b}	6.7(±0.75)^{a}
F33	*F. oxysporum*	75(±5.7)^{abc}	0.51(±0.03)^{abc}	1.87(±0.26)^{ab}	50(±5.8)^{b}	7.8(±0.75)^{a}
F83		66.7(±5.7)^{abcd}	0.5(±0.03)^{abc}	2.26(±0.26)^{ab}	65(±5.8)^{b}	6(±0.75)^{a}
F249		65(±5.7)^{abcd}	0.46(±0.03)^{c}	1.47(±0.26)^{b}	57.6(±5.8)^{b}	6.4(±0.75)^{a}
F126	*F. tonkinense*	64.2(±5.7)^{abcd}	0.53(±0.03)^{abc}	2.11(±0.26)^{ab}	61.7(±5.8)^{b}	6.5(±0.75)^{a}
F3	*F. vanettenii*	67.5(±5.7)^{abcd}	0.5(±0.03)^{abc}	1.51(±0.26)^{b}	60(±5.8)^{b}	6.7(±0.75)^{a}
F260		56.7(±5.7)^{abcd}	0.48(±0.03)^{bc}	1.67(±0.26)^{b}	55.8(±5.8)^{b}	6.8(±0.75)^{a}
F65	*F. solani*	41.7(±5.7)^{d}	0.65(±0.03)^{a}	2.66(±0.26)^{ab}	68.3(±5.8)^{ab}	4.8(±0.75)^{a}
F71		76.7(±5.7)^{ab}	0.49(±0.03)^{abc}	1.51(±0.26)^{b}	59.2(±5.8)^{b}	6.3(±0.75)^{a}
F80		49.2(±5.7)^{bcd}	0.5(±0.03)^{abc}	1.61(±0.26)^{b}	58.8(±5.8)^{b}	6.5(±0.75)^{a}
F238		59.2(±5.7)^{abcd}	0.5(±0.03)^{abc}	1.74(±0.26)^{b}	64.2(±5.8)^{b}	5.4(±0.75)^{a}
F251		50(±5.7)^{bcd}	0.55(±0.03)^{abc}	1.95(±0.26)^{ab}	59.2(±5.8)^{b}	6.4(±0.75)^{a}
F301		55.8(±5.7)^{abcd}	0.53(±0.03)^{abc}	1.82(±0.26)^{b}	59.2(±5.8)^{b}	5.7(±0.75)^{a}

*** Data were the mean from two independent experiments with three independent replicates. Means in the same column with different lowercase letters are statistically significant at p = 0.05 according to Tukey-Kramer test.**

- Isolates with pathogenicity scores <3, between 3 and 6, or >6 were considered to have a low, moderate, or high level of pathogenicity, respectively.

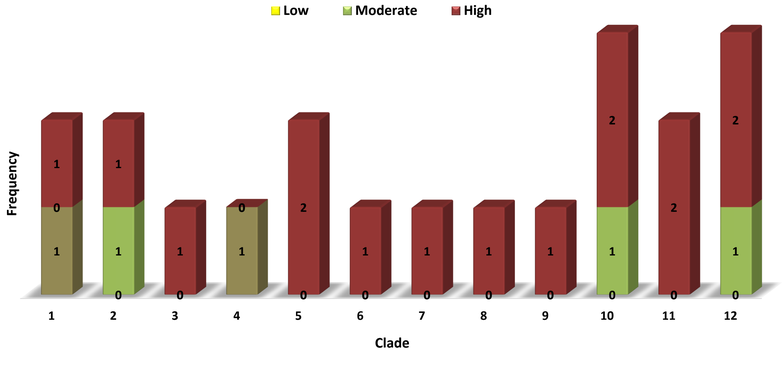

Figure 3. Frequency of isolates categorized as low, moderate, and high disease severity groups as related to the 12 phylogenetic clades.

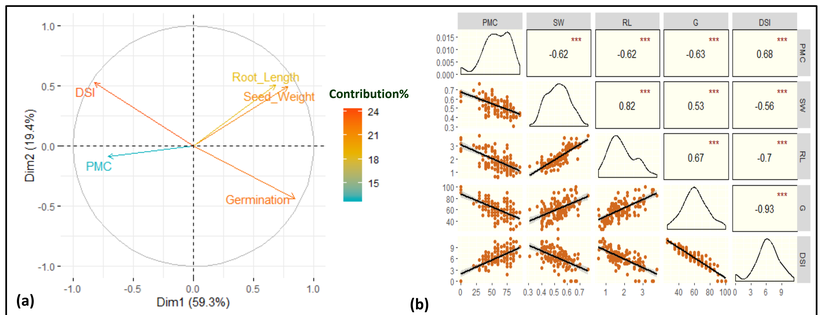

Figure 4. (a) Results of Principal component analysis showing the contribution of each measured parameter to the observed variability in disease occurrence for tested isolates and (b) correlation coefficients between the measured disease parameters and disease severity index (PMC = percent mycelial cover%, SW = seed weight, RL = root length, G = germination%, DSI = disease severity index).

DISCUSSION

- Based on the principal component analysis, the observed variability in disease data was largely explained by disease severity index, germination%, and seed weight, while root length and percent mycelial coverage played a minor role in determining pathogenicity of the isolates (Figure 4a).
- Few clades included isolates that grouped into more than one pathogenicity group (Figure 3) while isolates from all 12 clades showed differing degrees of pathogenicity. As expected, seed weight, root weight and germination% showed significant and negative correlations with DSI while the opposite was observed for PMC (Figure 4b) .
- Highest pathogenicity resulted for both *F. commune* isolates (F164 and F226), one FSSC isolate (F71), and one *F. oxysporum* isolate (F33), while lowest pathogenicity was observed for four isolates representing FSSC (F10, F65, F80, and F251), one from *F. incarnatum-equiseti*, and one *F. armeniacum* isolate (F173) (Table 1 and Figure 3).
- Results provided important directions towards deploying subsequent fungicide sensitivity assays for soilborne *Fusarium* spp. and should provide important insight on disease management strategies in PA soybean fields.

CONCLUSIONS

- All 20 isolates representing 12 phylogenetic clades were shown to be pathogenic while four of the isolates were highly pathogenic.
- A fungicide sensitivity assay on the isolates with commonly used fungicides should provide important information towards improving recommendations for managing pathogenic *Fusarium* spp. in PA field crops.

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Effectiveness of seed-applied fungicides for managing soybean seedling diseases in Pennsylvania

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INTRODUCTION

- Evidence on soybean response to different seed treatments is inconsistent.
- Bradley et al. (2001) reported metalaxyl-applied seed increased soybean stands in one of the two years tested, but not seed yield. Cox et al. (2008) reported no differences in both stand establishment and seed yield between untreated and fungicide treated soybean seed. Bierman et al. (2006) assessed six fungicide seed treatments in multiple environments but did not observe a seed yield increase in any environment. Dorrance et al. (2009) reported the benefit of metalaxyl and mefenoxam seed treatments to have been highly variable across 11 location-year combinations across six US states.
- However, due to the perceived protection it offers against major soilborne diseases accompanied by other benefits such as the capacity to use reduced seeding rates to compensate increasing seed and commodity costs (Esker and Conley, 2012), more and more farmers are compelled to use soybean seed treatments for their crop at present time.
- The current study focuses on evaluating the efficacy and necessity of ApronMaxx fungicide seed treatment on seedling diseases and plant performance in 11 different environments of Pennsylvania during 2018 and 2019 growing seasons.
- Findings from this study will inform on the need for fungicide seed treatments in Pennsylvania soybean fields and any probable agronomic or yield advantages, while also considering soil physicochemical properties and soilborne pathogen profiles at farm scale.

OBJECTIVE

- To investigate the impact of ApronMaxx (Mefenoxam + Fludioxonil) fungicide seed treatment on seedling diseases, seedling vigor, and yield of soybean grown in Pennsylvania.

MATERIALS AND METHODS

- Field trials were conducted in seven and four different counties in Pennsylvania during Summer 2018 and 2019, respectively.
- About a week prior to planting, soil samples were collected from each plot at a depth of 6-8 inches using a soil probe.
- To determine density of *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora* spp. soil was plated on modified Nash and Snyder, Ko and Hora, P5ARP, P5ARP with added hymexazol media, respectively.

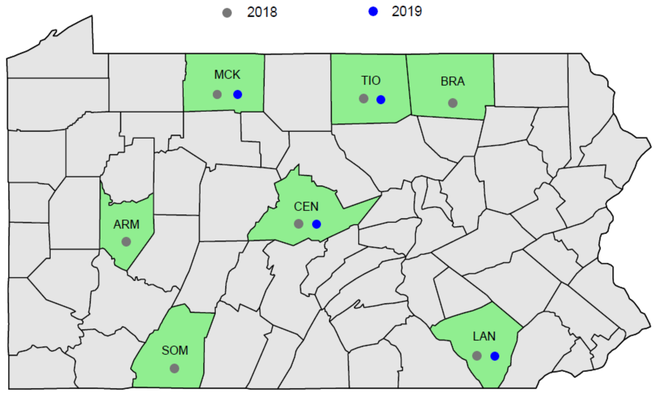

Figure 1. Pennsylvania state map showing counties where seed treatment trials were conducted during 2018 and during 2019. County abbreviations; ARM=Armstrong, BRA=Bradford, CEN=Centre, LAN=Lancaster, MCK=McKean, SOM=Somerset, TIO=Tioga.

Table 1. Planting date and soil temperature for each experimental location.

County	Planting Date		Soil Temperature (°F)	
	2018	2019	2018	2019
ARM	5/18	N/A	59.0	N/A
BRA	5/23	N/A	57.4	N/A
CEN	5/15	5/3	56.8	58.0
LAN	5/3	5/2	55.6	56.8
MCK	6/9	6/15	63.0	64.2
SOM	6/18	N/A	68.4	N/A
TIO	6/6	6/5	61.4	62.4

- At three weeks after planting, initial plant stand was recorded.
- Normalized difference vegetation index was measured at V4 (4th trifoliate), R1 (flowering) and R6 (full seed).
- Plant height (PH), tap root length (TRL), and root weight to shoot weight ratio (RW/SW) at R1 and V4 growth stages during 2018 and 2019, respectively.
- Plot yield was recorded at maturity. Soil nematode profiles and nutrient profiles were also determined.
- Data were analyzed using the PROC GLIMMIX procedure in SAS (v. 9.4, SAS Institute, 2017). For analysis of pathogen density data, a negative binomial model was used to account for overdispersion in the response variable. Pearson correlation and multivariate analysis were performed using Corrplot and factoextra packages in R, respectively (Wei and Simko, 2017; Kassambara and Mundt, 2020).

RESULTS

- Despite changing the seedling growth stage used for seedling disease and vigor assessments in 2018 and 2019 (R1 vs. V4), there were no statistical differences between control and ApronMaxx treated plots for PH, TRL and RW/SW ratio at all locations.

Table 2. Results for seedling parameter mean comparisons between ApronMaxx treated and control plots at R1 and V4 growth stages during 2018 and 2019 trial years, respectively.

County	Plant Height (cm)		Taproot length (cm)		Root to shoot biomass ratio (dry basis)		Initial plant stand count (no. of plants per 1m)	
	Control	ApronMaxx	Control	ApronMaxx	Control	ApronMaxx	Control	ApronMaxx
2018								
Armstrong	30.4 (±1.7)a	29.8(±1.7)a	16.7(±0.5)a	15.7(±0.5)a	0.28(±0.02)a	0.29(±0.02)a	11.5(±0.39)b	13.3(±0.39)a
Bradford	21.6 (±0.6)a	21.3 (±0.6)a	19.1 (±0.6)a	18.6(±0.6)a	0.44(±0.02)a	0.38(±0.02)a	20.7(±0.67)b	23.0(±0.67)a
Centre	21.3(±0.7)a	20.72(±0.7)a	18.1(±0.5)a	17.1(±0.5)a	0.41(±0.01)a	0.41(±0.01)a	25.3(±1.49)a	25.0(±1.49)a
Lancaster	18.9(±0.8)a	21.5(±0.8)a	12.8(±0.4)a	13.3(±0.44)a	0.30(±0.02)a	0.27(±0.02)a	12.0(±0.39)a	12.5(±0.39)a
McKean	29.9(±0.7)a	30.4(±0.7)a	22.4(±0.9)a	20.8(±0.96)a	0.20(±0.01)a	0.21(±0.01)a	8.3(±0.38)a	8.3(±0.38)a
Somerset	19.0(±0.9)a	17.8(±0.9)a	17.6(±0.7)a	17.3(±0.71)a	0.43(±0.04)a	0.44(±0.04)a	NA	NA
Tioga	41.8(±1.5)a	43.8(±1.5)a	21.7(±0.7)a	22.0(±0.73)a	0.23(±0.01)a	0.24(±0.01)a	13.0(±0.82)a	12.0(±0.82)a
2019								
Centre	16.0(±0.44)a	16.8(±0.44)a	13.9(±0.68)a	14.2(±0.68)a	0.53(±0.04)a	0.61(±0.04)a	NA	NA
Lancaster	13.8(±0.39)a	14.2(±0.39)a	9.4(±0.46)a	9.0(±0.46)a	0.36(±0.03)a	0.35(±0.03)a	9.7(±0.41)a	9.9(±0.41)a
McKean	15.4(±1.30)a	15.3(±1.30)a	12.0(±0.77)a	12.2(±0.77)a	0.38(±0.02)a	0.39(±0.02)a	23.3(±3.52)a	26.3(±3.52)a
Tioga	13.5(±0.51)a	14.4(±0.51)a	13.9(±0.45)a	14.9(±0.45)a	0.61(±0.05)a	0.54(±0.05)a	22.0(±2.07)a	22.3(±2.07)a

*The standard error of the mean is shown in parenthesis. Means followed by the same letter superscripts are not statistically different.

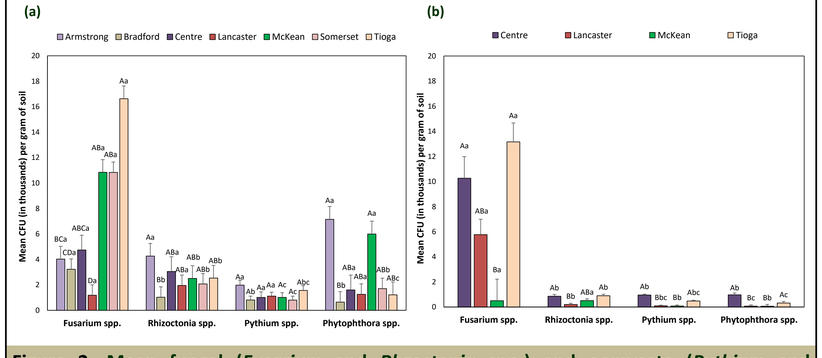

Figure 2. Mean fungal (*Fusarium* and *Rhzoctonia* spp.) and oomycete (*Pythium* and *Phytophthora* spp.) density (colony forming units per g of soil) at plot level observed for farm sites used for sampling during a). 2018 and b). 2019. The uppercase letters above the bars represent mean comparison results among farm sites for a certain pathogen group whereas lower case letters above the bars represent mean comparison results among pathogen groups found in a particular farm site. Means with the same letter do not significantly differ. Error bars indicate standard errors.

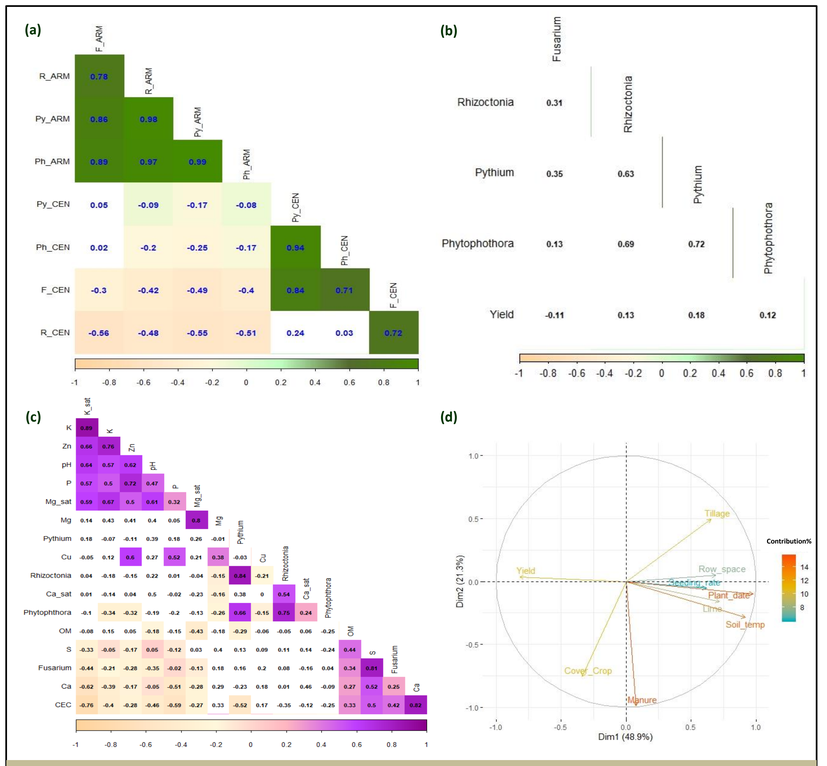

Figure 3. Pearson correlation coefficients for *Fusarium* (F), *Rhizoctonia* (R), *Pythium* (Py) and *Phytophthora* (Ph) spp. densities, resulted for a). soil samples collected during 2018 from Armstrong and Centre counties representing central Pennsylvania, and b). grain yield resulted for soil samples collected during 2018 and 2019 c). and soil physicochemical properties measured in soil samples collected during 2018 and 2019. Non-significant correlation coefficients at p-value = 0.05 are shown with white color background. d). The contribution different crop management factors as related to the observed variability in yield data. The first two dimensions explained 48.9% and 21.3% of the observed variability.

DISCUSSION

- The observed variability in soilborne fungal and oomycete density seemed to have been mostly contributed by unique ecological properties of each environment as reflected by Figures 2 and 3a, while there was no apparent relationship between early planting dates and pathogen densities.
- All experimental locations in both years showing non-significant differences for all measured parameters between control and ApronMaxx treated plots could be a function of pathogenic capabilities of investigated fungal and oomycete groups, long-term crop management practices and soil properties, weather and other related factors that have contributed to low disease pressure (Table 2 and Figures 3b and 3d).
- Soil physicochemical properties showed complex relationships with pathogen densities (Figure 3c) while soil nematodes profiles did not contain any harmful nematode groups.

CONCLUSIONS

- Apron Maxx fungicide seed treatment used on different Pennsylvania farmer fields during both 2018 and 2019 did not have significant impact on crop growth or yield performance
- The outcome of this study should help Pennsylvania farmers to re-consider management decisions on the necessity of fungicide seed treatments.

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