Project FY22-TS-007: Metagenomics Informed Trait Development for Breeders

1. What are the major goals and objectives of the research project?

In this research, we aimed to unravel the complex interactions between barley genotypes and microbial communities in the context of Fusarium Head Blight (FHB) infection. Our overall objective has been to **identify microbes that are recruited by FHB and those that are responsive to different barley genotypes** with the long term aim of enhanced breeding for FHB-protective microbial communities.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

The major activities involved, first, an extensive sampling from four U.S. nurseries over two years (2021 and 2022), measuring Fusarium biomass and DON levels, and profiling the microbial communities associated with diseased and non-diseased spikes in 10 breeding lines from a training population using amplicon sequencing. This involved 16S rRNA amplicon sequencing for bacteria and ITS amplicon sequencing for fungi. Using these data we investigated the influence of genotype and disease on microbial community structure.

Second, we performed a shotgun metagenomics study using a nursery from Langdon, ND in 2023 to complement the amplicon sequencing study and gain genome-resolved information about the responsiveness of microbes to genotype and disease. This study involved deep shotgun metagenomics sequencing of heads from two genotypes (AAC Synergy Explorer) and grouped into disease severity categories, R0 = 0%, R1 = 1-10%, R2 = 11-20%, and R3 = 21-100%. The high depth shotgun sequencing allowed us to correlate *Fusarium* read abundance to disease levels, visualize the impact of disease and genotype on the microbiome taxonomic and functional profile, and identify taxa metagenome-assembled genomes that respond to disease and to genotype.

What were the significant results?

1) Amplicon sequencing study

- Amplicon sequencing first revealed that location had a dominating effect on both bacterial and fungal community structure (i.e., beta-diversity) - with the ID location showing the most divergent community structure compared to the more eastern locations (**Figure 1**).

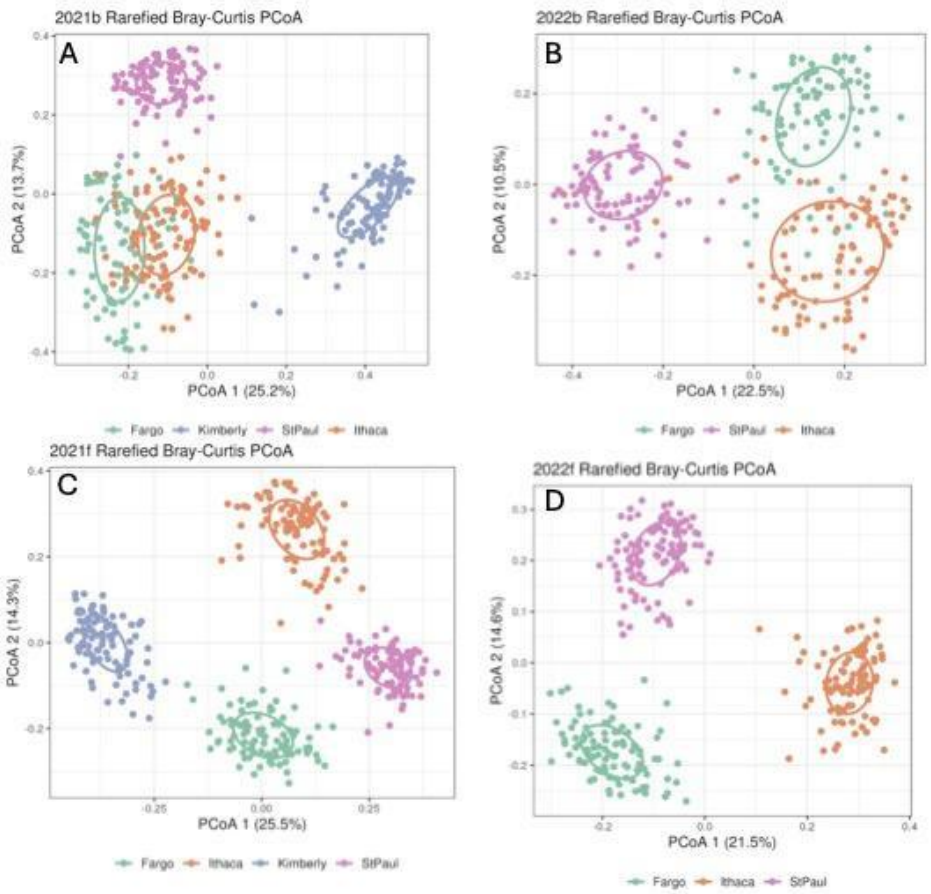

Figure 1. Location is a major driver of microbiome structure across years. A. and B show the bacterial microbiome from 2021 and 2022 respectively. C and D show the fungal microbiome from 2021 and 2022 respectively.

- Because location was such a driving force in the community structure of both microbiomes, we separated the data by location to further assess the importance of disease and line on community structure. We found a significant two-way interaction of disease and line on microbiome structure for all locations and kingdoms except for the bacteria in the NY samples (**Figure 2**). These data supported our hypothesis that the barley head microbiome is responsive to disease in a genotype-dependent manner.

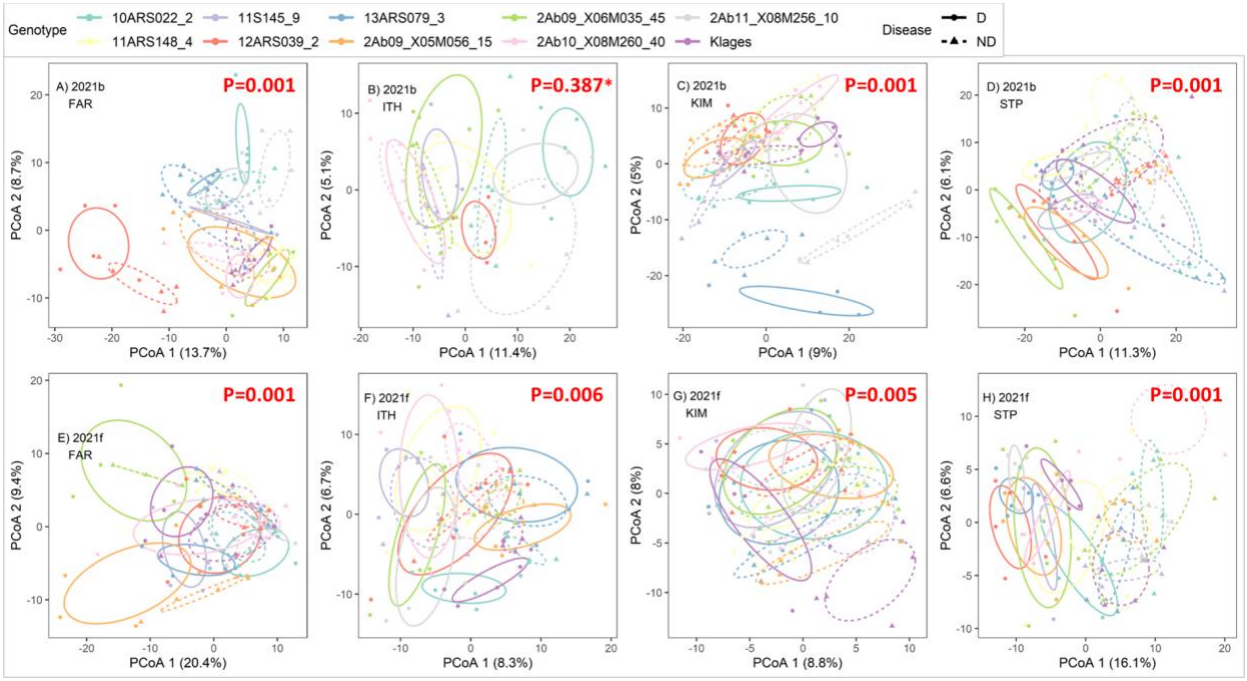

Figure 2. PCoA community structure analysis of metabarcoding data for the 16S bacterial communities (A, B, C, D) and the ITS fungal communities (E, F, G, H). Data from four locations are shown: A,E) Fargo ND; B,F) Ithaca NY; C,G) Kimberly ID; and D,H) St Paul MN. Each panel is colored by plant genotype, with point shapes and line styles indicating diseased or non-diseased spikes. P-values indicate the significance of the Genotype by Disease interaction.

- Variance partitioning analysis indicated that genotype (barley line) generally accounted for more variance in the bacterial and fungal microbiomes (consistently ~10%) than disease, while disease effect on the microbial communities was most pronounced in the St. Paul nursery in both years, explaining up to 17% of variation in the fungal microbiome in 2021 (**Figure 3**).

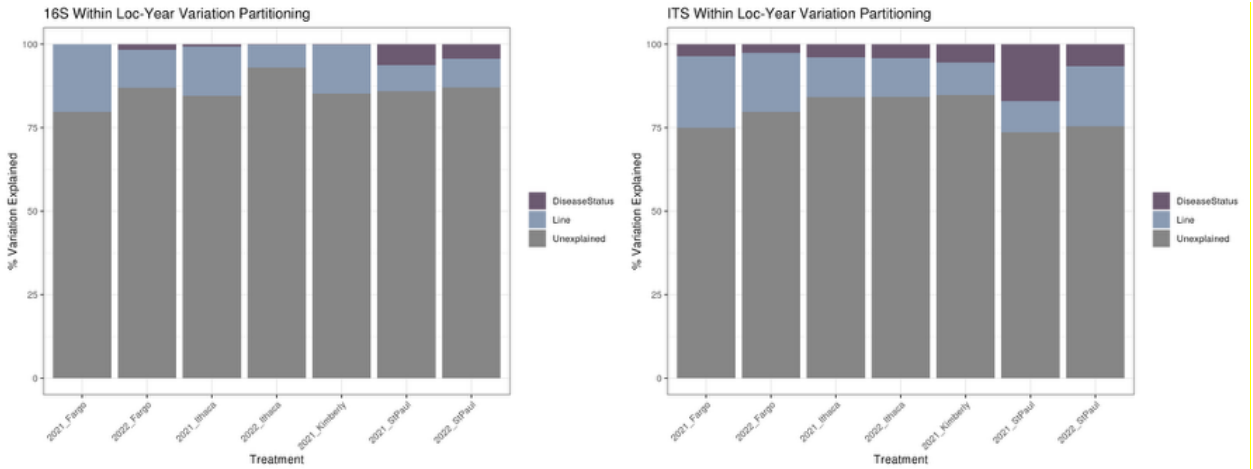

Figure 3. Variation partitioning analysis within locations shows higher impact of line than disease on microbial structure. The proportion of variance explained by line and disease status is shown within each location for the bacterial microbiome (left) and fungal microbiome (right)

- Disease had the strongest effect on microbial community structure in St. Paul, which was also the location with the highest disease pressure. Many taxa from both the bacterial and fungal microbiomes were found to be significantly enriched or depleted in response to disease by a DeSeq analysis **(Figure 4)**.

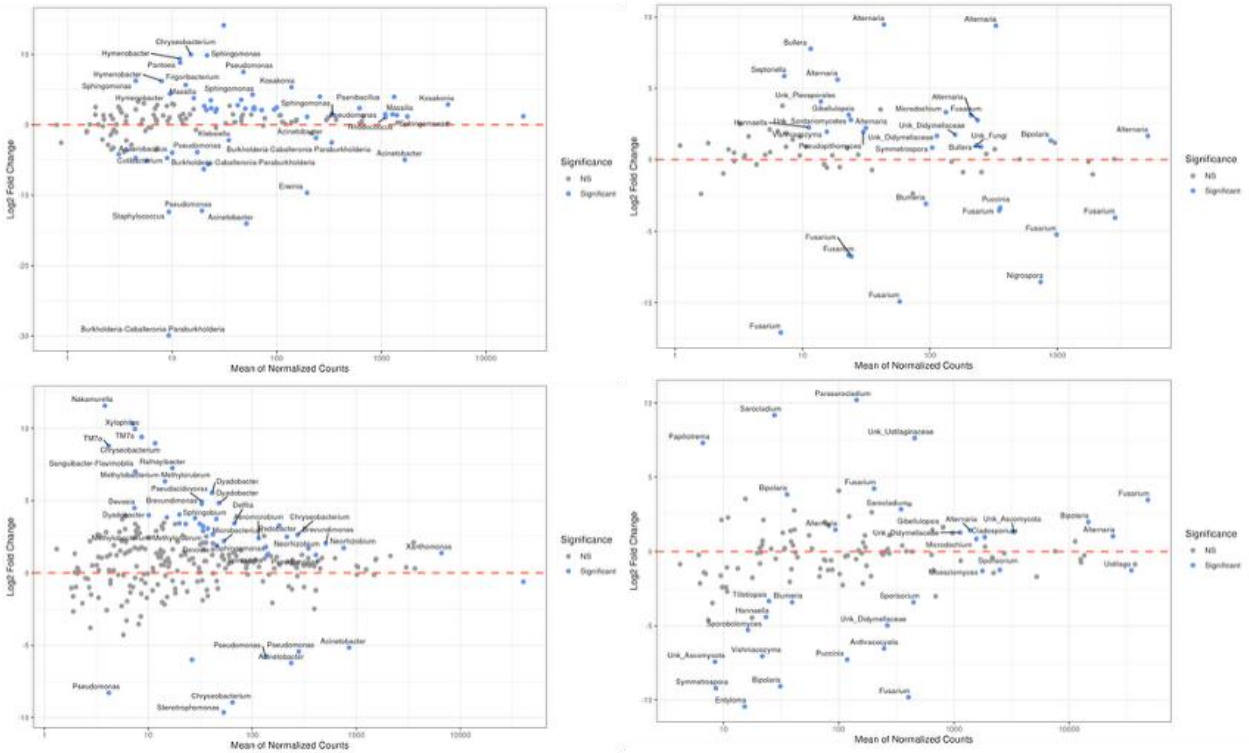

Figure 4. Significantly enriched or depleted taxa in the presence of FHB disease by amplicon sequencing analysis. Data are from DeSeq analysis of changes in taxa abundance in St Paul in response to disease. Disease-enriched taxa are shown below the dashed line, while disease-depleted taxa are shown above. Data are shown for the bacterial microbiome(left) as well as the fungal microbiome (right) in 2021 (top) and 2022 (bottom). Statistically significant enrichment or depletion is shown as blue dots with taxa labels for the genus name.

2) Metagenomics study

- In our third year of funding, we performed metagenomics on barley heads belonging to 2 genotypes. We found a linear correlation between sequencing reads belonging to *Fusarium* and disease severity of barley heads **(Figure 5)**, validating our **visual selection of disease symptoms**.

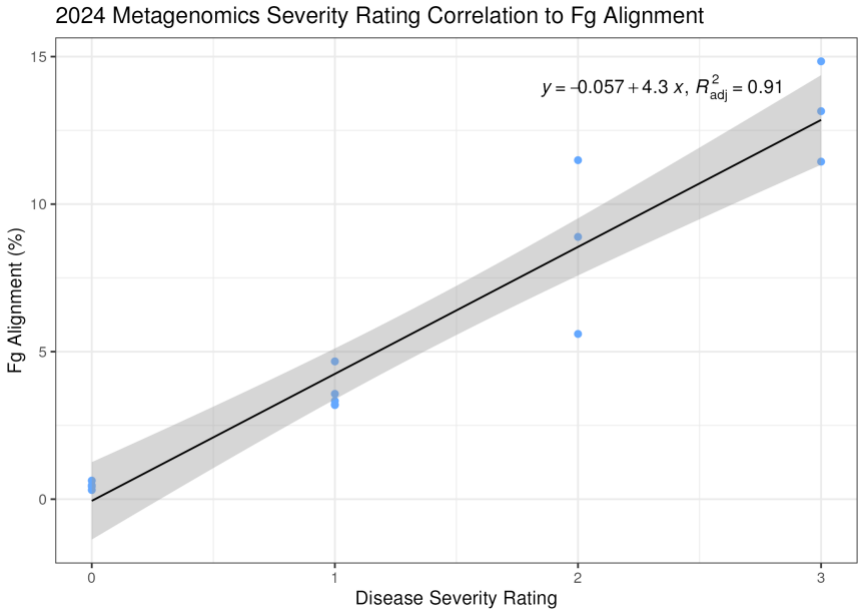

Figure 5. Disease severity rating predicts Fusarium read abundance in metagenomics dataset. The Y axis indicates the % of reads aligning to the *Fusarium graminearum* genome, while the X-axis indicates disease severity rating of the heads.

- Additionally, a community structure analysis performed on the metagenomics-derived bacterial and fungal reads indicated a clear impact of disease and genotype on microbial community structure **(Figure 6).**

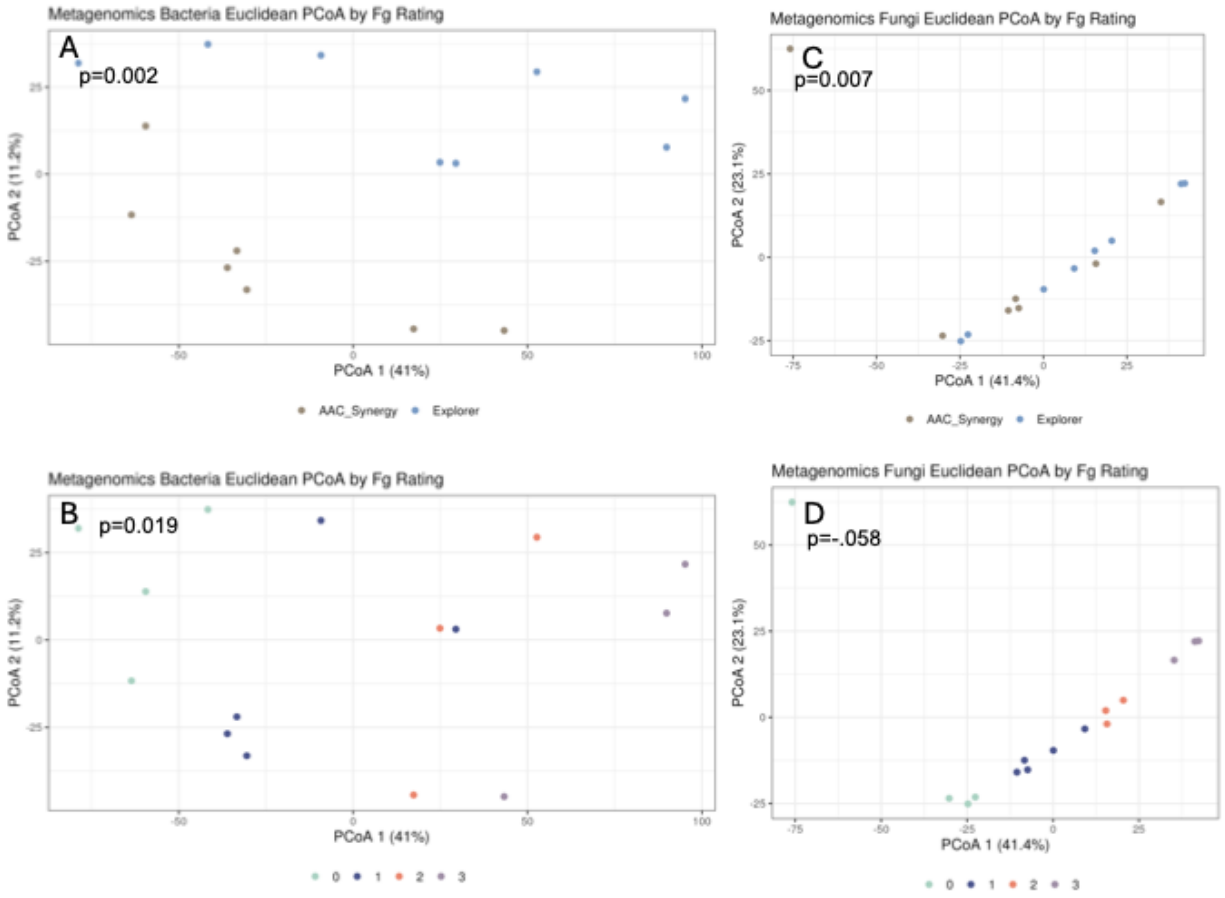

Figure 6. Genotype and disease structure the microbial communities of barley heads as measured by metagenomics. Shown are principal coordinate analysis of Euclidean distance (beta-diversity) of bacterial (A and B) and fungal (C and D) microbiome structure colored by genotype (top) (p = 0.019) or disease (bottom) (P= 0.058).

- Differential abundance analysis showed many taxa were significantly enriched or depleted in response to disease and genotype. Importantly, an overlap in genotype- and disease- responsive taxa was observed, with several taxa present in high abundance such as *Exiguobacterium* and *Stenotrophomonas* (Bacteria) and *Tilletiopsis* (Fungi) **(Figure 7)**

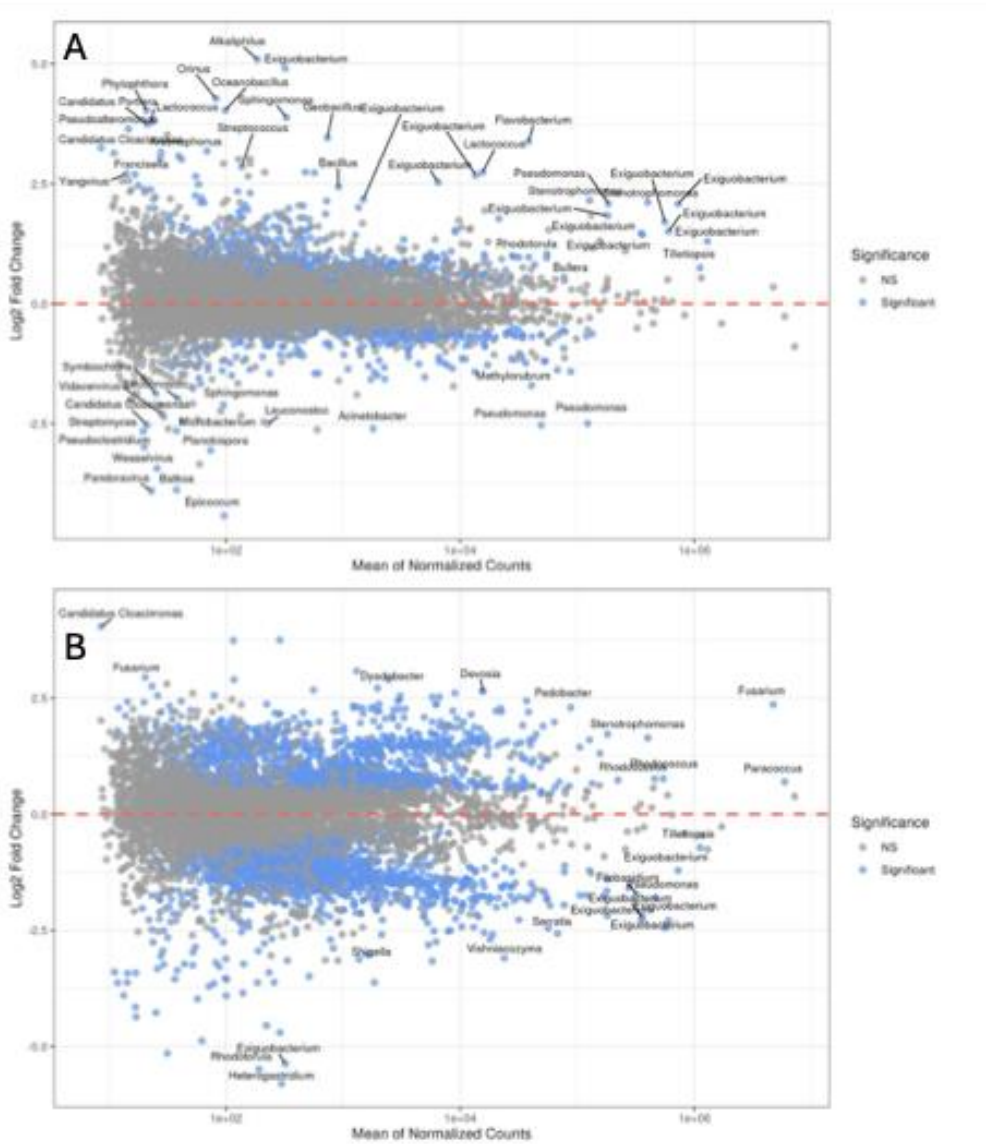

Figure 7. Significantly enriched or depleted taxa in the presence of FHB disease. The Y axis indicates the log2 fold change in abundance of taxa in disease or no disease (A), and between genotypes (B)., while the X axis reflects the abundance of reads from the taxa in the microbiomes. Blue dots indicate statistically significantly enriched or depleted taxa.

- We also used the metagenomics data for generation of metagenome-assembled genomes (MAGs) of bacteria. 17 MAGs showed greater than 90% completeness with

less than 10% contamination. 47% of these genomes show significant correlations with disease rating . Differences in responsiveness to disease were observed depending on genotype. For example, disease-enriched MAG250 (*Pedobacter* sp.) was more strongly enriched in the Explorer variety, while MAG95 (*Spirosoma* sp.) was more strongly enriched in AAC Synergy. (**Figure 8**)

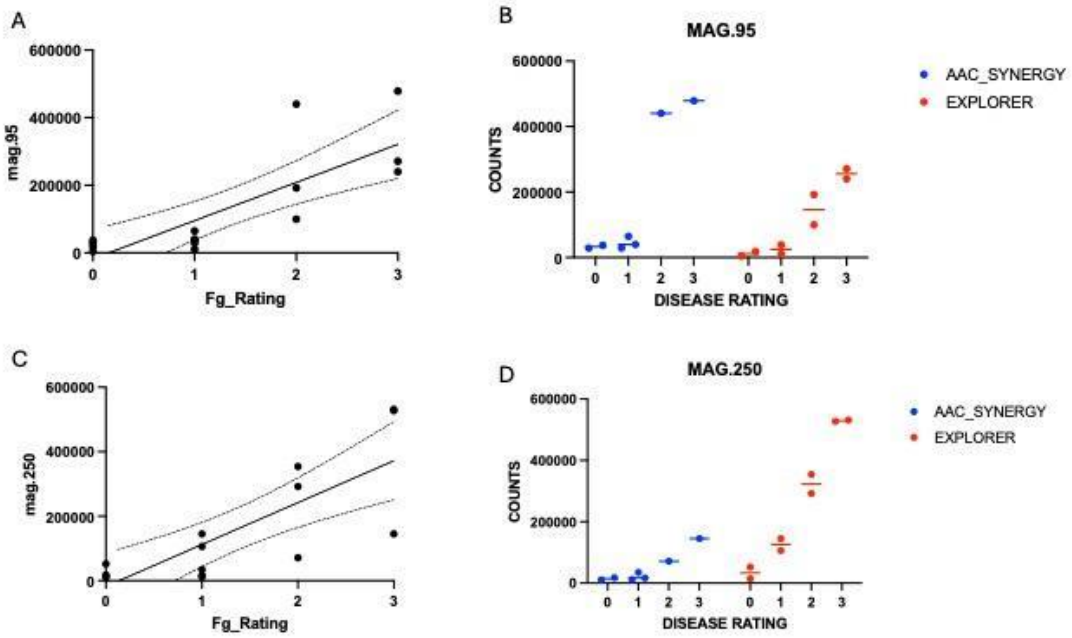

Figure 8. Changes in metagenome assembled genome abundance according to disease. A. and C. show correlations between the abundance of reads matching to MAG95 and MAG250 respectively to disease rating of barley heads in the samples. B and D compare the abundance of MAGs across disease ratings between the two genotypes.

List key outcomes or other achievements.

- Microbiome profiling of diseased and non-diseased heads across 4 U.S. barley nurseries over 2 years, constituting ~800 total samples, provides a significant boost to the understanding of the barley head microbiome structure across the continental US that we expect to be a foundational tool for developing microbial applications in barley production and malting.
- Evidence for disease and genotype-responsiveness of the barley head microbiome using two different methods and study systems supports our hypothesis that disease- and genotype-recruited microbes are present in the barley head microbiome. Once such members are validated for their ability to suppress FHB disease our study sets the stage for breeding towards their increased incorporation in the barley microbiome to assist in disease suppression.
- We received a USDA-NIFA Agricultural Microbiomes Grant (A1402) using these preliminary data to investigate microbial community assembly in barley in the context of FHB disease "Elucidating Transkingdom Microbial Community Assembly in the Barley Phyllosphere during *Fusarium* Infection"

3. What opportunities for training and professional development has the project provided?

- Presented at 2023 Scab initiative with an invited oral presentation by Dr. Barney Geddes "The Impact of Environment, Host Genotype and Fusarium Head Blight on Microbiome Assembly in a Barley Breeding Population"
- Oral presentation by Dr. Briana Whitaker at Ecological Society of America 2023 "Host Genotype & Fusarium head blight filtering in the barley head microbiome"
- Two 2023 Scab initiative posters by students "Experimentally Tractable Systems for Investigating Fusarium Head Blight-Microbiome Interactions on Barley: A Pilot Study" and "Host Genotype and Fusarium Head Blight Status Impact Microbiome Assembly of a Barley Breeding Population Across Four Locations"
- IS-MPMI 2023, Providence, RI (Poster)
- Plant Health 2023, Denver, CO (Poster and Travel Award for Microbiome workshop)

4. How have the results been disseminated to communities of interest?

The results have been disseminated to the scientific communities through the posters and oral presentations mentioned above. Farmers and industry have been approached in conversation from those meetings and commodity group meetings on the progress of this research. Letters of support for this research were received from both the American Malt Barley Association and North Dakota Barley Council to continue and expand this work by applying to federal grants. Two publications, one on amplicon sequencing and one on metagenomics are in preparation for publication for the research community.