Culturomics of *Andropogon gerardii* rhizobiome revealed nitrogen transforming capabilities of stress-tolerant *Pseudomonas* under drought conditions

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Abstract

Background: Climate change will result in more frequent droughts that impact soil-inhabiting microbiomes in the agriculturally vital North American perennial grasslands. In this study, we used the combination of culturomics and high-resolution genomic sequencing of microbial consortia isolated from the rhizosphere of a tallgrass prairie foundation grass, Andropogon gerardii. We cultivated the plant host-associated microbes under artificial drought-induced conditions and identified the microbe(s) that might play a significant role in the rhizobiome of Andropogon gerardii under drought conditions.

Results: Phylogenetic analysis of the non-redundant metagenome-assembled genomes (MAGs) identified the bacterial population of interest – MAG-Pseudomonas. Further metabolic pathway and pangenome analyses detected genes and pathways related to nitrogen transformation and stress responses in MAG-Pseudomonas.

Conclusions: Our data indicate that the metagenome-assembled MAG-Pseudomonas has the functional potential to contribute to the plant host’s growth during stressful conditions. This study provided insights into optimizing plant productivity under drought conditions.

Keywords: Pseudomonas, culturomics, drought, rhizobiome, stress, nitrogen
**Background**

Global climate change is a serious concern, resulting in soil degradation, soil erosion, and impacts on soil health [1]. Climate change has severe impacts worldwide including in the USA, resulting in more frequent and prolonged droughts [2], and is gradually degrading the plant diversity and ecosystem functions [3]. The rhizobiome, microbial communities that are intimately associated with the plant rhizosphere [4,5], is one of the key factors in maintaining ecosystem function, soil quality and plant health [6,7]. The plant rhizosphere is the primary site for plant-microbe and microbe-microbe interactions, governed primarily by root exudates [8]. Microbes in the rhizobiome can facilitate plant host nutrient and water uptake, element cycling (carbon, nitrogen, phosphorus) and other processes that are beneficial to plants [9–11].

Rhizobiomes are also instrumental in enhancing plant hosts’ resistance and resilience against abiotic stresses such as drought, salinity, and heavy metal exposure [12]. Therefore, with the more frequent and more extreme droughts events predicted in the global climate change scenarios in the future, it is even more urgent to provide insights into the mechanisms of how the rhizobiome may promote plant host resilience and response to stress. Although there are various studies that have dissected how climate change impacts the rhizobiome [13–16], more concerted efforts are needed to provide insights into the mechanisms of how the rhizobiome can enhance the plant host resilience during drought-induced experimental stress.

Previous studies have reported a clear contribution from plant-associated microbial members to plant growth and resilience during drought conditions [17–19]. Plant growth-promoting bacteria (PGPB) reportedly enhance plant growth during drought [20,21], an observation attributed to the microbial nitrogen cycling and transformation in soil [22]. Therefore, candidate microbes capable of nitrogen transformation and increase nitrogen availability in the rhizosphere have been the key targets in a growing number of experimental and observational studies that focus on the assembly of plant health promoting Synthetic Communities (SynCom) [23,24]. SynComs have been successfully deployed to alter the plant phenotype, to enhance plant disease resistance
and productivity [25,26]. However, it is challenging and tedious to select optimal members of SynComs because of the lack of knowledge of the microorganisms that could impart favorable functions under stressful conditions [27]. Therefore, in identifying candidate microbes for SynComs, it may be more expedient to identify specific microbial functions and mechanisms rather than to depend solely on taxonomy.

Our long-term, ongoing research on the microbiome of dominant Great Plains prairie grass *Andropogon gerardii* (Big Bluestem) provided an excellent opportunity to acquire deeper insights into the microbial functional potential under abiotic stress [28–30]. There are three *A. gerardii* ecotypes (dry, mesic, and wet) that originated in Hays, Kansas (rainfall ~500 mm/year), Manhattan, Kansas (rainfall ~870 mm/year) and Carbondale, Illinois (rainfall ~1,200 mm/year), respectively [28,29]. In this study, we attempted to elucidate the rhizosphere microbial functional potential from *A. gerardii* growing in Colby, Kansas, where the low precipitation defines a margin of the environment suitable for *A. gerardii* survival and growth. We aimed to identify the *A. gerardii* rhizobiome associated microbial population(s) that are drought resistant or resilient, and to acquire insights into the microbial functions by: (1) isolating and cultivating microbes that existed in the ecotypes using media that promote drought-induced stress [28]; (2) obtaining genomic insights into drought resilient bacterial populations that can contribute to the nitrogen transformation. In this study, we combined culturomics and high-resolution genomic sequencing to identify microbial populations and their functional potential to enhance *A. gerardii* resistance and resilience during drought stress.

**Results and discussion**

**MAGs analysis, phylogenetic analysis, and identification of MAG-Pseudomonas**

We cultured the *A. gerardii* rhizosphere microbial populations using the following samples and media - dry ecotype in R2A, dry ecotype in R2A with PEG, wet ecotype in R2A, and wet ecotype in R2A with PEG. We expected that the PEG-amended media would yield bacterial populations enriched with drought resistant gene functions. We recovered a total of 125 MAGs and generated a total of 63 non-redundant MAGs from
the four conditions (Figure 1A, Supplementary Table S1). We recovered an average of 173,480 ± 22,383 contigs, with N50 of 33,485 ± 2,526. The resolved MAGs had completion values of 89.4% ± 2.0%, and ~92.8% were annotated to the genus level (Supplementary Table S1). Among the 63 non-redundant MAGs, the dominant phyla were Proteobacteria (n=20), Firmicutes (n=36) and Actinobacteria (n=7).

Among the 63 non-redundant MAGs that we resolved, one of the clusters (consisting of four MAGs having > 95% ANI identity) was assigned to the genus *Pseudomonas*. We obtained 40 closest related *Pseudomonas* genomes using the Similar Genome Finder service, and confirmed that the MAG had the greatest similarity to *Pseudomonas sp. NFACC52* (Figure 1B, Supplementary Table S1). We observed that the representative MAG (MAG_001; hereafter referred to as MAG-*Pseudomonas*) for this cluster was highly detected in all the culture conditions, and with their ubiquitous presence in the soil irrespective of the ecotype and drought stress, we hypothesized that MAG-*Pseudomonas* might be an important contributor in the rhizobiome associated with *A. gerardii*. *Pseudomonas spp.* are common in the rhizosphere and reported to have important functions in modulating host performance [54–56]. *Pseudomonas thivervalensis* were isolated from the roots of *Brassica napus* and *Arabidopsis thaliana* [57], and is a functionally significant member of soil microbial communities [55]. *Pseudomonas* have also been implicated to be a plant growth-promoting rhizobacteria (PGPR) and have been associated with plant growth, control of pathogenicity [55] and aid in plant resilience under drought-stressed conditions [54,56].

**Stress response genes identified in MAG-Pseudomonas enhanced drought tolerance**

MAG-*Pseudomonas* has a total length of 6,777,975 bp, with 99 contigs and an N50 of 146,692 bp. The GC content of MAG-*Pseudomonas* is 61.1%. When annotated with the COG database, we noticed that it yielded 5,953 gene calls, and 4,924 were assigned at least one COG categorical function (Table 1).
Previous studies [54,56] based on 16S rRNA gene sequences have identified *Pseudomonas* in aiding the plant host to become more resilient under drought-stressed conditions. This led us to ask what might be some potential functions of *Pseudomonas* that 1) enabled the survival of *Pseudomonas* under stressful conditions; 2) provided cues to how the *Pseudomonas* might assist in the stress tolerance of the associated plant host. We observed 14 COG functions that were assigned to stress responses, with 6 gene functions that were in the universal stress protein (UspA) family, that might enhance the survivability of *Pseudomonas* during stressful conditions. There were 3 COG functions that were assigned to Ser/Thr protein kinase RdoA which was involved in the Cpx stress response. The other gene functions that were identified related to stress responses were putative negative regulator of RcsB (n=1), acid stress-induced BolA-like protein IbaG/YrbA (n=1), stress-induced morphogen (n=1), ribosomal protein L25 (n=1), various environmental stresses-induced protein Ves (n=1) and predicted membrane GTPase involved in stress response (n=1) (Figure 2; Supplementary Table S2). Universal stress protein (USP) superfamily has an important role in the survivability of bacteria under a wide range of stress conditions [58]. In our study, the amendment of PEG in R2A for cultivation not only mimicked the drought conditions, but also generated moderate levels of osmotic shock for the cells [59], the conditions that often accompany drought conditions [60]. Furthermore, we identified Ves proteins during drought/osmotic stress in MAG-*Pseudomonas*. Our results suggest that MAG-*Pseudomonas* had the potential to be more resilient and tolerant against drought stress, demonstrating moderate tolerance to dehydration and water limiting conditions [61–64]. Under stress, most microbes will restructure their metabolism and specifically activate various stress pathways [17]. *Pseudomonas* can utilize a range of mechanisms such as alginate [65] and trehalose production [66] to be more resilient during drought conditions. Besides the 14 stress related gene functions, we also identified DNA-binding transcriptional regulator YbjK, (Figure 2; Supplementary Table S2) which is involved in the regulation of stress response [67]. Drought conditions often result in the generation of oxidative stresses in both plants and their associated microbes [68,69]. There is established documentation about the beneficial role of *Pseudomonas* to alleviate the oxidative
stress [70,71]. Our results illustrated that our MAG-*Pseudomonas* had several regulatory responses that might enhance its drought stress resilience and fitness.

*Nitrogen transformation potential of MAG-Pseudomonas could enhance A. gerardii growth*

Initial genomic analysis showed that our resolved MAG-*Pseudomonas* harbored several stress-response related gene functions. Besides understanding microbial mechanisms of MAG-*Pseudomonas* resilience during drought-induced stress, we were also interested in gaining a deeper understanding of how the plant host could benefit from the *A. gerardii* and MAG-*Pseudomonas* interactions. We detected several gene functions that demonstrated the nitrogen transformation potential in our resolved MAG-*Pseudomonas*, which could contribute to the growth enhancement of the associated plant host, *A. gerardii*.

We detected a number of gene functions that are related to nitrogen transformation - nitrogen fixation protein NifU and related proteins; signal transduction histidine kinase NtrY involved in nitrogen fixation and metabolism regulation (NtrY); signal transduction histidine kinase NtrB, nitrogen specific (NtrB), two-component system; NtrC family (nitrogen regulation response regulator GlnG, nitrogen PTS system EIIA component, and nitrogen regulatory protein PII, GlnK) (Figure 2, Supplementary Table S2). These genes have been previously reported in *Pseudomonas spp* [72–74]. All the nitrogen transformation gene functions that were detected in our MAG-*Pseudomonas* can be essential in helping to fulfill the plant host’s need for nitrogen, especially in N-depleted soils [75–78]. Bacterial Nif genes transform the atmospheric nitrogen to the form that can be utilized by the plants [79,80], whereas NtrY encodes for the sensory kinase of the two-component regulatory system of NtrY/NtrX associated with nitrogen metabolism [81]. NtrB also plays a role in nitrogen metabolism and can regulate the nitrogen dynamics under nitrogen-deprived and enriched environments [82]. NtrC is another nitrogen metabolism regulator that contributes to nitrogen assimilation [73]. Similarly, nitrogen regulatory protein PII (GlnK) and nitrogen PTS system EIIA components are
also involved in regulating nitrogen metabolism [83]. Besides the Nif genes, we further detected gene functions in the MAG-*Pseudomonas* that corresponded to nitrogen assimilation and nitrogen dissimilation (nitrification and denitrification) (Figure 2, Supplementary Table S2), which contributes to the nitrogen cycle [84]. Assimilatory nitrate reductase catalytic subunit was identified in this study that catalyzes the process from nitrate to nitrite [85]. NAD(P)H-nitrite reductase, a large subunit (NirB) was detected that can catalyze nitrite reduction, and forms ammonia [86]. We also detected nitrite reductase (NADH) large and small subunits that can carry out similar processes and contribute to the nitrogen cycle [86].

We used the comparative pathway tool in PATRIC, and identified 138 potential pathways of MAG-*Pseudomonas* based on genomic information from 3 *Pseudomonas* genomes - *Pseudomonas chlororaphis subsp. aurantiaca* strain ARS 38 isolated from the cotton rhizosphere, *Pseudomonas sp.* DR208 and *Pseudomonas sp.* DR48 isolated from the soybean rhizosphere. The identified pathway classes included carbohydrate metabolism, lipid metabolism, metabolism of cofactors and vitamins, energy metabolism, nucleotide metabolism, biosynthesis of secondary metabolites, amino acid metabolism, xenobiotics biodegradation and metabolism, metabolism of other amino acids, glycan biosynthesis and metabolism, translation, signal transduction, and immune system (Supplementary Table S3). We further analyzed the differential occurrence of the genes in MAG-*Pseudomonas* and the 3 *Pseudomonas* genomes, and observed that there was a higher occurrence of nitrate reductase, nitrate reductase (NO-forming), and ubiquinol-cytochrome-c reductase in the MAG-*Pseudomonas* than in the other 3 *Pseudomonas* genomes (Figure 3A). Given the importance of nitrogen-mediated microbial processes on plant growth [87], we analyzed the annotated MAG-*Pseudomonas* nitrogen transformation processes and showed that there were 79 annotated genes that were involved in the nitrogen metabolism pathway with 100% coverage. We identified several important genes in MAG-*Pseudomonas* associated with nitrogen transformation processes such as nitrate reductase (n=14), nitrate reductase (NO-forming) (n=6), ubiquinol-cytochrome-c reductase (n=4), NADH:ubiquinone reductase (H (+) - translocating (n=2), cytochrome-c oxidase (n=2), carbamate kinase
(n=2), glutamin-(aspargin-)ase (n=2), formate dehydrogenase (n=1), glutamate synthase (NADPH) (n=1), glutamate dehydrogenase (n=1), glutamate dehydrogenase (NADP(+)) (n=1), glutamate synthase (ferredoxin) (n=1), aminomethyltransferase (n=1), aspariginase (n=1), glutaminase (n=1), nitrilase (n=1), carbonate dehydratase (n=1), cyanase (n=1), aspartate ammonia-lyase (n=1), glutamate-ammonia ligase (n=1), and aspargine synthase (glutamine-hydrolyzing) (n=1) (Figure 3B). We observed a high number of genes that were related to nitrate reductase, suggesting its importance to the nitrogen metabolism in our MAG-Pseudomonas. The nitrate reductase is critical in reducing nitrate to nitrite for several crop plants, as this reaction leads to the production of proteins that are necessary to maintain plant health [88]. Glutamate syntheses are actively involved in ammonia assimilation pathways in bacteria [89], while glutamate dehydrogenase has a prominent role in nitrogen assimilation and is capable of maintaining the balance of carbon and nitrogen [90]. Putting it all together, our resolved MAG-Pseudomonas with its potential in microbial-driven nitrogen transformation processes could play a critical role in the regulation of primary productivity of its plant host, A. gerardii, even during times of drought-induced stress.

MAG-Pseudomonas is essential to understand the resilience of the host plant under abiotic stress

Our genomic analysis revealed several stress response and nitrogen transformation functional potential, but are there any niche specificity for MAG-Pseudomonas? We used a pangeomic analysis to compare the shared and unique gene functional potential of MAG-Pseudomonas and 6 closely-related Pseudomonas genomes. Our analysis yielded 39,798 genes across the 7 genomes, with a total of 12,473 gene clusters. We used hierarchical clustering to group the gene clusters, showing similar distribution patterns across the 7 genomes (Figure 4, Supplementary Table S4). Our pangenomic analyses identified a collection of 15,605 core gene clusters that occurred in all Pseudomonas genomes, and 752 gene clusters that only occurred in the MAG-Pseudomonas genome. The proportion of genes with functional annotation varies between the core and accessory clusters of MAG-Pseudomonas. We noticed that there
were 94.4% of core gene clusters annotated with gene functions, using NCBI’s Clusters of Orthologous Groups (COGs) database, while only 63.6% of the gene clusters in the accessory clusters of MAG-\textit{Pseudomonas} were annotated (Figure 4, Supplementary Table S4).

We identified several stress response and nitrogen transformation genes in the core cluster in the pangenomic analysis which again reiterated our hypotheses that MAG-\textit{Pseudomonas} might have microbial mechanisms that enhanced its survivability and would contribute to the plant hosts’ well-being under abiotic stress conditions (Figure 4, Supplementary Table S4). The genes that were identified were predicted membrane GTPase TypA/BipA involved in stress response (TypA), putative negative regulator of RcsB-dependent stress response, UPF0070 family (YfgM), universal stress protein E, nucleotide-binding universal stress protein UspA family (UspA), acid stress protein IbaG/YrbA, BolA-like family (IbaG), Ser/Thr protein kinase RdoA involved in Cpx stress response, MazF antagonist (SrkA), desiccation stress tolerance protein with LEA/WHy domain (LEA), BolA family transcriptional regulator, general stress-responsive regulator, uncharacterized stress-responsive protein, DNA-binding protein YaaA associated with the oxidative stress response (YaaA), universal stress protein A, and Ribosomal protein L25 (general stress protein Ctc) (RplY) (Figure 4, Supplementary Table S4). Desiccation stress tolerance proteins with LEA/WHy domain (LEA) is suggested to confer a broad range of stress response function to bacteria such as \textit{Escherichia coli} \cite{91}, while genes corresponding to a WHy protein homologue have been identified in both archaea and bacteria including \textit{Pseudomonas} \cite{92,93} although the specific function in \textit{Pseudomonas} is still incomprehensible. Our findings in MAG-\textit{Pseudomonas} and 6-closely related genomes provided insights into potential gene functions in \textit{Pseudomonas} that could be instrumental in providing resilience against drought induced stress. We also identified a set of universal stress proteins (UspA, UspE), which belonged to bacterial universal stress proteins, and were produced under stressful conditions \cite{94}. We also identified other genes - YaaA (oxidative stress); TypA/BipA (general stress-response regulator, \cite{95}; BolA (family transcriptional regulator, \cite{96}; and Ribosomal protein L25 (general stress protein Ctc) (RplY) \cite{97}, that demonstrated the
potentiality of *Pseudomonas* to elicit one or more microbial mechanisms to become more resilient when subjected to abiotic stresses. Similar to stress response genes, our findings, which identified numerous nitrogen transformation gene functions (Figure 4, Supplementary Table S4), in the pangenome analysis corroborate with our MAG-*Pseudomonas* genome analysis that *Pseudomonas* might have the capability to contribute to the resilience and well-being of the plant host under environmental stresses.

Besides the core-clusters gene functions, we also observed genes related to chemotaxis in the MAG-*Pseudomonas* accessory gene-clusters. We detected genes corresponding to methyl-accepting chemotaxis protein and chemotaxis protein CheD (Figure 4, Supplementary Table S4). Our resolved MAG-*Pseudomonas* might show chemotaxis towards certain amino acids by using methyl-accepting chemotaxis proteins [98], as these bacterial cells are known to methylate the methyl-accepting chemotaxis proteins when adapting to environmental repellents and attractants [99]. Similarly, CheD chemotaxis proteins might be used by MAG-*Pseudomonas* to attract or evade various environmental stimuli [100–102]. Our MAG-*Pseudomonas* also had a gene corresponding to insecticidal toxin complex protein TccC. These proteins exhibit toxicity to a wide range of insects that could be utilized in designing strategies for crop protection [103]. Interestingly, we also identified the LuxR family transcriptional regulator, quorum-sensing system regulator ExpR in MAG-*Pseudomonas*, suggesting that MAG-*Pseudomonas* might use the LuxR proteins to communicate with neighboring bacteria [104] involving Acyl homoserine lactone (AHL)-dependent Quorum Sensing mechanism [105]. MAG-*Pseudomonas*, therefore, has a plethora of gene functions that might enable the microbe to show phenomena such as chemotaxis and quorum sensing.

Tailoring SynComs is an important approach to provide insights into plant host-microbe interactions. Understanding the mechanisms and functions of host-associated microbial populations is particularly relevant in the construction of these plant-associated
SynComs. Our study showed that MAG-*Pseudomonas* not only possessed the resiliency to survive in drought-induced conditions, but were also able to perform essential microbial functions for generating products related to the nitrogen cycle [106], which could be exploited by plant host and other host-associated microbes [107]. A SynCom consisting of six *Pseudomonas* strains isolated from the garlic rhizosphere has been reported to promote plant growth [108]. Apart from the potential to contribute to the plant host’s well-being, our MAG-*Pseudomonas* might also be able to influence and interact with other bacteria, [109], contributing to its role as an important member of the core rhizobiome along with other members such as *Streptomyces*, *Rhizobium*, *Burkholderia*, *Nitrosomonas*, *Nitrospira*, *Azospirillum*, *Bradyrhizobium*, and *Azotobacter* [110]. Overall, our study emphasized that the understanding of the MAG-*Pseudomonas* mechanism and functional potential might contribute to the successful construction of a SynCom that can benefit the plant-host during drought-induced stress [27].

**Conclusion**

In this study, we used culturomics and metagenomic strategy to identify bacterial populations in the *A. gerardii* rhizobiome, and identified MAG-*Pseudomonas* as the candidate microbe that had significant functional potential in nitrogen transformation and stress response. In support of other studies, our study verified the abundance of MAG-*Pseudomonas* in the rhizobiome and suggested its potential pivotal role under drought conditions. In a continuing effort to understand the contributions of different microbiota in the plant rhizobiome, it is important to remember that identity and relative abundance alone may not truly reflect the relative functional importance of the bacterial population. Instead, understanding the functional role of the microbe during host-microbe and microbe-microbe interactions might provide more insights. The functional potential of our resolved MAG-*Pseudomonas*, resulting from a combination of conventional culturing and high-throughput analysis, showed the immense potential to inform and refine our efforts to dissect the mechanistic interaction taking place in the rhizobiome.
**Materials and Methods**

*Plot design, sampling, and cultivation of rhizosphere communities from soil samples*

We collected *Andropogon gerardii* rhizosphere samples from a common garden in Colby at the Kansas State University Agricultural Research Center located in Thomas County (39°23′N, 101°04′W). Further information on the experimental layout, ecotypes, and sampling collections has been described previously [30]. In this comparative study, we selected rhizosphere samples from native dry (Hays, Kansas) and wet (Carbondale, Illinois) ecotypes for microbial cultivation. We separated bulk soil from the soil attached to the rhizosphere by handshaking the roots gently. We dissolved 0.1 g of the rhizosphere samples in 0.9 ml of Phosphate-Buffered Saline (PBS) [pH 7] buffer, serially diluted the samples (10^{-1} - 10^{-6}), and spread 100μl solution onto the Petri plates. We designed two culture conditions - 0.315% R2A media (Teknova, USA) [31] and 0.315% R2A media amended with a 36% Polyethylene Glycol 8000 (PEG) (Ψ = -1.54 MPa) to alter the media osmotic potential and to mimic absence and presence of water limitation, respectively [32,33]. A similar range of PEG concentrations has been used to simulate dry environments in other studies [34,35]. To prepare the R2A-PEG media, we 36% (w:v) dissolved PEG powder in autoclaved MilliQ water, allowing the mixed solution (20 mL) to sit on top of a pre-made R2A plate for 24 hours to diffuse throughout the agar. After 24 hours, we removed excess solution and spread 100μl of the diluted soil culture on the surface of the agar. The prepared plates were incubated at 37°C for 24-48 hours until the appearance of the colonies. After the incubation period, we scraped all colonies by flooding the plate with 2 mL of sterile PBS buffer, transferred the liquid that contained microbes and stored at -20°C until genomic DNA extraction. We were interested in capturing the microbial consortia that grew together in different conditions, so instead of picking individual colonies, we scraped all colonies from the individual plates to sequence the full genome(s) [36,37]. Rhizosphere microbial communities were cultivated from dry (R2A; n=10 and R2A+PEG; n=10) and wet ecotypic *A. gerardii* rhizosphere samples (R2A; n=10 and R2A+PEG; n=10).
DNA extraction, shotgun sequencing, and analyses

We extracted the microbial DNA with the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) following the manufacturer’s protocol. Shotgun metagenomes were sequenced from the extracted samples on the Illumina NovaSeq 6000 (Illumina, San Diego, CA, United States), with a 150 bp paired-end sequencing strategy, with Nextera DNA Flex for library preparation and S1 flow cell. We used the program ‘iu-filer-quality-minoche’ [38] to process the short metagenomic reads, and the quality-filtered short reads were assembled into longer contiguous sequences (contigs) using MetaHit [39] with a minimum contig length of 1000 bp. We then identified open reading frames (ORFs) in the contigs, and recruited metagenomic short reads to the contigs. We used CONCOCT [40] to bin the metagenomes, and used anvi’o ver 7.0 [41] to manually curate the bins into metagenome-assembled genomes (MAGs) that satisfied the conditions of >70% completion and <10% redundancy based on single copy genes. NCBI’s Cluster of Orthologous Groups (COGs) [42] was used to assign functions to the ORFs. The MAGs were assigned to taxa using the single-copy core genes of bacteria and archaea. We further compared the resolved MAGs using Average Nucleotide Identity (ANI) [43] to identify non-redundant MAGs based on 95%ANI [44].

Phylogenetic, pathway, and pangenomic analyses

Among the resolved MAGs, there was a MAG of interest for this study: MAG-Pseudomonas. The selected non-redundant MAG was analyzed by Similar Genome Finder service that uses the MinHash on the Pathosystems Resource Integration Center (PATRIC) web portal [45,46]. Similar genomes deposited in public databases were obtained and used to estimate the genome distances to the MAG-Pseudomonas. We constructed a phylogenetic tree for the selected non-redundant MAG and 40 closely related genomes. The workflow used the PATRIC Codon Tree Service which used the amino acid and nucleotide sequences from a well-defined database of global protein families [47]. Then, we used the RAxML program [48] to construct a tree based on the pairwise differences between the aligned protein families of the selected sequences.
We used the comparative pathway tool of PATRIC to predict the metabolic pathways in our selected MAG. To compare the pathways, we selected *Pseudomonas* genomes from rhizospheres of cotton and soybean. KEGG maps and heat maps of the nitrogen metabolism pathway were generated in the PATRIC portal.

We downloaded 6 closely-related *Pseudomonas* genomes from NCBI RefSeq [49] and performed pangenomic analyses using anvi’o workflow [41,50]. The workflow uses BLASTP [51] to compute amino acid level similarities between all possible ORF pairs. We then used Markov Cluster Algorithm (MCL) [52] to group ORFs into homologous gene clusters and aligned amino acid sequences in each gene cluster using MUSCLE for visualization [53]. We determined the core gene clusters of the MAG-*Pseudomonas* and the 6 additional, available *Pseudomonas* genomes, as well as the accessory gene cluster of MAG-*Pseudomonas*.

**Availability of data**

The raw data used in this study are publicly available at NCBI under the project accession PRJNA844897. Analyzed data in the form of databases and fasta files can be found at figshare https://doi.org/10.6084/m9.figshare.20005550.
**Figures and Tables legend:**

Figure 1: (A) Detection of non-redundant metagenome-assemble genomes (MAGs) in the rhizosphere of dry and wet *Andropogon gerardii* ecotypes when cultivated in normal precipitation (without PEG) and under drought-induced conditions (with PEG). MAG-*Pseudomonas* was highly detected in all growing media conditions of both dry and wet ecotypic rhizosphere samples. (B) Phylogenetic analysis of MAG-*Pseudomonas* with closely related 40 genomes. *Pseudomonas* sp. NFACC52 is the most closely related genomes to MAG-*Pseudomonas*.

Figure 2: Stress response and nitrogen transformation genes identified in MAG-*Pseudomonas*. Each layer represents a sample (ecotype x media used) and each split represents a contig. There were 14 genes annotated to stress responses and 11 genes were annotated to nitrogen transformation capability.

Figure 3: (A) Differential occurrence of the genes in MAG-*Pseudomonas* with *Pseudomonas chlororaphis* subsp. *aurantiaca* strain ARS 38, *Pseudomonas* sp. DR208 and *Pseudomonas* sp. DR48. The darker the highlight represents higher occurrences in the MAG-*Pseudomonas*. MAG-*Pseudomonas* showed high differential occurrences in nitrate reductase, nitrate reductase (NO-forming), and ubiquinol-cytochrome-c reductase as compared to the other 3 genomes. (B) Nitrogen metabolism pathways in MAG-*Pseudomonas* were detected based on a comparative pathway tool in PATRIC. MAG-*Pseudomonas* had 79 annotated genes involved in the nitrogen metabolism pathway with 100% coverage.

Figure 4: Pangenomic analysis of MAG-*Pseudomonas* with 6 closely-related *Pseudomonas* genomes. The closely-related genomes include *Pseudomonas thiivervalensis* strain DSM 13194, *Pseudomonas synxantha* strain R6 28 08, *Pseudomonas stutzeri* strain F2a, *Pseudomonas fluorescens* strain ATCC 13525, *Pseudomonas chlororaphis* strain qlu-1, and *Pseudomonas brassicacearum* strain
The pangenomic analyses show the core gene clusters and MAG-
*Pseudomonas* only accessory gene clusters.

Table 1: Assembly statistics of MAG-*Pseudomonas*: GC-content, N-50, number of contigs, percent completion, percent redundancy, and total length.

Supplementary Table S1: Non-redundant MAGs and taxonomic identity.

Supplementary Table S2: Gene functions of MAG-*Pseudomonas*.

Supplementary Table S3: Pathways identified in MAG-*Pseudomonas*.

Supplementary Table S4: Shared and unique gene clusters identified in pangenomic analysis of MAG-*Pseudomonas* with 6 closely-related *Pseudomonas* genomes.
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**Author’s contributions**

S.S. conceptualized, conducted the experiments, performed the data analysis, and wrote the manuscript. A.K., K.W., and E.H. conducted the experiments. Q.R. and B.F. analyzed the data. M.G., A.J., and L.J. reviewed the writing for the manuscript. S.L. conceptualized, performed the data analysis, supervised, was responsible for resource acquisitions, and wrote the manuscript. All the authors contributed to the article and approved the submitted version.

**Ethics approval and consent to participate**

Not Applicable.

**Competing Interests**

The authors declare no competing interests.
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Figure 1
Figure 2
Figure 3
Table 1.

|                       | MAG-Pseudomonas |
|-----------------------|-----------------|
| GC-content            | 61.11%          |
| N-50                  | 146,692 bp      |
| Number of contigs     | 99              |
| Percent Completion    | 100%            |
| Percent redundancy    | 1.41%           |
| Total length          | 6,777,975 bp    |