Pseudomonas cultivated from *Andropogon gerardii* rhizosphere show functional potential for promoting plant host growth and drought resilience

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Abstract

Background: Climate change will result in more frequent droughts that can impact soil-inhabiting microbiomes (rhizobiomes) in the agriculturally vital North American perennial grasslands. Rhizobiomes have contributed to enhancing drought resilience and stress resistance properties in plant hosts. In the predicted events of more future droughts, how the changing rhizobiome under environmental stress can impact the plant host resilience needs to be deciphered. There is also an urgent need to identify and recover candidate microorganisms along with their functions, involved in enhancing plant resilience, enabling the successful development of synthetic communities.

Results: In this study, we used the combination of cultivation and high-resolution genomic sequencing of bacterial communities recovered 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. Phylogenetic analysis of the non-redundant metagenome-assembled genomes (MAGs) identified a bacterial genome of interest – MAG-*Pseudomonas*. Further metabolic pathway and pangenome analyses recovered genes and pathways related to stress responses including ACC deaminase; nitrogen transformation including assimilatory nitrate reductase in MAG-*Pseudomonas,* which might be associated with enhanced drought tolerance and growth for *Andropogon gerardii*.

Conclusions: Our data indicated that the metagenome-assembled MAG-*Pseudomonas* has the functional potential to contribute to the plant host's growth during stressful conditions. Our study also suggested the nitrogen transformation potential of *MAG-Pseudomonas* that could impact *Andropogon gerardii* growth in a positive way. The cultivation of MAG-*Pseudomonas* sets the foundation to construct a successful synthetic community for *Andropogon gerardii*. To conclude, stress resilience mediated through genes ACC deaminase, nitrogen transformation potential through assimilatory nitrate reductase in MAG-*Pseudomonas* could place this microorganism as an important candidate of the rhizobiome aiding the plant host resilience under environmental stress. This study, therefore, provided insights into the MAG-*Pseudomonas* and its potential to optimize plant productivity under ever-changing climatic patterns, especially in frequent drought conditions.

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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], 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 one of the sites 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 such as inducing plant growth, and enhancing plant drought tolerance that is beneficial to plants [9–11].

Rhizobiomes are 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 essential to provide new insights into the mechanisms of how the rhizobiome may promote plant host resilience and response to stress. Reports show that bacteria populations can modulate the associated plant stress responses to environmental stresses [13, 14]. Plants respond to the above-mentioned stressors by modulating the level of various hormones, such as ethylene, which in turn induce the expression of stress-related proteins [15, 16]. However, when ethylene is produced more than its threshold level, it becomes unfavorable in terms of root/shoot proliferation and other growth parameters, hindering plant growth and development [17]. Bacteria-mediated 1-aminocyclopropane-1-carboxylate deaminase (ACCD) is able to mediate the enhanced resistance to biotic stressors and increased tolerance to abiotic stresses in their associated plant hosts [18–24], by breaking down ACC, an immediate precursor of ethylene resulting in plants resuming root/shoot growth [25, 26]. Although there are various studies that have dissected how climate change impacts the rhizobiome [13, 27–29], more concerted efforts are needed to provide insights into the mechanisms of how the rhizobiome can enhance the plant host resilience during drought-induced stress.

Previous studies have reported a clear contribution from plant-associated microbial members to plant growth and resilience during drought conditions [30–32].

Plant growth-promoting bacteria (PGPB) reportedly enhance plant growth during drought [33, 34], an observation attributed to the microbial nitrogen cycling and transformation in soil [35]. Therefore, candidate microbes capable of nitrogen transformation and increasing 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) [36, 37]. SynComs have been successfully deployed to alter the plant phenotype, to enhance plant disease resistance and productivity [38, 39]. 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 [40]. 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 [41–43]. There are three *A. gerardii* ecotypes (dry, mesic, and wet) that originated in Hays, Kansas (averaged annual precipitation ~ 500 mm), Manhattan, Kansas (averaged annual precipitation ~ 870 mm) and Carbondale, Illinois (averaged annual precipitation ~ 1,200 mm), respectively [41, 42]. In this study, we attempted to elucidate the rhizosphere microbial functional potential from *A. gerardii* dry and wet ecotypes growing in Colby, Kansas, where the low precipitation defines a margin of the environment suitable for *A. gerardii* survival and growth. In Colby, the averaged annual precipitation regime is comparable to Hays, Kansas. 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) recovering and cultivating microbes that existed in the ecotypes using media that promote drought-induced stress [41]; (2) obtaining genomic insights into drought resilient bacterial populations that can contribute to the nitrogen transformation. In this study, we combined cultivation and high-resolution genomic sequencing to identify microbial populations and their functional potential to enhance *A. gerardii* resistance and resilience during drought stress. The ultimate goal of this study is to identify bacteria populations and their functional potentials in synthetic communities

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(SynComs). In the previous study, we recovered gDNA directly from the soil to identify the bacterial and fungal populations under abiotic environmental stress [44]. The current study takes one step further to undertake a strategy to potentially construct operative SynComs in the future, by cultivating and selecting for a few potential candidate microorganisms that could be engineered for the benefit of the host.

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 an average of $173,480 \pm 22,383$ contigs, with N50 of $33,485 \pm 2,526$. The resolved metagenome-assembled genomes (MAGs) were 4.1 ± 1.3 Mbp had completion values of $89.4\% \pm 2.0\%$, and $\sim 92.8\%$ were annotated to the genus level (Supplementary Table S1). We recovered a total of 125 MAGs and generated a total of 63 non-redundant MAGs from the four conditions (Fig. 1A, Supplementary Table S1). MAGs that share > 95% average nucleotide identity were considered to be redundant MAGs. We identified 62 redundant MAGs, and those were excluded from downstream analyses. The dominant phyla among the non-redundant MAGs 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 observed that the MAG was phylogenetically close to *Pseudomonas sp.* NFACC52 (Fig. 1B, Supplementary Table S1). The MAG-*Pseudomonas* was found to belong to the *Pseudomonas corrugata* subgroup within the *Pseudomonas fluorescence* species complex [45, 46]. 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*. Not commonly found, but rarely *Pseudomonas* has been identified in the *A.gerardii* rhizosphere [47]. *Pseudomonas spp.* are common in the rhizosphere of other plants, and reported to have important functions in modulating host performance [48–50]. MAG-*Pseusomonas* was found to be relatively close to the *Pseudomonas thivervalensis*. The species *P. thivervalensis* was reported to be isolated from the roots of *Brassica napus* and *Arabidopsis thaliana* [51], and is an important member of soil microbial communities [49]. *Pseudomonas* have also been implicated to be a plant growth-promoting rhizobacteria (PGPR) and have been associated with plant growth, control of pathogenicity [49] and aid in plant resilience under drought-stressed conditions [48, 50].

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 [48, 50] based on 16S rRNA gene sequences have identified *Pseudomonas* in aiding the plant host to become more resilient under drought-stressed conditions. *Pseudomonas* is highly diverse in phylogeny and functions [45]. With the observation from the phylogenetic tree analysis that MAG-*Pseudomonas* belonged to the *Pseudomonas corrugata* phylogenomic subgroup, we 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 identified the universal stress protein (UspA) family (Table 2), which suggested that it had an important putative functional role in the survivability of MAG-*Pseudomonas* under a wide range of stress conditions [52, 53] which includes starvation

(See figure on next page.)

Fig. 1 **A** Detection, showing reads recruited to the contigs 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). The darker the highlight represents higher detection in the samples. MAG-*Pseudomonas* was highly detected in all growing media conditions of both dry and wet ecotypic rhizosphere samples. **B** Phylogenetic analysis of MAG-*Pseudomonas* based on the 40 closely related whole genomes with small set of potential outgroup genomes. Tree scale indicated the length of the branches of the tree in terms of evolution. Note that *Pseudomonas fluorescens* F113 and *Pseudomonas fluorescens* FR1 are now known to be *Pseudomonas ogarae* sp. nov., nom rev., type strain F113^T (= DSM 112162^T = CECT 30235.^{T}). *Pseudomonas* sp. NFACC52 was the most closely related genome to MAG-*Pseudomonas*. The outgroup in the phylogenetic tree is *Pseudomonas* sp. NFACC23-1, *Pseudomonas* sp. NFACC16-2, and *Pseudomonas* sp. NFACC17-2
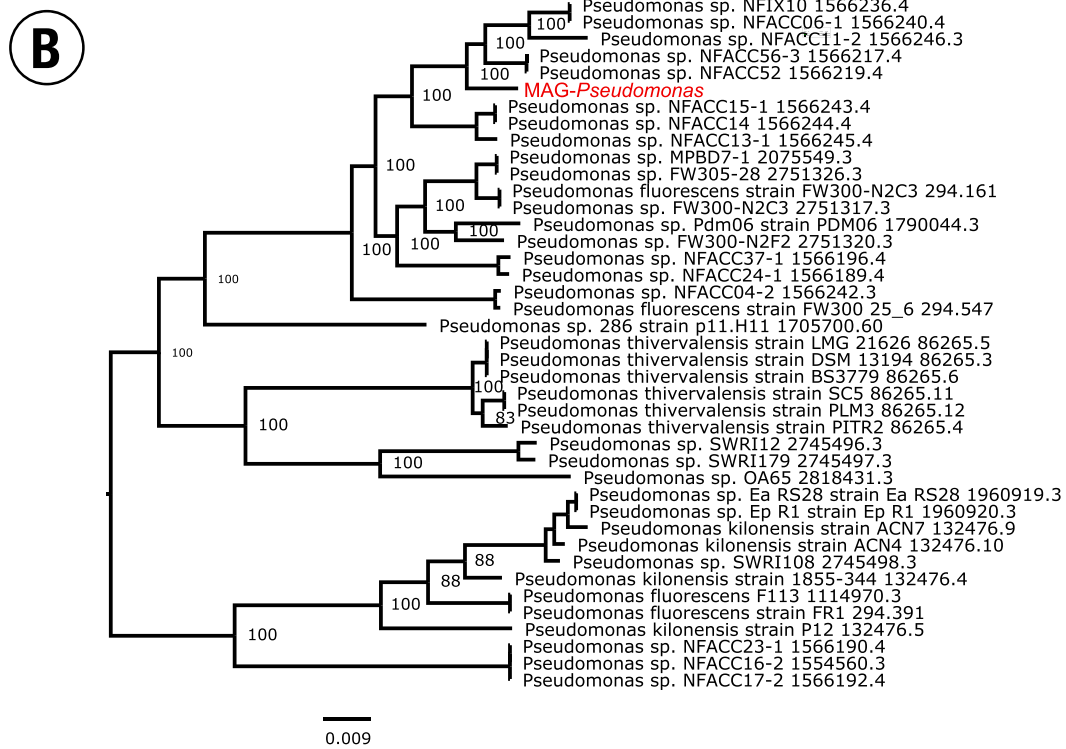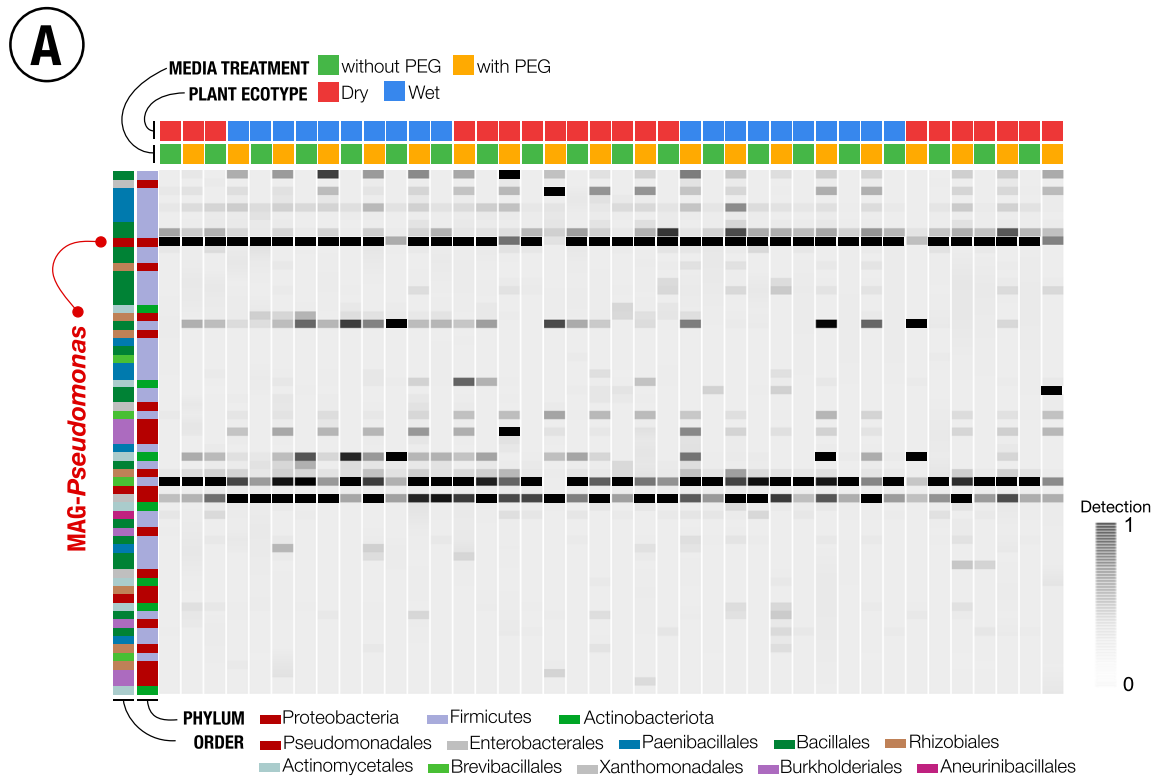
Fig. 1 (See legend on previous page.)

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

	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

of elements like nitrogen, carbon, phosphate, sulfate as well as heat exposures, etc. [53]. Drought might reduce growth yields [54], and UspA has been found to be abundant in growth-arrested cells [55]. We further identified aminocyclopropane-1-carboxylic acid (ACC) deaminase ($n = 3$) in the MAG-*Pseudomonas*, implying the putative gene could be essential in the modulation of the associated plant's stress responses during drought conditions (Table 2, Supplementary Table S2). ACC deaminase is an enzyme known to enhance plant growth by cleaving plant-produced ACC, which decreases the ethylene production in the plant, thereby stimulating plant growth [18]. Moreover, the ACC deaminase has been extensively studied in *Pseudomonas* [56], and its role in stress tolerance is well documented [20, 21]. Putting it all together—1) MAG-*Pseudomonas* was closely related to *P. corrugata* in our phylogenomic analyses; 2) both *P. corrugata* [45] and MAG-*Pseudomonas* possess ACC deaminase; our results strongly suggested that MAG-*Pseudomonas* had functional potential in enhancing the associated plant's resilience during drought stress.

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 putative 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.*

Nitrogen transformation genes—nitrogen regulation response regulator GlnG, nitrogen PTS system EIIA component, nitrogen regulatory protein PII, GlnK, and nitrogen regulation protein NtrB, that were detected in our resolved MAG-*Pseudomonas* (Table 2, Supplementary Table S2), has also been previously reported in other *Pseudomonas spp* [57–59]. 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 [60–63]. NtrB also plays a role in nitrogen metabolism and can regulate the nitrogen dynamics under nitrogen-deprived and enriched environments [64]. NtrC is another nitrogen metabolism regulator that contributes to nitrogen assimilation [58]. Similarly, nitrogen regulatory protein PII (GlnK) and nitrogen PTS system EIIA components are also involved in regulating nitrogen metabolism [65]. Assimilatory nitrate reductase catalytic subunit was also identified in this study which catalyzes the process from nitrate to nitrite [66] (Table 2).

We selected the 3 genomes from the Pathosystems Resource Integration Center (PATRIC) web portal. 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 criteria for the selection of these genomes were: 1) genomes were obtained from the rhizosphere; 2) genomes were complete and of high quality. 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 high occurrence of nitrate reductase and glutamate synthase in the MAG-*Pseudomonas* genome when compared with the other genomes (Fig. 2A). Nitrate reductase plays a key role in nitrogen transformation processes [66]. Given the importance of nitrogen-mediated microbial processes on plant growth [67], 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 in the nitrogen metabolism pathway (Table 2, Fig. 2B). Glutamate synthases, identified in MAG-*Pseudomonas* are actively involved in ammonia

Table 2 Annotated gene features in MAG-*Pseudomonas*. The gene features were annotated using the PATRIC portal. The table lists genes from stress, nitrogen transformation, and pathogenic behavior detected in MAG-*Pseudomonas*. This includes assimilatory nitrate reductase large subunit, ACC deaminase and LPQ island features

Putative gene function	Categories
NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain J (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain I (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain H (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain G (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain F (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain E (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain C (EC 1.6.5.3) / NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain B (EC 1.6.5.3)	Nitrogen
Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	Nitrogen
Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	Nitrogen
Plant-induced nitrilase (EC 3.5.5.1), hydrolyses beta-cyano-L-alanine	Nitrogen
Glutamine synthetase type III (EC 6.3.1.2)	Nitrogen
Aminomethyltransferase	Nitrogen
Glutamate synthase [NADPH] putative GlxC chain (EC 1.4.1.13)	Nitrogen
Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	Nitrogen
L-asparaginase (EC 3.5.1.1)	Nitrogen
Aminomethyltransferase	Nitrogen
Cytochrome c oxidase polypeptide III (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase polypeptide I (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	Nitrogen
Carbonic anhydrase, beta class (EC 4.2.1.1)	Nitrogen
L-asparaginase I, cytoplasmic (EC 3.5.1.1)	Nitrogen
Aspartate ammonia-lyase (EC 4.3.1.1)	Nitrogen
Glutaminase (EC 3.5.1.2)	Nitrogen
Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)	Nitrogen
Assimilatory nitrate reductase large subunit (EC 1.7.99.4)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoP (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoQ (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoO (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoN (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoP (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoQ (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoO (EC 1.9.3.1)	Nitrogen
Cytochrome c oxidase (cbb3-type) subunit CcoN (EC 1.9.3.1)	Nitrogen
Glutamine synthetase (EC 6.3.1.2), putative	Nitrogen
NAD-specific glutamate dehydrogenase (EC 1.4.1.2), large form	Nitrogen
Ubiquinol-cytochrome C reductase iron-sulfur subunit (EC 1.10.2.2)	Nitrogen
Ubiquinol-cytochrome C reductase, cytochrome B subunit (EC 1.10.2.2)	Nitrogen
Formamidase (EC 3.5.1.49)	Nitrogen
Carbonic anhydrase, beta class (EC 4.2.1.1)	Nitrogen
Carbonic anhydrase, beta class (EC 4.2.1.1)	Nitrogen
Cyanate hydratase (EC 4.2.1.104)	Nitrogen
Carbamate kinase (EC 2.7.2.2)	Nitrogen
Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)	Nitrogen
Ferredoxin-dependent glutamate synthase (EC 1.4.7.1)	Nitrogen
Glutamine synthetase type I (EC 6.3.1.2)	Nitrogen
NADH-ubiquinone oxidoreductase chain N (EC 1.6.5.3)	Nitrogen
NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)	Nitrogen
Nitrogen regulatory protein P-II, GlnK	Nitrogen
PTS IIA-like nitrogen-regulatory protein PtsN	Nitrogen
Nitrogen regulation protein NtrB (EC 2.7.13.3)	Nitrogen
Nitrogen regulation protein NR(I), GlnG (=NtrC)	Nitrogen
Two-component transcriptional response regulator, LuxR family	Pathogenic
Acyl-homoserine lactone-binding transcriptional activator, LuxR family	Pathogenic
DNA-binding response regulator, LuxR family, near polyamine transporter	Pathogenic
Sensory box transcriptional regulator, LuxR family	Pathogenic
transcriptional regulator, LuxR family	Pathogenic
DNA-binding response regulator, LuxR family, near polyamine transporter	Pathogenic
Homoserine/homoserine lactone efflux protein	Pathogenic
1-aminocyclopropane-1-carboxylate deaminase (EC 3.5.99.7)	Stress
Universal stress protein family	Stress
Universal stress protein family 7	Stress
Universal stress protein family 5	Stress

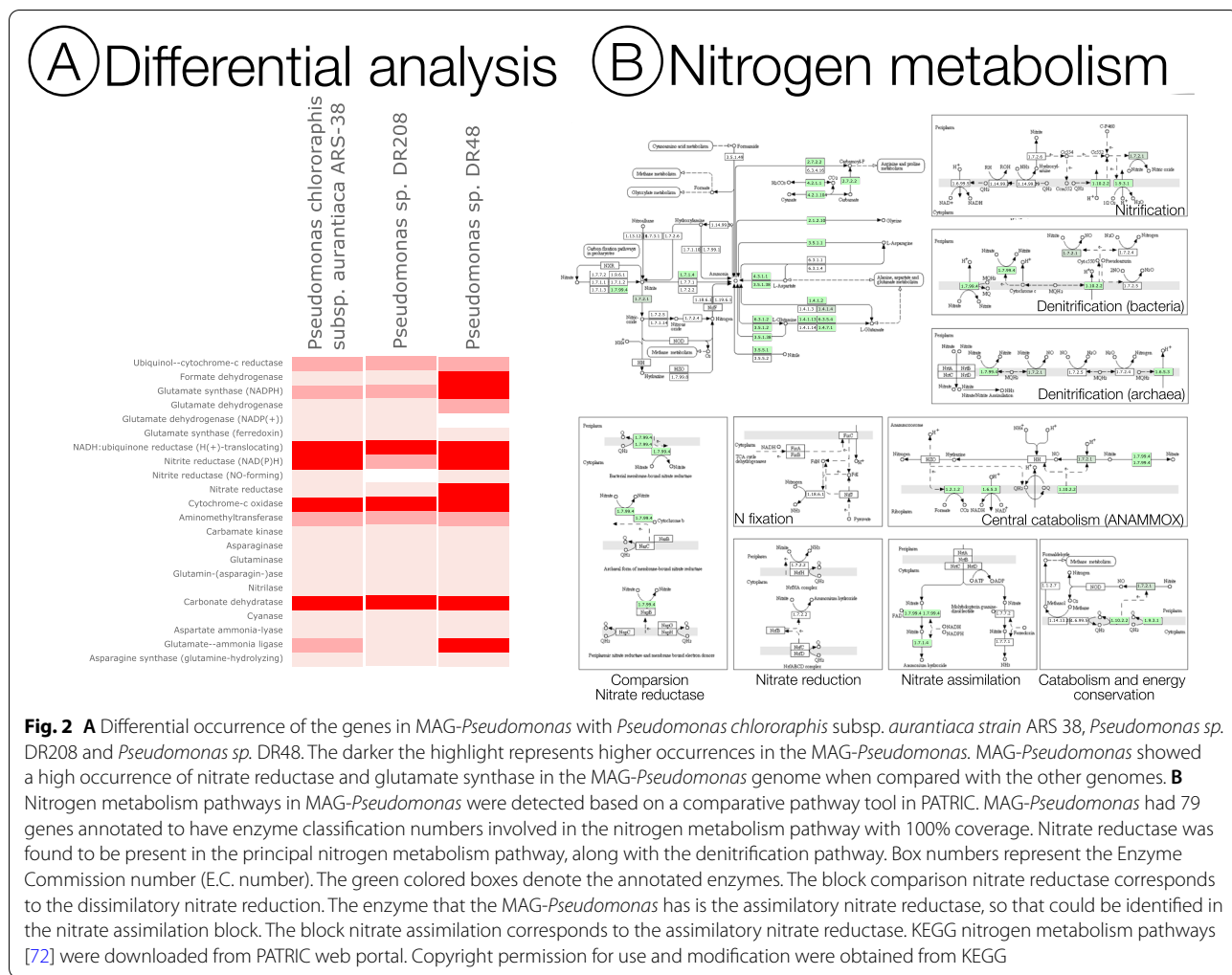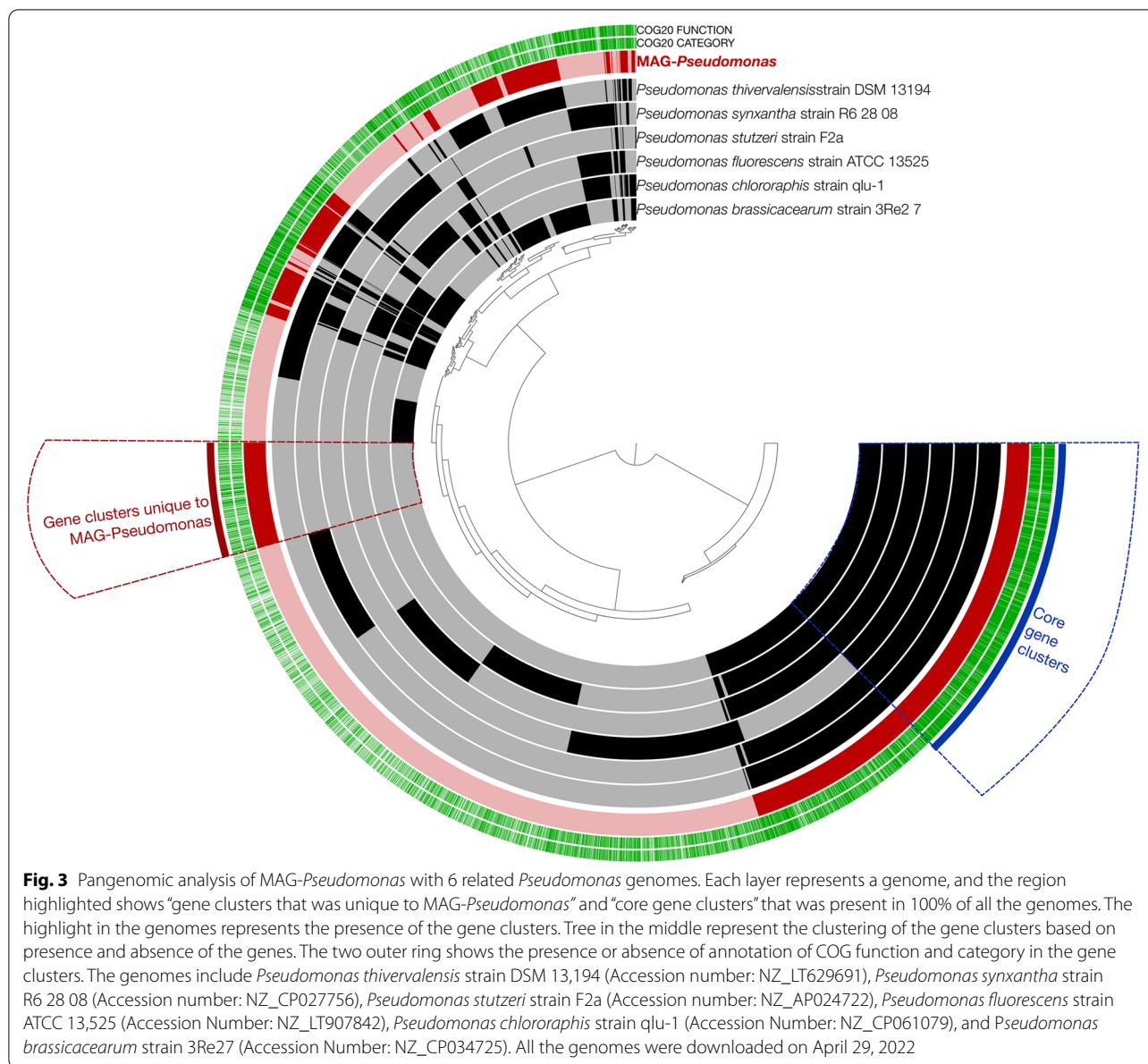Sarkar *et al. BMC Genomics*   (2022) 23:784

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Fig. 2 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 a high occurrence of nitrate reductase and glutamate synthase in the MAG-*Pseudomonas* genome when compared with the other genomes. **B** Nitrogen metabolism pathways in MAG-*Pseudomonas* were detected based on a comparative pathway tool in PATRIC. MAG-*Pseudomonas* had 79 genes annotated to have enzyme classification numbers involved in the nitrogen metabolism pathway with 100% coverage. Nitrate reductase was found to be present in the principal nitrogen metabolism pathway, along with the denitrification pathway. Box numbers represent the Enzyme Commission number (E.C. number). The green colored boxes denote the annotated enzymes. The block comparison nitrate reductase corresponds to the dissimilatory nitrate reduction. The enzyme that the MAG-*Pseudomonas* has is the assimilatory nitrate reductase, so that could be identified in the nitrate assimilation block. The block nitrate assimilation corresponds to the assimilatory nitrate reductase. KEGG nitrogen metabolism pathways [72] were downloaded from PATRIC web portal. Copyright permission for use and modification were obtained from KEGG

assimilation pathways in bacteria [68] (Table 2), while glutamate dehydrogenase has a prominent role in nitrogen assimilation and is capable of maintaining the balance of carbon and nitrogen [69] (Table 2). We identified a wide range of genes involved in nitrogen transformation processes in MAG-*Psuedomonas* that could increase the nitrogen availability to the plant host [70, 71]. 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 is 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 related *Pseudomonas* genomes. We selected *Pseudomonas* genomes from RefSeq that have complete chromosomes [73], and had been subjected to rounds of quality check and consistent gene annotation [74], in order to ensure more conclusive pangenome analysis. 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 (Fig. 3, Supplementary Table S4). Our pangenomic analyses identified a collection of 2,112 core gene clusters that occurred in 100% of all *Pseudomonas* genomes, and 719 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

Fig. 3 Pangenomic analysis of MAG-*Pseudomonas* with 6 related *Pseudomonas* genomes. Each layer represents a genome, and the region highlighted shows "gene clusters that was unique to MAG-*Pseudomonas*" and "core gene clusters" that was present in 100% of all the genomes. The highlight in the genomes represents the presence of the gene clusters. Tree in the middle represent the clustering of the gene clusters based on presence and absence of the genes. The two outer ring shows the presence or absence of annotation of COG function and category in the gene clusters. The genomes include *Pseudomonas thivervalensis* strain DSM 13,194 (Accession number: NZ_LT629691), *Pseudomonas synxantha* strain R6 28 08 (Accession number: NZ_CP027756), *Pseudomonas stutzeri* strain F2a (Accession number: NZ_AP024722), *Pseudomonas fluorescens* strain ATCC 13,525 (Accession Number: NZ_LT907842), *Pseudomonas chlororaphis* strain qlu-1 (Accession Number: NZ_CP061079), and P*seudomonas brassicacearum* strain 3Re27 (Accession Number: NZ_CP034725). All the genomes were downloaded on April 29, 2022

accessory clusters of MAG-*Pseudomonas* were annotated (Fig. 3, 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-*Pseudomonas* might have microbial mechanisms that enhanced its survivability and would contribute to the plant hosts' well-being under abiotic stress conditions (Fig. 3, Supplementary Table S4). The genes that were identified were predicted universal stress protein E, nucleotide-binding universal stress protein UspA family (UspA), desiccation stress tolerance protein with LEA/WHy domain (LEA), and universal stress protein A. (Fig. 3, 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 *Escherichia coli* [75], while genes corresponding to a WHy protein homologue have been identified in both archaea and bacteria including *Pseudomonas* [76, 77] although the specific function in *Pseudomonas* is still incomprehensible. Our findings in MAG-*Pseudomonas* and 6 related genomes provided insights into potential gene functions in *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 [55]. We also identified other genes—YaaA (oxidative stress); TypA/BipA (general stress-response regulator, [78]; BolA (family transcriptional regulator, [79]; and Ribosomal protein L25 (general stress protein Ctc) (RplY) [80], 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 (Fig. 3, 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 (Supplementary Table S4). Our resolved MAG-*Pseudomonas* might show chemotaxis towards certain amino acids by using methyl-accepting chemotaxis proteins [81], as these bacterial cells are known to methylate the methyl-accepting chemotaxis proteins when adapting to environmental repellents and attractants [82]. Similarly, CheD chemotaxis proteins might be used by MAG-*Pseudomonas* to attract or evade various environmental stimuli [83–85]. 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 [86]. Interestingly, we also identified the pathogenicity LPQ (lipopeptide/quorum-sensing) island that is also present in the closely related *Pseudomonas sp.* [45, 87–90]. This genetic island is characterized by the presence of luxR genes and acyl-homoserine lactone (AHL) efflux protein [45]. Both these features were identified in our MAG-*Pseudomonas* (Table 2, Supplementary Table S2)*. The existence of such quorum-sensing machinery could be used by these specific groups of bacteria to respond to quorum sensing signals and demonstrate as markers of pathogenic behavior to plants or antifungal activity [87].

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* possessed putative genes that were involved in the function enhancing the resilience during drought-induced conditions, and might performed essential microbial functions for

generating products related to the nitrogen cycle [91], which could be exploited by plant host and other host-associated microbes [92]. A SynCom consisting of six *Pseudomonas* strains isolated from the garlic rhizosphere has been reported to promote plant growth [93]. Thus, 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 [94], 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* [95]. 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 [40].

Conclusion
In this study, we used cultivation 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
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 [43]. In this comparative study, we selected rhizosphere samples from native dry (Hays, Kansas) and wet (Carbondale, Illinois) ecotypes growing in Colby for microbial cultivation. We separated

bulk soil from the soil attached to the rhizosphere by handshaking the roots gently. We resuspended 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 100 µl solution onto the Petri plates. We designed two culture conditions—R2A media (Teknova, USA) [96] and R2A media amended with a 36% Polyethylene Glycol 8000 (PEG) ($\Psi = $ -1.54 MPa) to alter the media osmotic potential and to mimic absence and presence of water limitation, respectively [97, 98]. A similar range of PEG concentrations has been used to simulate dry environments in other studies [99, 100]. To prepare the R2A-PEG media, we dissolved 36% (w:v) PEG powder in autoclaved MilliQ water, allowing the mixed solution (20 mL) to sit on top of a pre-made R2A plate for 24 h to diffuse throughout the agar. After 24 h, we removed excess solution and spread 100 µl of the diluted soil culture on the surface of the agar. The prepared plates were incubated at 37℃ for 24–48 h 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℃ until genomic DNA extraction. We were interested in capturing the bacterial communities 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) [44, 101]. Rhizosphere bacterial 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' [102] to process the short metagenomic reads and removed low-quality reads following criteria outlined in Minoche et al. ([103). We organized the samples into 4 metagenomic groups (R2A + Wet ecotype; R2A + PEG + Wet ecotype; R2A + Dry ecotype; R2A + PEG + Dry ecotype) based on the cultivation conditions and ecotypes for co-assembling strategy. The quality-filtered short reads were co-assembled into longer contiguous sequences (contigs) using MetaHit v1.2.9 [104] with a minimum contig length of 1000 bp. We then used 'anvi-gen-contigs-database' in anvio ver 7.0 [105] to compute k-mer frequencies and identify

open reading frames (ORFs) in the contigs using Prodigal v 2.6.3 [106], and recruited metagenomic short reads to the contigs. We then annotate the bacterial and archaeal single-copy genes using HMMER v3.2.1 [107]. NCBI's Cluster of Orthologous Groups (COGs) [108] was used to assign functions to the ORFs. We mapped the metagenomic short reads to the contigs with Bowtie2 v2.3.5 [109], and converted mappings to BAM tiles with samtools v. 1.9 [110]. The converted BAM files were then profiled using 'anvi-profile' with a minimum contig length of 1,000 bp. We used CONCOCT v 1.1.0 [111] to bin the metagenomes, and used anvi'o ver 7.0 [105] 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. The MAGs were assigned to taxa using the single-copy core genes of bacteria and archaea. We further used 'anvi-compute-genome-similarity' to calculate average nucleotide identity (ANI) [112], using PyANI v0.2.9 [113] to compare the resolved MAGs and identify non-redundant MAGs based on 95% ANI [114]. We analyzed the resolved MAG occurence in a sample with the "detection" metric, detailing how much of the contig recruited reads to it. We considered a MAG as detected in a metagenome if the detection was > 0.25, which is an appropriate cutoff to eliminate false-positive signals in read recruitment results.

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 the Similar Genome Finder service that uses the MinHash on the Pathosystems Resource Integration Center (PATRIC) web portal [115, 116]. 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 genome status of the 40 genomes was either complete or at the levels of whole genome sequencing. The workflow used the PATRIC Codon Tree Service which used the amino acid sequences from a well-defined database of global protein families [117]. In our workflow, we used amino acid sequence files for each genome. For tree construction, genomes were used with small set of potential outgroup genomes. It was a two-step process that we followed to identify the sets of homologous proteins. For the first step, a single genome from each distinct species was selected, and then aligned against each other using the BLAT alignment tool [118]. We clustered the top-scoring hits with Markov Cluster (MCL) Algorithm which defined the initial seed

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sets for determining the homologous groups [119]. In the second step, Multiple Sequence Comparison by Log-Expectation (MUSCLE) was used to align the seed sets [120]. Hidden Markov Model (HMMs) were built using the hmmbuild. All genomes including the outgroup pool were searched with hmmsearch. Homologous groups were then defined by hmmsearch top hits. Outgroup genomes were selected from the pool of outgroup candidates that were based on the total hmmsearch score. In the next stage, homologous groups were filtered for the taxon coverage, and MUSCLE was used to align the groups. Gblocks eliminated the poorly aligned regions, and the remaining well-aligned regions were concatenated into a long single alignment. The main phylogenetic tree was then estimated from the long single alignment using FastTree [121]. Bootstrap values can often be overly optimistic for long fragments. In our case, we used random samples of 50% of the homologous groups that were used for the main tree. This workflow was termed gene-wise jackknifing. In the final step, hundreds of the 50% gene-wise jackknife trees with the support values indicated the times a particular branch was observed in the support trees. We used the RAxML program v 8 [122] to construct a tree based on the pairwise differences between the aligned protein families of the selected sequences. For inferring the trees, the maximum likelihood method was used. We used the comparative pathway tool of PATRIC to predict the metabolic pathways in our selected MAG. The comparative pathway service of the PATRIC portal allowed us to identify the set of pathways that was based on EC number, taxonomy, pathway name, pathway ID and/or the annotation type. In the comparative analysis tool, we compared the MAG-*Pseudomanas* genome with the genome group that contained the *Pseudomonas* genomes isolated from rhizospheres of cotton and soybean using the select genome and select genome group options available under the comparative pathway service. The rationale behind using these *Pseudomonas* genomes was because these genomes were also isolated from the rhizosphere, giving us the opportunity to compare different *Pseudomonas* strains that were isolated from rhizospheres from different plant hosts. The criteria that we used for the genome selections were (1) genomes must be complete (2) genomes were of high quality (3) genomes isolated from the rhizosphere. That would allow us to have a comparative study between the *Pseudomonas* isolated from the rhizosphere of *A. gerardii* with *Pseudomonas chlororaphis subsp. aurantiaca* strain ARS 38 isolated from cotton rhizosphere and *Pseudomonas sp.* DR208 and *Pseudomonas sp.* DR48 from the soybean rhizosphere. We focused on the nitrogen metabolism pathway that we selected from the list of pathways available for MAG-*Pseudomonas* and the other *Pseudomonas* genomes. KEGG maps and heat maps of the nitrogen metabolism pathway were generated in the PATRIC portal.

We downloaded 6 related *Pseudomonas* genomes from NCBI RefSeq [74] and performed pangenomic analyses using anvi'o workflow [105, 123]. The criteria that we set up for efficient pangenomic analyses is to download only the genomes of *Pseudomonas* that were complete. The workflow uses BLASTP [124] to compute amino acid level similarities between all possible ORF pairs. We then used Markov Cluster Algorithm (MCL) [119] to group ORFs into homologous gene clusters and aligned amino acid sequences in each gene cluster using MUSCLE for visualization [120]. 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.*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-09019-0.

> **Additional file 1: Supplementary Table S1.** Non-redundant MAGs and taxonomic identity.
>
> **Additional file 2: Supplementary Table S2.** Gene functions of MAG-Pseudomonas.
>
> **Additional file 3: Supplementary Table S3.** Pathways identified in MAG-Pseudomonas.
>
> **Additional file 4: Supplementary Table S4.** Shared and unique gene clusters identified in pangenomic analysis of MAG-Pseudomonas with 6 related Pseudomonas genomes.

Authors' 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. analyzed the data and drafted the work. A.J., and L.J. drafted the work and substantively revised 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.

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Availability of data and materials
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.

Declarations

Ethics approval and consent to participate
Samples were collected on experimental grounds belonging to Kansas State University. No permission was necessary to collect the samples used in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Borrelli P, Robinson DA, Panagos P, Lugato E, Yang JE, Alewell C, et al. Land use and climate change impacts on global soil erosion by water (2015–2070). Proc Natl Acad Sci U S A. 2020;117:21994–2001.
2. Epa US, OAR. Climate change indicators: drought. 2016.
3. Sintayehu DW. Impact of climate change on biodiversity and associated key ecosystem services in Africa: a systematic review. Ecosystem Health Sustainability. 2018;4:225–39.
4. Turner TR, James EK, Poole PS. The plant microbiome. Genome Biol. 2013;14:209. https://doi.org/10.1186/gb-2013-14-6-209.
5. Vukanti RVNR. Structure and Function of Rhizobiome. In: Varma A, Tripathi S, Prasad R, editors. Plant Microbe Symbiosis. Cham: Springer; 2020. https://doi.org/10.1007/978-3-030-36248-5_13.
6. Bhaduri D, Pal S, Purakayastha TJ, Chakraborty K, Yadav RS, Akhtar MS. Soil Quality and Plant-Microbe Interactions in the Rhizosphere. In: Lichtfouse E, editor. Sustainable Agriculture Reviews, vol. 17. Cham: Springer International Publishing; 2015. p. 307–35.
7. Olanrewaju OS, Ayangbenro AS, Glick BR, Babalola OO. Plant health: feedback effect of root exudates-rhizobiome interactions. Appl Microbiol Biotechnol. 2019;103:1155–66.
8. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol. 2006;57:233–66.
9. Barea J-M, Pozo MJ, Azcón R, Azcón-Aguilar C. Microbial co-operation in the rhizosphere. J Exp Bot. 2005;56:1761–78.
10. Adl S. Rhizosphere, food security, and climate change: a critical role for plant-soil research. Rhizosphere. 2016;1:1–3.
11. Ahkami AH, Allen White R, Handakumbura PP, Jansson C. Rhizosphere engineering: enhancing sustainable plant ecosystem productivity. Rhizosphere. 2017;3:233–43.
12. Shahzad R, Khan AL, Bilal S, Waqas M, Kang S-M, Lee I-J. Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa. Environ Exp Bot. 2017;136:68–77.
13. Compant S, Van Der Heijden MGA, Sessitsch A. Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiol Ecol. 2010;73:197–214.
14. Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JA, Cregger MA, Patterson CM. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead. 2015.
15. Ravanbakhsh M, Sasidharan R, Voesenek LACJ, Kowalchuk GA, Jousset A. Microbial modulation of plant ethylene signaling: ecological and evolutionary consequences. Microbiome. 2018;6:52.
16. Khan NA, Khan MIR, Ferrante A, Poor P. Editorial: ethylene: a key regulatory molecule in plants. Front Plant Sci. 2017;8:1782.
17. Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. Ethylene role in plant growth, development and senescence: interaction with other phytohormones. Front Plant Sci. 2017;8:475.
18. Glick BR. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett. 2005;251:1–7.
19. Arshad M, Shaharoona B, Mahmood T. Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (Pisum sativum L.). Pedosphere. 2008;18(5):611–20.
20. Saleem M, Arshad M, Hussain S, Bhatti AS. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol. 2007;34:635–48.
21. Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res. 2016;184:13–24.
22. Liu, Duan, Li, Chen, Su-Zhou, Li, et al. ACC Deaminase-producing PGPRs Improve Drought Stress Tolerance in Grapevine (Vitis vinifera L.). Front Plant Sci. https://doi.org/10.3389/fpls.2021.706990.
23. Orozco-Mosqueda M del C, Kumar A, Glick BR, Santoyo G. Chapter 3 - The role of bacterial ACC deaminase and trehalose in increasing salt and drought tolerance in plants. In: Santoyo G, Kumar A, Aamir M, Uthandi S, editors. Mitigation of Plant Abiotic Stress by Microorganisms: Academic Press; 2022. p. 41–52. https://doi.org/10.1016/B978-0-323-90568-8.00003-1.
24. Ali SZ, Sandhya V, Venkateswar RL. Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent Pseudomonas sp. Ann Microbiol. 2014;64:493–502.
25. Honma M, Shimomura T. Metabolism of 1-Aminocyclopropane-1-carboxylic Acid. Agric Biol Chem. 1978;42:1825–31.
26. Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res. 2014;169:30–9.
27. Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA, et al. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere. 2015;6:art130.
28. Alshaal T, El-Ramady H, Al-Saeedi AH, Shalaby T, Elsakhawy T, Omara AED, et al. The rhizosphere and plant nutrition under climate change. In: Naeem M, Ansari AA, Gill SS, editors., et al., Essential plant nutrients: uptake, use efficiency, and management. Cham: Springer International Publishing; 2017. p. 275–308.
29. Pugnaire FI, Morillo JA, Peñuelas J, Reich PB, Bardgett RD, Gaxiola A, et al. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. Sci Adv. 2019;5:eaaz1834.
30. Naylor D, Coleman-Derr D. Drought stress and root-associated bacterial communities. Front Plant Sci. 2017;8:2223.
31. Monohon SJ, Manter DK, Vivanco JM. Conditioned soils reveal plant-selected microbial communities that impact plant drought response. Sci Rep. 2021;11:21153.
32. Poudel M, Mendes R, Costa LAS, Bueno CG, Meng Y, Folimonova SY, et al. The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. Front Microbiol. 2021;12: 743512.

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33. Wang S, Ouyang L, Ju X, Zhang L, Zhang Q, Li Y. Survey of plant drought-resistance promoting bacteria from Populus Euphratica tree living in arid area. Indian J Microbiol. 2014;54:419–26.

34. Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, et al. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environ Microbiol. 2015;17:316–31.

35. Lu X, Taylor AE, Myrold DD, Neufeld JD. Expanding perspectives of soil nitrification to include ammonia-oxidizing archaea and comammox bacteria. Soil Sci Soc Am J. 2020;84:287–302.

36. Zhang J, Liu YX, Zhang N, Hu B, Jin T, Xu H, et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol. 2019;37:676–84.

37. Marín O, González B, Poupin MJ. From microbial dynamics to functionality in the rhizosphere: a systematic review of the opportunities with synthetic microbial communities. Front Plant Sci. 2021;12: 650609.

38. Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, et al. Functional overlap of the Arabidopsis leaf and root microbiota. Nature. 2015;528:364–9.

39. Castrillo G, Teixeira PJP, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, et al. Root microbiota drive direct integration of phosphate stress and immunity. Nature. 2017;543:513–8.

40. Jansson JK, Hofmockel KS. The soil microbiome-from metagenomics to metaphenomics. Curr Opin Microbiol. 2018;43:162–8.

41. Gray MM, St. Amand P, Bello NM, Galliart MB, Knapp M, Garrett KA, et al. Ecotypes of an ecologically dominant prairie grass (Andropogon gerardii) exhibit genetic divergence across the U.S. Midwest grasslands' environmental gradient. Molecular Ecology. 2014;23:6011–28.

42. Galliart M, Bello N, Knapp M, Poland J, St Amand P, Baer S, et al. Local adaptation, genetic divergence, and experimental selection in a foundation grass across the US great plains' climate gradient. Glob Change Biol. 2019;25:850–68.

43. Sarkar S, Kamke A, Ward K, Rudick AK, Baer SG, Ran Q, et al. Bacterial but not fungal rhizosphere community composition differ among perennial grass ecotypes under abiotic environmental stress. Microbiol Spectr. 2022;10(3):e0239121. https://doi.org/10.1128/spectrum.02391-21. Epub 2022 Apr 20.

44. Sarkar S, Ward K, Kamke A, Ran Q, Feehan B, Richie T, et al. Perspective: simple state communities to study microbial interactions: examples and future directions. Front Microbiol. 2022;13: 801864.

45. Garrido-Sanz D, Redondo-Nieto M, Martin M, Rivilla R. Comparative genomics of the Pseudomonas corrugata subgroup reveals high species diversity and allows the description of Pseudomonas ogarae sp. nov. Microb Genom. 2021;7(6):000593. https://doi.org/10.1099/mgen.0.000593.

46. Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. Genomic and genetic diversity within the pseudomonas fluorescens complex. PLoS ONE. 2016;11: e0150183.

47. Rosenzweig N, Bradeen JM, Tu ZJ, McKay SJ, Kinkel LL. Rhizosphere bacterial communities associated with long-lived perennial prairie plants vary in diversity, composition, and structure. Can J Microbiol. 2013;59:494–502.

48. Niu X, Song L, Xiao Y, Ge W. Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol. 2017;8:2580.

49. Sah S, Singh R. Phylogenetical coherence of Pseudomonas in unexplored soils of Himalayan region. 3 Biotech. 2016;6:170.

50. Chandra D, Srivastava R, Glick BR, Sharma AK. Drought-tolerant pseudomonas spp. improve the growth performance of finger millet (Eleusine coracana (L.) Gaertn.) under non-stressed and drought-stressed conditions. Pedosphere. 2018;28:227–40.

51. Achouak W, Sutra L, Heulin T, Meyer JM, Fromin N, Degraeve S, et al. Pseudomonas brassicacearum sp. nov. and Pseudomonas thivervalensis sp. nov., two root-associated bacteria isolated from brassica napus and Arabidopsis thaliana. Int J Syst Evol Microbiol. 2000;50(pt1):9–18.

52. Siegele DA. Universal stress proteins in Escherichia coli. J Bacteriol. 2005;187:6253–4.

53. Nachin L, Nannmark U, Nyström T. Differential roles of the universal stress proteins of Escherichia coli in oxidative stress resistance, adhesion, and motility. J Bacteriol. 2005;187:6265–72.

54. Malik AA, Bouskill NJ. Drought impacts on microbial trait distribution and feedback to soil carbon cycling. Funct Ecol. 2022;36:1442–56.

55. Kvint K, Nachin L, Diez A, Nyström T. The bacterial universal stress protein: function and regulation. Curr Opin Microbiol. 2003;6:140–5.

56. Arshad M, Shaharoona B, Mahmood T. Inoculation with Pseudomonas spp. Containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (Pisum sativum L.)*1 *1project supported by the higher education commission, Islamabad, Pakistan (No. PIN 041 211534 A-031). Pedosphere. 2008;18:611–20.

57. Yan Y, Yang J, Dou Y, Chen M, Ping S, Peng J, et al. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated Pseudomonas stutzeri A1501. Proc Natl Acad Sci U S A. 2008;105:7564–9.

58. Hervás AB, Canosa I, Little R, Dixon R, Santero E. NtrC-dependent regulatory network for nitrogen assimilation in Pseudomonas putida. J Bacteriol. 2009;191:6123–35.

59. García-González V, Jiménez-Fernández A, Hervás AB, Canosa I, Santero E, Govantes F. Distinct roles for NtrC and GlnK in nitrogen regulation of the Pseudomonas sp. strain ADP cyanuric acid utilization operon. FEMS Microbiol Lett. 2009;300:222–9.

60. Setten L, Soto G, Mozzicafreddo M, Fox AR, Lisi C, et al. Correction: Engineering Pseudomonas protegens Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. PLOS ONE. 2013;8(10). https://doi.org/10.1371/annotation/279fe0d7-d9b1-4d05-a45a-5ff00b4606b7.

61. Fox AR, Soto G, Valverde C, Russo D, Lagares A Jr, Zorreguieta Á, et al. Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium Pseudomonas protegens Pf-5 X940. Environ Microbiol. 2016;18:3522–34.

62. Pankievicz VCS, Irving TB, Maia LGS, Ané J-M. Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops. BMC Biol. 2019;17:99.

63. Mahmud K, Makaju S, Ibrahim R, Missaoui A. Current progress in nitrogen fixing plants and microbiome research. Plants (Basel). 2020;9(1):97. https://doi.org/10.3390/plants9010097.

64. Kramer G, Weiss V. Functional dissection of the transmitter module of the histidine kinase NtrB in Escherichia coli. Proc Natl Acad Sci U S A. 1999;96:604–9.

65. Huergo LF, Chandra G, Merrick M. P(II) signal transduction proteins: nitrogen regulation and beyond. FEMS Microbiol Rev. 2013;37:251–83.

66. Cabello P, Roldán, Castillo F, Moreno-Vivián C. Nitrogen Cycle. In: Schaechter M, editor. Encyclopedia of Microbiology (3rd Edition). Oxford: Academic Press; 2009. p. 299–321. https://doi.org/10.1016/B978-012373944-5.00055-9.

67. Robertson GP, Groffman PM. Nitrogen transformations. In: Soil microbiology, ecology and biochemistry. 4th ed. Burlington: Academic Press; 2015. p. 421–46.

68. Temple SJ, Vance CP, Stephen GJ. Glutamate synthase and nitrogen assimilation. Trends Plant Sci. 1998;3:51–6.

69. Miflin BJ, Habash DZ. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot. 2002;53:979–87.

70. Di Benedetto NA, Corbo MR, Campaniello D, Cataldi MP, Bevilacqua A, Sinigaglia M, et al. The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. AIMS Microbiol. 2017;3:413–34.

71. Dellagi A, Quillere I, Hirel B. Beneficial soil-borne bacteria and fungi: a promising way to improve plant nitrogen acquisition. J Exp Bot. 2020;71:4469–79.

72. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28:27–30.

73. Sutton G, Fogel GB, Abramson B, Brinkac L, Michael T, Liu ES, et al. A pan-genome method to determine core regions of the Bacillus subtilis and Escherichia coli genomes. F1000Res. 2021;10:286. https://doi.org/10.12688/f1000research.51873.2.

74. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44:D733–45.

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75. Mertens J, Aliyu H, Cowan DA. LEA Proteins and the Evolution of the WHy Domain. Appl Environ Microbiol. 2018;84(15):e00539–18. https://doi.org/10.1128/AEM.00539-18.

76. Ciccarelli FD, Bork P. The WHy domain mediates the response to desiccation in plants and bacteria. Bioinformatics. 2005;21:1304–7.

77. Anderson D, Ferreras E, Trindade M, Cowan D. A novel bacterial Water Hypersensitivity-like protein shows in vivo protection against cold and freeze damage. FEMS Microbiol Lett. 2015;362:fnv110.

78. Liu P, Myo T, Ma W, Lan D, Qi T, Guo J, et al. TaTypA, a ribosome-binding GTPase protein, positively regulates wheat resistance to the stripe rust fungus. Front Plant Sci. 2016;7:873.

79. Weber H, Polen T, Heuveling J, Wendisch VF, Hengge R. Genome-wide analysis of the general stress response network in Escherichia coli: sigmaS-dependent genes, promoters, and sigma factor selectivity. J Bacteriol. 2005;187:1591–603.

80. Schmalisch M, Langbein I, Stülke J. The general stress protein Ctc of Bacillus subtilis is a ribosomal protein. J Mol Microbiol Biotechnol. 2002;4:495–501.

81. Hida A, Oku S, Miura M, Matsuda H, Tajima T, Kato J. Characterization of methyl-accepting chemotaxis proteins (MCPs) for amino acids in plant-growth-promoting rhizobacterium Pseudomonas protegens CHA0 and enhancement of amino acid chemotaxis by MCP genes overexpression. Biosci Biotechnol Biochem. 2020;84:1948–57.

82. Kehry MR, Dahlquist FW. The methyl-accepting chemotaxis proteins of Escherichia coli. Identification of the multiple methylation sites on methyl-accepting chemotaxis protein I. J Biol Chem. 1982;257:10378–86.

83. Muff TJ, Ordal GW. The CheC phosphatase regulates chemotactic adaptation through CheD. J Biol Chem. 2007;282:34120–8.

84. Glekas GD, Plutz MJ, Walukiewicz HE, Allen GM, Rao CV, Ordal GW. Elucidation of the multiple roles of CheD in Bacillus subtilis chemotaxis. Mol Microbiol. 2012;86:743–56.

85. Yuan W, Glekas GD, Allen GM, Walukiewicz HE, Rao CV, Ordal GW. The importance of the interaction of CheD with CheC and the chemoreceptors compared to its enzymatic activity during chemotaxis in Bacillus subtilis. PLoS ONE. 2012;7: e50689.

86. Yang G, Waterfield NR. The role of TcdB and TccC subunits in secretion of the Photorhabdus Tcd toxin complex. PLoS Pathog. 2013;9: e1003644.

87. Melnyk RA, Hossain SS, Haney CH. Convergent gain and loss of genomic islands drive lifestyle changes in plant-associated Pseudomonas. ISME J. 2019;13:1575–88.

88. Licciardello G, Strano CP, Bertani I, Bella P, Fiore A, Fogliano V, et al. N-acyl-homoserine-lactone quorum sensing in tomato phytopathogenic Pseudomonas spp. is involved in the regulation of lipodepsipeptide production. J Biotechnol. 2012;159:274–82.

89. Licciardello G, Bertani I, Steindler L, Bella P, Venturi V, Catara V. Pseudomonas corrugata contains a conserved N-acyl homoserine lactone quorum sensing system; its role in tomato pathogenicity and tobacco hypersensitivity response. FEMS Microbiol Ecol. 2007;61:222–34.

90. Berry CL, Nandi M, Manuel J, Brassinga AKC, Fernando WGD, Loewen PC, et al. Characterization of the Pseudomonas sp. DF41 quorum sensing locus and its role in fungal antagonism. Biol Control. 2014;69:82–9.

91. Rascio N, La Rocca N. Biological Nitrogen Fixation. In: Jørgensen SE, Fath BD, editors. Encyclopedia of Ecology. Oxford: Academic Press; 2008. p. 412–9.

92. Takai K. The nitrogen cycle: a large, fast, and mystifying cycle. Microbes Environ. 2019;34:223–5.

93. Zhuang L, Li Y, Wang Z, Yu Y, Zhang N, Yang C, et al. Synthetic community with six Pseudomonas strains screened from garlic rhizosphere microbiome promotes plant growth. Microb Biotechnol. 2021;14:488–502.

94. Sitaraman R. Pseudomonas spp. as models for plant-microbe interactions. Front Plant Sci. 2015;6:787.

95. Castellano-Hinojosa A, Strauss SL. Insights into the taxonomic and functional characterization of agricultural crop core rhizobiomes and their potential microbial drivers. Sci Rep. 2021;11:10068.

96. de Raad M, Li Y, Andeer P, Kosina SM, Saichek NR, Golini A, et al. A defined medium based on R2A for cultivation and exometabolite profiling of soil bacteria. bioRxiv. 2021. https://doi.org/10.1101/2021.05.23.445362.

97. Michel BE. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiol. 1983;72:66–70.

98. Meher, Shivakrishna P, Ashok Reddy K, Manohar Rao D. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi J Biol Sci. 2018;25:285–9.

99. Marulanda A, Barea JM, Azcón R. Stimulation of plant growth and drought tolerance by native microorganisms (AM Fungi and Bacteria) from dry environments: mechanisms related to bacterial effectiveness. J Plant Growth Regul. 2009;28:115–24.

100. Nelson SK, Oliver MJ. A soil-plate based pipeline for assessing cereal root growth in response to polyethylene glycol (PEG)-induced water deficit stress. Front Plant Sci. 2017;8:1272.

101. Maritz JM, Sullivan SA, Prill RJ, Aksoy E, Scheid P, Carlton JM. Filthy lucre: a metagenomic pilot study of microbes found on circulating currency in New York City. PLoS ONE. 2017;12: e0175527.

102. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using illumina paired-end technology. PLoS ONE. 2013;8: e66643.

103. Minoche AE, Dohm JC, Himmelbauer H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and genome analyzer systems. Genome Biol. 2011;12:R112.

104. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, et al. MEGAHIT v1. 0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods. 2016;102:3–11.

105. Eren AM, Murat Eren A, Esen ÖC, Quince C, Vineis JH, Morrison HG, et al. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ. 2015;3: e1319.

106. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.

107. HMMER. http://hmmer.org/. Accessed 7 Sept 2022.

108. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, et al. CDD: NCBI's conserved domain database. Nucleic Acids Res. 2015;43(Database issue):222–6.

109. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.

110. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V. Twelve years of SAMtools and BCFtools. Gigascience. 2021;10(2):008 PubMed Abstract| Publisher Full.

111. Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, et al. Binning metagenomic contigs by coverage and composition. Nat Methods. 2014;11:1144–6.

112. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek. 2017;110:1281–6.

113. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal Methods. 2016;8:12–24.

114. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A. 2009;106:19126–31.

115. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, et al. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol. 2016;17(1):132. https://doi.org/10.1186/s13059-016-0997-x.

116. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, et al. The PATRIC bioinformatics resource center: expanding data and analysis capabilities. Nucleic Acids Res. 2020;48:D606–12.

117. Davis JJ, Gerdes S, Olsen GJ, Olson R, Pusch GD, Shukla M, et al. PATtyFams: protein families for the microbial genomes in the PATRIC Database. Front Microbiol. 2016;7:118.

118. Kent WJ. BLAT–the BLAST-like alignment tool. Genome Res. 2002;12:656–64.

119. Enright AJ, Van Dongen S, Ouzounis CA. An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res. 2002;30(7):1575–84.

120. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.

Sarkar *et al. BMC Genomics*   (2022) 23:784

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121. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009;26:1641–50.
122. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3.
123. Eren AM, Murat Eren A, Kiefl E, Shaiber A, Veseli I, Miller SE, et al. Community-led, integrated, reproducible multi-omics with anvi'o. Nat Microbiol. 2021;6:3–6.
124. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.

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