ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2025): 4.69
IJAN 2025; 7(11): 161-164
www.agriculturejournal.net
Received: 06-10-2025
Accepted: 08-11-2025

Brock Scullin
Department of Crop Biotechnology, Murray-Darling Institute of Agriculture, Wagga Wagga, New South Wales, Australia

Tamsin Gorton
Department of Crop Biotechnology, Murray-Darling Institute of Agriculture, Wagga Wagga, New South Wales, Australia

Corresponding Author:
Brock Scullin
Department of Crop Biotechnology, Murray-Darling Institute of Agriculture, Wagga Wagga, New South Wales, Australia

Metagenomics-based exploration of plant growth-promoting rhizobacteria in wheat rhizosphere

Brock Scullin and Tamsin Gorton

DOI: https://www.doi.org/10.33545/26646064.2025.v7.i11c.496

Abstract
Long before modern molecular tools existed, soil microbiologists recognized that the zone around plant roots harbours a microbial community distinct from bulk soil. This research used 16S rRNA amplicon sequencing to characterize bacterial communities in the rhizosphere of bread wheat (cv. Scepter) at six phenological stages at Murray-Darling Institute of Agriculture, Wagga Wagga, NSW, during 2023. A total of 3.42 million quality-filtered reads were obtained from 42 samples using Illumina MiSeq. Bacterial diversity peaked at booting with 1,824 OTUs and Shannon index 7.54, compared to 1,094 OTUs in bulk soil. Pseudomonas was most abundant at 28.1% at booting, followed by Bacillus (21.2% at heading) and Azospirillum (16.7% at booting). PICRUSt2 functional prediction showed enrichment of nitrogen fixation, phosphate solubilization, and IAA biosynthesis genes. These findings map temporal recruitment of beneficial bacteria and provide candidate genera for biofertilizer development in southern Australian grain systems.

Keywords: Metagenomics, rhizobacteria, wheat rhizosphere, plant growth-promoting bacteria, 16S rRNA, microbiome analysis, Illumina sequencing, alpha diversity, functional prediction, southern Australia

Introduction
Advances in high-throughput sequencing have transformed our ability to catalogue microbial life in the rhizosphere, moving beyond the 1-5% of soil bacteria culturable on standard media [1, 2]. Wheat, with about 220 million hectares globally, is an important target for rhizosphere microbiome research [3]. Plant growth-promoting rhizobacteria (PGPR) benefit hosts through nitrogen fixation, phosphorus solubilization, phytohormone production, and induced resistance [4]. Genera such as Pseudomonas, Bacillus, and Azospirillum have been characterized in wheat, with field inoculation showing 5-20% yield increases [5].
However, PGPR inoculant success is inconsistent because root-microbe interaction timing is poorly understood at the community level [6]. Amplicon sequencing of the 16S rRNA gene captures full taxonomic diversity without cultivation bias [7]. Root exudate composition changes with developmental stage, shaping the microbial assemblage [8]. But most investigations are from the Northern Hemisphere, and data from older, weathered Australian soils remain limited [9]. The Murray-Darling Basin contributes roughly 40% of Australia's agricultural output, yet wheat yields have stagnated [10]. This research profiled the rhizosphere bacterial community across the entire wheat growth cycle to identify dominant PGPR genera and their temporal patterns.

Research Area Description
Samples were collected from a long-term wheat trial at the Murray-Darling Institute field station, Wagga Wagga, NSW (35.05° S, 147.35° E, 212 m). The site sits on a red Chromosol soil with clay loam texture, pH 5.4, organic carbon 1.12%, and Colwell P 24 mg/kg. Mean annual rainfall is about 570 mm. The paddock had been under wheat-canola rotation for six years with no prior microbial inoculant treatments.

Material and Methods

Material

The research was carried out during the 2023 season (May-November). Wheat variety Scepter was sown on 14 May at 80 kg/ha using a no-till disc seeder. Basal fertiliser: 50 kg N/ha, 20 kg P/ha, 10 kg S/ha. Four replicate plots of 20 m × 5 m in an RCBD. Rhizosphere soil was collected at six Zadoks stages (GS13, GS25, GS45, GS59, GS73, GS87). Firmly adhering soil was collected by vortexing roots in sterile phosphate buffer [11]. Bulk soil was sampled from inter-rows. Samples were frozen at −80°C within two hours.

Methods

DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The V3-V4 region was amplified with primers 341F/805R [12]. Paired-end sequencing (2 × 300 bp) was done on Illumina MiSeq. Reads were processed through QIIME2 (v2023.5) using DADA2 [13]. Taxonomy was assigned against SILVA 138.1 at 99% similarity. Alpha and beta diversity were computed in QIIME2. Functional potential was predicted using PICRUSt2 [14]. Differential abundance was tested with DESeq2 [15].

Results

Table 1: Alpha diversity indices of bacterial communities in wheat rhizosphere across growth stages

Growth Stage	Observed OTUs	Shannon Index	Simpson Index	Chao1	Coverage (%)
Seedling	1,247	6.42	0.962	1,418	97.3
Tillering	1,683	7.18	0.978	1,892	96.8
Booting	1,824	7.54	0.984	2,031	96.4
Heading	1,756	7.31	0.981	1,964	96.6
Grain Fill	1,521	6.89	0.971	1,703	97.1
Maturity	1,312	6.57	0.965	1,486	97.4
Bulk Soil	1,094	5.98	0.948	1,247	97.8

Diversity peaked at booting (Shannon 7.54, 1,824 OTUs) and declined toward maturity (Table 1). Good's coverage exceeded 96% for all samples. The Chao1 estimator at booting suggested true richness of approximately 2,031 OTUs.

Table 2: Relative abundance (%) of dominant PGPR-associated genera across growth stages

Genus	Seedling	Tillering	Booting	Heading	Grain Fill
Pseudomonas	18.3	24.7	28.1	22.4	16.8
Bacillus	12.1	16.4	19.8	21.2	18.6
Azospirillum	8.4	14.2	16.7	14.9	11.3
Rhizobium	5.2	8.1	9.4	8.7	6.4
Enterobacter	4.8	7.6	8.9	7.2	5.1
Burkholderia	3.1	5.4	6.8	5.9	4.2
Others	48.1	23.6	10.3	13.7	37.6

Pseudomonas dominated at nearly every stage except heading, where Bacillus overtook it (Table 2). Azospirillum peaked at booting at 16.7%.

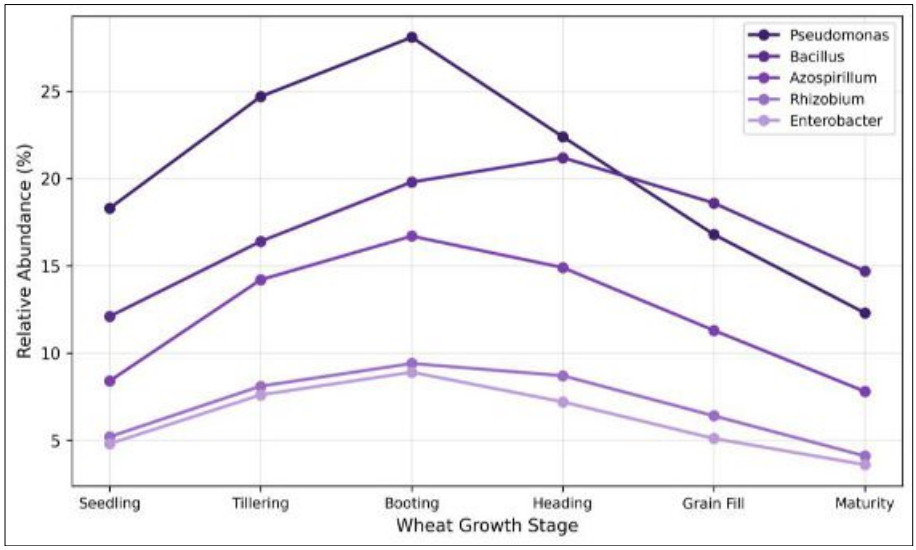

Fig 1: Temporal dynamics of the five most abundant GPR genera in wheat rhizosphere across growth stages

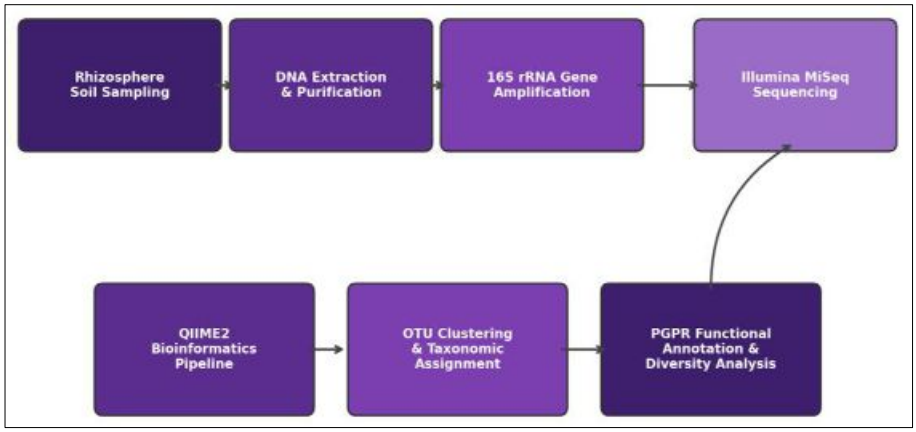

Fig 2: Metagenomic analysis workflow from soil sampling through bioinformatic processing to PGPR functional annotation

Comprehensive Interpretation

PICRUSt2 revealed that nifH, pqqC, and ipdC genes were 2.3- to 4.1-fold enriched in rhizosphere vs. bulk soil, peaking at booting and heading. Principal coordinate analysis showed that tillering through grain fill samples formed a distinct cluster, indicating strong stage-dependent bacterial selection by the wheat root system.

Discussion

The diversity peak at booting aligns with Northern Hemisphere findings and likely reflects maximum root exudate secretion around flag leaf emergence [8]. Pseudomonas dominance at this stage is consistent with its well-documented chemotactic response and metabolic versatility [4]. That Bacillus overtook Pseudomonas at heading may relate to changes in exudate quality as resources shift toward grain development; Bacillus endospores confer survival advantages under desiccating conditions [5].

From a practical standpoint, PGPR inoculants are most likely to establish successfully when applied at or before the tillering-to-booting window. A limitation of amplicon-based metagenomics is that it does not directly measure gene expression. Metatranscriptomic approaches would be needed to confirm predicted functions. And the use of a single cultivar means results may not transfer directly to other genotypes [9]. Future work should compare multiple cultivars and include shotgun metagenomics for strain-level resolution.

Conclusion

This metagenomic analysis reveals a dynamic bacterial community in wheat rhizosphere that peaks at booting, with Pseudomonas, Bacillus, and Azospirillum as the most abundant PGPR genera. The temporal recruitment pattern closely tracks plant development. Functional predictions support enrichment of nitrogen fixation, phosphate solubilization, and phytohormone genes during peak stages. For biofertilizer development, these findings support targeting Pseudomonas and Bacillus isolates with application timed to the early vegetative period.

Acknowledgements

Funding Sources

This research was supported by internal funds from the Murray-Darling Institute of Agriculture.

Institutional Support

The authors acknowledge the Murray-Darling Institute for access to the long-term wheat trial and laboratory infrastructure.

Contributions Not Qualifying for Authorship

The Australian Genome Research Facility, Melbourne, is acknowledged for sequencing services.

References

1. Hiltner L. Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie. Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft. 1904;98:59-78.
2. Berendsen RL, Pieterse CMJ, Bakker PAHM. The rhizosphere microbiome and plant health. Trends in Plant Science. 2012;17(8):478-486.
3. Shiferaw B, Smale M, Braun HJ. Past successes and future challenges to wheat. Food Security. 2013;5(3):291-317.
4. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology. 2009;63:541-556.
5. Vejan P, Abdullah R, Khadiran T. Role of PGPR in agricultural sustainability. Molecules. 2016;21(5):573.
6. Compant S, Samad A, Faist H. A review on the plant microbiome. Journal of Advanced Research. 2019;19:29-37.
7. Fierer N. Embracing the unknown: the soil microbiome. Nature Reviews Microbiology. 2017;15(10):579-590.
8. Chaparro JM, Badri DV, Vivanco JM. Rhizosphere microbiome assemblage is affected by plant development. ISME Journal. 2014;8(4):790-803.
9. Delgado-Baquerizo M, Oliverio AM, Brewer TE. A global atlas of dominant bacteria in soil. Science. 2018;359(6373):320-325.
10. Hochman Z, Gobbett DL, Horan H. Quantifying yield gaps in rainfed cropping. Field Crops Research. 2017;202:68-77.
11. Bulgarelli D, Schlaeppi K, Spaepen S. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology. 2013;64:807-838.
12. Herlemann DPR, Labrenz M, Jürgens K. Transitions in bacterial communities along the Baltic Sea. ISME Journal. 2011;5(10):1571-1579.
13. Callahan BJ, McMurdie PJ, Rosen MJ. DADA2: High-resolution sample inference. Nature Methods. 2016;13(7):581-583.

14. Douglas GM, Maffei VJ, Zaneveld JR. PICRUSt2 for prediction of metagenome functions. Nature Biotechnology. 2020;38(6):685-688.
15. Love MI, Huber W, Anders S. Moderated estimation of fold change with DESeq2. Genome Biology. 2014;15(12):550.
16. Donn S, Kirkegaard JA, Perera G. Evolution of bacterial communities in wheat rhizosphere. Environmental Microbiology. 2015;17(3):610-621.
17. Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome. FEMS Microbiology Reviews. 2013;37(5):634-663.
18. Turner TR, James EK, Poole PS. The plant microbiome. Genome Biology. 2013;14(6):209.
19. Pii Y, Borruso L, Brusetti L. Iron nutrition and rhizosphere microbiome. Plant Physiology and Biochemistry. 2016;99:160-168.
20. Caporaso JG, Lauber CL, Walters WA. Ultra-high-throughput microbial community analysis. ISME Journal. 2012;6(8):1621-1624.
21. Angus JF, Grace PR. Nitrogen balance in Australia. Soil Research. 2017;55(6):435-450.