AMERICAN SOCIETY FOR MICROBIOLOGY | JMBE Journal of Microbiology & Biology Education

Check for updates

| Tips and Tools

Climate change and plant rhizosphere microbiomes: an experiential course-embedded research project

James A. Parejko[1]

AUTHOR AFFILIATION See affiliation list on p. 4.

ABSTRACT The current and ongoing challenges brought on by climate change will require future scientists who have hands-on experience using advanced molecular techniques, can work with large data sets, and can make correlations between metadata and microbial diversity. A course-embedded research project can prepare students to answer complex research questions that might help plants adapt to climate change. The project described herein uses plants as a host to study the impact of climate change-induced drought on host-microbe interactions through next-generation DNA sequencing and analysis using a command-line program. Specifically, the project studies the impact of simulated drought on the rhizosphere microbiome of Fast Plants rapid cycling *Brassica rapa* using inexpensive greenhouse supplies and 16S rRNA V3/V4 Illumina sequencing. Data analysis is performed with the freely accessible Python-based microbiome bioinformatics platform QIIME 2.

KEYWORDS microbiomes, climate change, rhizosphere, drought, QIIME 2, plant research, host-microbe interactions

Climate change-induced droughts have broad-ranging implications for global agriculture production and food security (1, 2). Under drought stress, plants exude unique organic molecules into their rhizosphere thereby changing the microbial community colonizing the plant's roots as compared to normal conditions (3–6). By studying the rhizosphere microbiome of water-stressed plants, we can prospect for novel plant growth-promoting rhizosphere microbes to ameliorate the negative effects of drought on crop productivity (7). Furthermore, adapting to climate change will require scientists with experience in advanced molecular techniques and complex data analysis. Finally, course-based microbial ecology research projects effectively engage students and train them in skillsets that create successful scientists and motivated lifelong learners (8).

The methods described here were developed to fit into 6 weeks and investigate the effects of simulated drought on the Wisconsin Fast Plants rapid cycling *Brassica rapa* rhizosphere microbiome (Wisconsin Fast Plants Program of the Department of Plant Pathology, University of Wisconsin—Madison, https://fastplants.org). It was tested in an upper-level special topics course laboratory that consisted of nine biology students with varying microbiology backgrounds. The course lecture discussed current microbiome topics, sequencing platforms, and microbiome-related primary literature. This project is best fit for upper-level courses with class sizes of 20 or fewer students where one-on-one instructor-student interactions allow for troubleshooting QIIME 2 Python commands.

The adaptability of this experiment allows the instructor to cater the experiment to student interests and input beyond drought. This might include variables like extremes in temperature or flooding. The experiment could also explore changes to the rhizosphere microbiome of Fast Plants mutants, changes to the fungal microbiome, changes

Editor Arpita Bose, Washington University in St. Louis, St. Louis, Missouri, USA

Address correspondence to James A. Parejko, jparejko@gustavus.edu.

The author declares no conflict of interest.

Received 26 March 2024
Accepted 28 May 2024
Published 18 June 2024

to the rhizosphere of other plant species, or explore the unique rhizosphere microbial species found in geographically distinct soils.

PROCEDURE

Fast Plants drought simulation

The effect of drought can be reliably simulated in a growth chamber or greenhouse with inexpensive and accessible supplies and equipment (Fig. 1A and Appendix S1) (9). As described in Appendix S2, seeds are germinated in autoclaved potting mix and transplanted into a mixture of natural soil from a source of interest (e.g., campus arboretum) and autoclaved potting mix in larger pots with enlarged drainage holes plugged with sterile loose cotton or nylon mesh. Two plants are transplanted per pot and watered regularly for 7 days. Each pot of two plants represents one "sample" to be harvested for baseline (pre-drought), control (watered), and drought-treated plants. The baseline samples are harvested (3–5 samples are recommended), and the remaining pots are divided up into the control and drought-treated groups. Control and drought-treated pots are placed on pre-soaked 3″ × 4″ × 9″ green floral foam bricks in plastic containers with 1 cm of water (Fig. 1A). Control pots are watered every 1–2 days. Drought-treated pots only receive water through capillary action by maintaining the 1 cm of water. Plants are grown for a minimum of 2 weeks and harvested in the same manner as the baseline plants. In addition, soil moisture sensors could be used throughout the experiment to monitor drought conditions.

The roots of a single sample are harvested as described in Appendix S3. Both roots are placed into one sterile 50 mL screw cap tube, to which 40 mL of sterile epiphyte removal buffer is added (6.75 g KH_2PO_4, 8.75 g K_2HPO_4, 1 mL Triton X-100 per liter) (10). A brief vortex followed by sonicating in a sonicating water bath promotes the removal of microbes from the root surface, without damaging the root tissue. Roots are removed from the tubes, and the suspension is centrifuged to create a rhizosphere microbiome pellet. Total microbial DNA is extracted from 250 mg of the pellet using

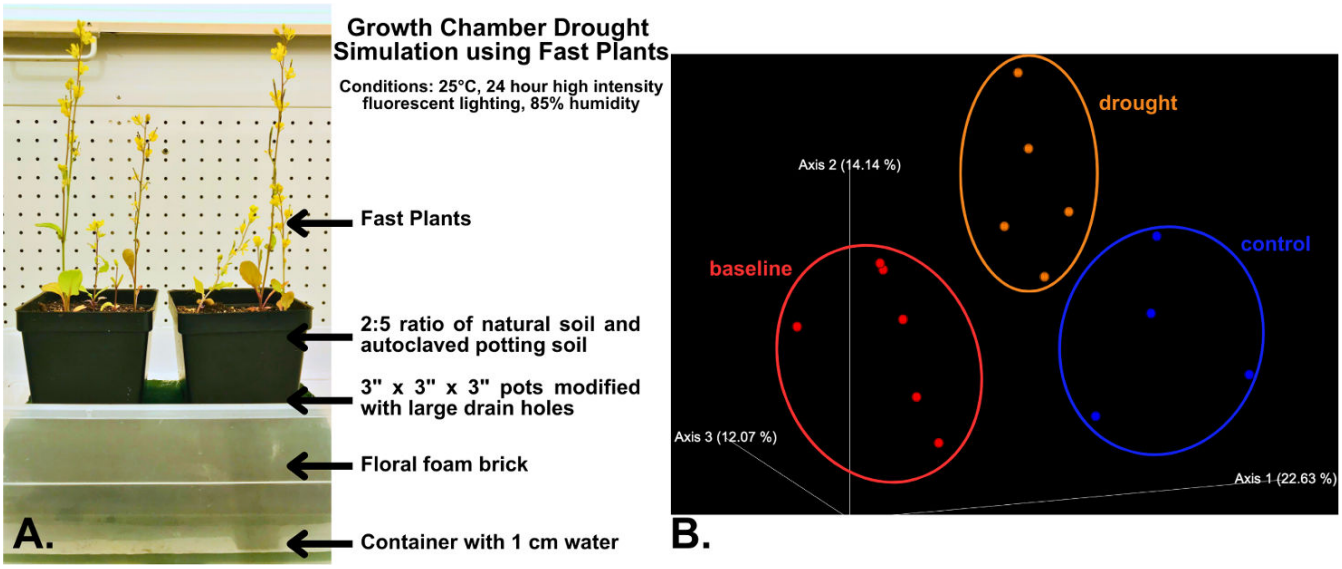

FIG 1 Impact of climate change-induced drought on the rhizosphere bacterial microbiome of Fast Plants (*Brassica rapa*). (A) A basic inexpensive drought-simulation experimental setup using a floral foam brick that allows water from a container to reach potted plants only by capillary action. Rhizosphere microbiomes of pre-drought baseline plants and regularly watered control plants can be compared by students to drought-treated plants. (B) Weighted UniFrac, a beta diversity index, was visualized on a principal coordinates analysis plot and showed statistically significant differences between all treatment groups (overall permutational multivariate ANOVA, or PERMANOVA, with 999 permutations: *P*-value = 0.001; pairwise PERMANOVA with 999 permutations: baseline to control *P*-value = 0.006, *q*-value = 0.009; baseline to drought *P*-value = 0.003, *q*-value = 0.009; control to drought *P*-value = 0.017, *q*-value = 0.017). Illumina V3/V4 16S rRNA raw data were analyzed, and plots were created in QIIME 2 by students in the BIO 344: Exploring Microbiomes course.

bead-beating and the ZymoBIOMICS DNA Miniprep kit (Zymo Research, Irvine, CA, USA). Purified DNA can be quantified and sent to an off-site sequencing lab for 16S rRNA V3/V4 Illumina sequencing. Sequencing labs like SeqCenter (Pittsburgh, PA, USA, https://www.seqcenter.com) provide affordable bacterial 16S rRNA V3/V4 or fungal ITS2 sequencing. In our experience, the smallest 16S rRNA sequencing package (minimum 10K read pairs guaranteed; our lowest median read number was 74,214 paired-end reads after quality control was performed) provided enough data to draw significant conclusions between treatments. Metadata like stem length, root length, and dried root weight can be measured and averaged (two plants per sample).

QIIME 2 tutorials and assignments

To analyze the 16S rRNA reads from the drought-simulation experiment, students learn Python commands from QIIME 2 tutorials while the plants are growing. QIIME 2 is a frequently cited command-line microbiome analysis package (11). A wealth of background and troubleshooting help is provided for students through the freely accessible and continually updated QIIME 2 website (https://QIIME 2.org/), and common implementation issues are defined in Appendix S4. Students work through the first three tutorials ("Moving Pictures," fecal microbiota transplant, and Atacama soil microbiome tutorials) to learn how to take raw .fastq sequencing files through quality control and processing to final manuscript quality figures and statistics. The goal is for students to be proficient with QIIME 2 by the time their own 16S rRNA sequence data are obtained (an example tutorial assignment with answers is provided in Appendix S5). For the final project, each student applies the practiced QIIME 2 skills to create a section of a collaborative poster to communicate the results of the experiment to a broader audience. Emphases should be placed on defining significant differences in microbiome beta diversity (Fig. 1B), taxonomic composition (Fig. S1 in Appendix S6), and bacterial genera enriched or depleted when comparing control and drought samples (Fig. S2 in Appendix S6).

Safety issues

Basic safety precautions should be followed and personal protective equipment (e.g., gloves and safety glasses) should be worn when working with soils and DNA extraction kit reagents.

CONCLUSION

Next-generation DNA sequencing, as applied in this plant rhizosphere microbiome project, has become inexpensive enough to allow its broad application in the laboratory classroom to train future scientists and policymakers with advanced research and data analysis skills to combat the effects of climate change. This laboratory addresses ASM Curriculum Guidelines by applying the scientific method, quantitative computational skills and bioinformatics (Scientific Thinking), investigating the role that the plant rhizosphere has in surviving drought (Impact of Microbes), and understanding the impact that the environment has on microbial communities (Microbial Ecology). In addition, rhizosphere bacteria or fungi could be isolated and characterized from the rhizosphere pellet for experience in aseptic technique, dilutions, and media preparation (Microbiology Laboratory Skills).

ACKNOWLEDGMENTS

Funding for this course was graciously provided by the Gustavus Adolphus College Department of Biology.

I am grateful to the fall 2023 BIO 344 Gustavus Adolphus College students for their patience and understanding in this project's initial attempt.

AUTHOR AFFILIATION

[1]Department of Biology, Gustavus Adolphus College, Saint Peter, Minnesota, USA

AUTHOR ORCIDs

James A. Parejko http://orcid.org/0009-0009-5282-1090

DATA AVAILABILITY

Microbiome data from this initial attempt can be accessed via NCBI using BioProject ID PRJNA1091725.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Appendixes 1 to 6 (jmbe00046-24-S0001.docx). Materials, methods, learning objectives and guidance for instructors, tutorial assignment example, and extra figures.

REFERENCES

1. Stanke C, Kerac M, Prudhomme C, Medlock J, Murray V. 2013. Health effects of drought: a systematic review of the evidence. PLoS Curr 5. https://doi.org/10.1371/currents.dis.-7a2cee9e980f91ad7697b570bcc4b004
2. Orimoloye IR. 2022. Agricultural drought and its potential impacts: enabling decision-support for food security in vulnerable regions. Front Sustain Food Syst 6. https://doi.org/10.3389/fsufs.2022.838824
3. Ait-El-Mokhtar M, Meddich A, Baslam M. 2023. Plant-microbiome interactions under drought—insights from the molecular machinist's toolbox. Front Sustain Food Syst 7:1253735. https://doi.org/10.3389/fsufs.2023.1253735
4. Bhattacharyya A, Pablo CHD, Mavrodi OV, Weller DM, Thomashow LS, Mavrodi DV. 2021. Rhizosphere plant-microbe interactions under water stress. Edited by G. M. Gadd and S. Sariaslani. Advances in Applied Microbiology 115:65–113. https://doi.org/10.1016/bs.aambs.2021.03.001
5. Xie J, Dawwam GE, Sehim AE, Li X, Wu J, Chen S, Zhang D. 2021. Drought stress triggers shifts in the root microbial community and alters functional categories in the microbial gene pool. Front Microbiol 12:744897. https://doi.org/10.3389/fmicb.2021.744897
6. Gargallo-Garriga A, Preece C, Sardans J, Oravec M, Urban O, Peñuelas J. 2018. Root exudate metabolomes change under drought and show limited capacity for recovery. Sci Rep 8:12696. https://doi.org/10.1038/s41598-018-30150-0
7. Benmrid B, Ghoulam C, Zeroual Y, Kouisni L, Bargaz A. 2023. Bioinoculants as a means of increasing crop tolerance to drought and phosphorus deficiency in legume-cereal intercropping systems. Commun Biol 6:1016. https://doi.org/10.1038/s42003-023-05399-5
8. Parks S, Joyner JL, Nusnbaum M. 2020. Reaching a large urban undergraduate population through microbial ecology course-based research experiences. J Microbiol Biol Educ 21:30. https://doi.org/10.1128/jmbe.v21i1.2047
9. Marchin RM, Ossola A, Leishman MR, Ellsworth DS. 2019. A simple method for simulating drought effects on plants. Front Plant Sci 10:1715. https://doi.org/10.3389/fpls.2019.01715
10. Simmons T, Caddell DF, Deng S, Coleman-Derr D. 2018. Exploring the root microbiome: ebacterial community data from the soil, rhizosphere, and root endosphere. J Vis Exp:57561. https://doi.org/10.3791/57561
11. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9