

The soil microbiome associated with brown root rot pathogen (*Phellinus noxius*) in the Pacific Islands – Implications for disease management

Olga Kozhar¹, Mee-Sook Kim², Phil G. Cannon³, Jane E. Stewart^{1*}, and Ned B. Klopfenstein⁴

Introduction

Phellinus noxius, is a destructive, fast-growing pathogen that causes brown root rot disease of a very wide range of woody hosts (Cannon et al. 2022). Its devastating effects on fruit trees, cacao, ornamentals, coffee, and rubber have been documented across diverse global regions, such as Taiwan, Japan, Hong Kong, Southeast Asia, Australia, and various Pacific Islands (e.g., Guam, Saipan, Kosrae, Pohnpei, Palau, American Samoa, etc.) (Stewart et al. 2020; Cannon et al. 2022). To date, this pathogen has not been reported in some Pacific Islands and other U.S. states/territories (e.g., Hawaii, Florida, Puerto Rico), but it represents a major invasive threat based on our bioclimatic models (Stewart et al. 2020). Current concerns are that future human activities will exacerbate this disease or even potentially introduce it to other locations, where it has not been reported (e.g., Kozhar et al. 2022; Stewart et al. 2020).

With a broad host range of over 200 host species (e.g., Ann et al. 2002; Brooks 2002; Cannon et al. 2022), *P. noxius* can cause extensive damage to sub- and tropical forest ecosystems when present on a site. Further, as a root pathogen that can exist as a saprophyte for long periods (e.g., Chang 1996), *P. noxius* can be extremely difficult to eradicate from a site. In attempting to reduce inoculum density of *P. noxius*, some soil amendments (e.g., urea) were added and showed promising results (Chang and Chang 1999; Cannon et al. 2022). To assess the efficacy of treatments to reduce *P. noxius*, it is essential to monitor and determine changes of *P. noxius* inoculum density as influenced by different soil amendments over time. Monitoring inoculum density through traditional techniques (e.g., cultured-based) can be challenging, time consuming, and lack sensitivity. A molecular approach with microbiome sequencing and species-specific markers will enable monitoring of the management treatment effects over time more quickly and efficiently. The goal of this study was to characterize microbial communities associated with soils of trees infected with *P. noxius* on Guam and Pohnpei.

Methods

Sampling: Rhizosphere soils collected from healthy and *P. noxius*-infected trees including 3 healthy and 3 diseased *Meiogyne cylindrocarpa* (an evergreen rainforest plant also known as fingersop) trees in Guam, and 2 healthy and 2 diseased *Artocarpus altilis* (breadfruit) trees in Pohnpei, FSM.

Procedure: For each brown root rot diseased (*P. noxius*-infected/symptomatic) tree, soil samples were collected on the sides of infected lateral roots with symptoms/signs (encrusted fungal mat and/or soft-rot). Similar rhizosphere samples were collected from asymptomatic (healthy) trees that were at least 15 m (50 ft) away from any tree with brown root rot disease symptoms.

Soil collection, extraction, sequencing and analyses: To collect soil samples from the rhizosphere of healthy and diseased trees, the organic debris was first removed from the soil surface, and then ca. 50 g (3 tablespoons) of soil was collected at a depth of 7.5 cm (3 inches) and placed in LifeGuard Soil Preservation solution to protect the DNA. Additional soil associated with each sample was collected in bags and placed in a freezer for subsequent analyses of soil pH and % soil moisture. Total soil DNA was extracted using the Qiagen PowerSoil Kit. Libraries

¹Colorado State University, Department of Agricultural Biology; CO, USA

²USDA Forest Service, Pacific Northwest Research Station; Corvallis, OR, USA

³USDA Forest Service, State, Private, and Tribal Forestry, Pacific Southwest Region, Forest Health Protection; Vallejo, CA, USA

⁴USDA Forest Service, Rocky Mountain Research Station; Moscow, ID, USA

*jane.stewart@colostate.edu

were generated for each sample at the ITS for fungal communities and 16s for bacterial communities, and Illumina sequenced on a MiSeq. For investigating soil microbes associated with *P. noxius* inoculum density, reads from the pooled dataset were separated for each sample. Software mothur (Schloss et al. 2009) was used for reads identification and package vegan v.2.6-4 (implemented in R) (Oksanen et al. 2020) for overall fungal and bacterial diversity estimates and ordination plots for estimating changes occurring over time and with different treatments.

Preliminary Results

- Principal coordinates analyses showed that microbial communities (both fungi and bacteria) differ by geographic location, but not by rhizosphere samples from *P. noxius*-infected (symptomatic) and healthy (asymptomatic) trees (Figure 1).
- In Guam, fungal diversity decreases and bacterial diversity increases within the rhizosphere of symptomatic trees, but this trend was not apparent in rhizosphere samples from Pohnpei (Figure 2).
- Comparisons between the rhizospheres of symptomatic and asymptomatic trees from Guam revealed a greater shift in the fungal communities compared to the bacterial communities (Figure 3).
- Significantly higher abundances of Symptoventuriaceae and *Trechispora* were found in rhizospheres of symptomatic trees, whereas significantly higher abundances of *Leohumicola*, *Plenodomus*, and Didymosphaeriaceae were found in rhizospheres of healthy trees (Figure 4).

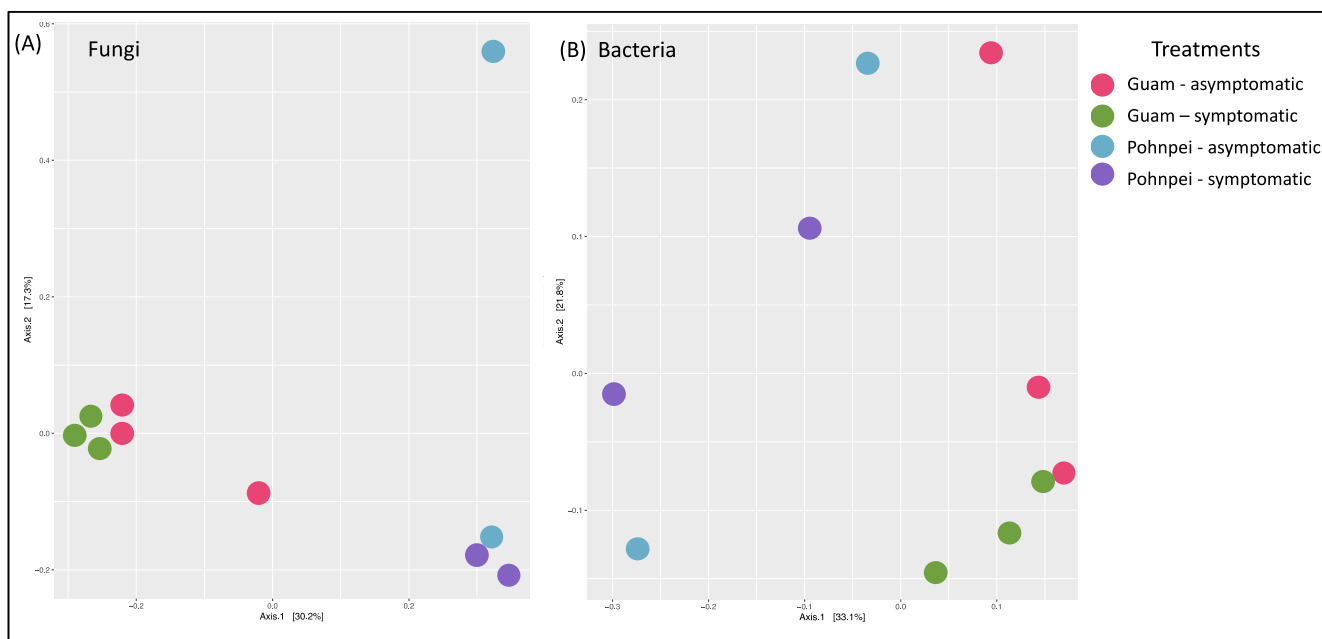


Figure 1: Principal Coordinates Analyses of microbial communities (A: Fungi and B: bacteria) from Guam and Pohnpei (FSM) associated with the rhizospheres of *Phellinus noxius*-infected (symptomatic) and healthy (asymptomatic) trees.

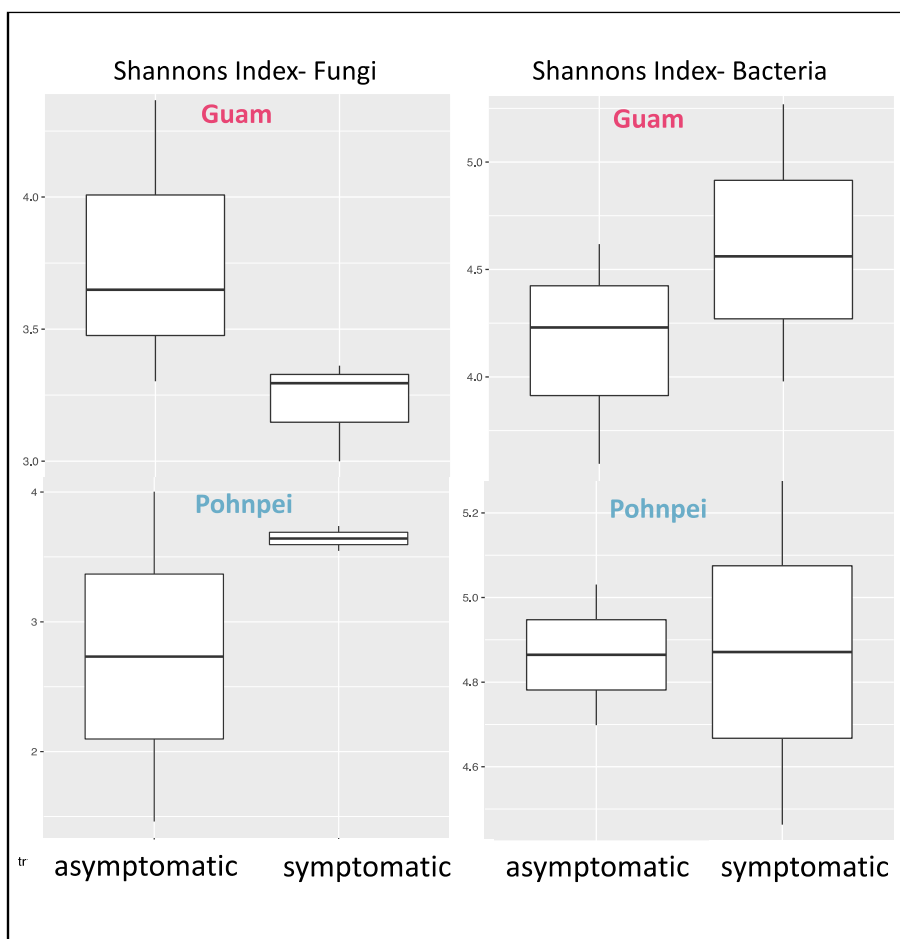


Figure 2: Shannon diversity index of microbial communities from Guam and Pohnpei associated with the rhizospheres of *Phellinus noxius*-infected (symptomatic) and healthy (asymptomatic) trees.

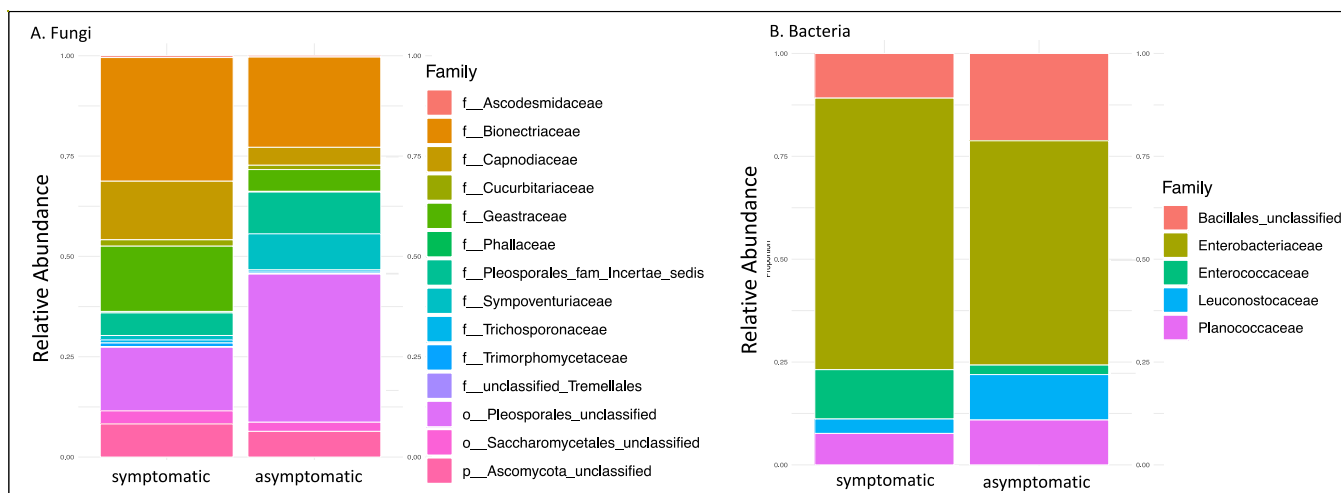


Figure 3: Top 1% of the relative abundance of fungal (A) and bacterial (B) taxa from the rhizospheres of *Phellinus noxius*-infected (symptomatic) and healthy (asymptomatic) trees from Guam.

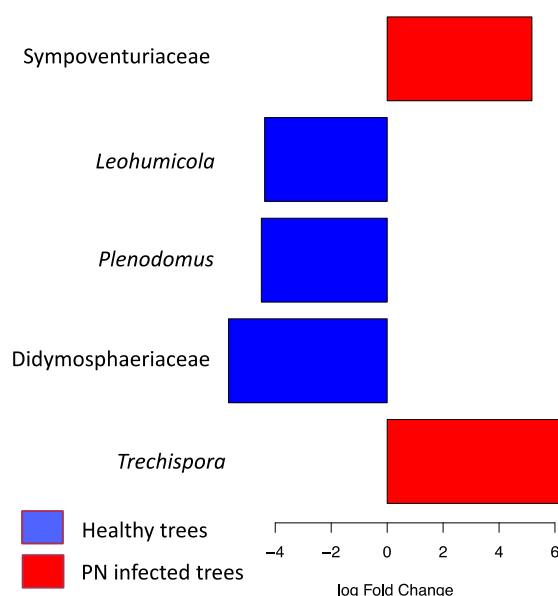


Figure 4: Significant log fold change of fungal communities from Guam. Red bars indicate taxa with significant higher abundance in rhizosphere samples from *Phellinus noxius*-infected (symptomatic) trees and blue bars indicate rhizosphere samples from healthy (asymptomatic) trees.

Discussion and Conclusions

This preliminary study obtained baseline data on the microbial communities associated with the rhizospheres of *P. noxius*-infected (symptomatic) and healthy (asymptomatic) trees in Guam and Pohnpei (FSM). In Guam, more change in the fungal community were observed in association with the rhizosphere of *P. noxius*-infected trees. This concurs with a recent study that showed one fungal taxa (*Cosmospora*) was associated with *P. noxius* infection (Liu et al. 2022). Dysbiosis is perhaps occurring in the rhizospheres of *P. noxius*-diseased (symptomatic) trees, whereby changes in the fungal communities are driven by pathogen pressure. Interestingly, little change was observed in the bacterial community of rhizospheres in response to *P. noxius* infection, both in Guam and Pohnpei. This work is the first step for developing novel approaches to reduce inoculum of *P. noxius* on the Pacific Island sites. Planned future studies include assessing *P. noxius* inoculum levels in the tree rhizosphere via quantitative PCR and analyzing microbial communities within the tree rhizosphere in association with different urea and lime soil treatments (e.g., urea, lime, urea + lime, and control). These planned studies will help determine effects of soil amendments on *P. noxius* abundance and microbiome composition in tree rhizospheres on sites prone to brown root rot disease.

Acknowledgments

We thank Robert L. Schlub, Julia Hudson, Michael Jordan, Roger Brown (deceased), and other field and laboratory personnel, who helped collect the soil samples in Guam, and Rodasio Samuel, Konrad Englberger, David Komorowski, and others who helped collect soil samples in Pohnpei, FSM. Funding sources include the USDA Forest Service (FS), State, Private and Tribal Forestry, Forest Health Protection, Special Technology Development Program (STDP R5-2019-02), USDA FS R&D Bipartisan Infrastructure Law (BIL) Ecosystem Restoration Research (ERR09), and USDA FS R&D, Rocky Mountain Research Station and Pacific Northwest Research Station. Any findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

Literature Cited

- Ann, P.-J.; Chang, T.-T.; Ko, W.-H. 2002. *Phellinus noxius* brown root rot of fruit and ornamental trees in Taiwan. Plant Disease. 86: 820–826.
- Brooks, F.E. 2002. Brown root rot disease in American Samoa's tropical rain forests. Pacific Science 56: 377–387.
- Cannon, P.G.; Klopfenstein, N.B.; Kim, M.-S.; Stewart, J.E.; Chung, C.-L. 2022. Brown root rot disease caused by *Phellinus noxius* in U.S.-Affiliated Pacific Islands. Gen. Tech. Rep. PNW-GTR-1006. U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR, USA. 99 p.
- Chang, T.-T. 1996. Survival of *Phellinus noxius* in soil and in the roots of dead host plants. Phytopathology. 86: 272–276.
- Chang, T.-T.; Chang, R.J. 1999. Generation of volatile ammonia from urea fungicidal to *Phellinus noxius* in infested wood in soil under controlled conditions. Plant Pathology. 48: 337–344.
- Kozhar, O.; Kim, M.-S.; Ibarra Caballero, J.; Klopfenstein, N.B.; Cannon, P.G.; Stewart, J.E. 2022. Long evolutionary history of an emerging fungal pathogen of diverse tree species in eastern Asia, Australia, and the Pacific Islands. Molecular Ecology 31: 2013–2031.
- Liu, T.Y.; Chen, C.H.; Yang, Y.L.; Tsai, I.J.; Ho, Y.N.; Chung, C.L. 2022. The brown root rot fungus *Phellinus noxius* affects microbial communities in different root-associated niches of Ficus trees. Environmental Microbiology 24: 276–297.
- Oksanen, J.; Simpson, G.L.; Guillaume Blanchet, F.; Kindt, R.; Legendre, P.; Minchin, P.R.; et al. 2020. Package ‘vegan’: Community Ecology Package.
- Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; Sahl, J.W.; Stres, B.; Thallinger, G.G.; Van Horn, D.J.; Weber, C.F. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75: 7537–7541.
- Stewart, J.E.; Kim, M.-S.; Ota, Y.; Sahashi, N.; Hanna, J.W.; Akiba, M.; Ata, J.P.; Atibalentja, N.; Brooks F.; Chung, C.-L.; Dann, E.K.; Farid, A.M.; Hattori, T.; Lee, S.S.; Otto, K.; Pegg, G.S.; Schlub, R.L.; Shuey, L.S.; Tang, A.M.C.; Tsai, J.-N.; Cannon, P.G.; Klopfenstein, N.B. 2020. Phylogenetic and population genetic analyses reveal three distinct lineages of the invasive brown root-rot pathogen, *Phellinus noxius*, and bioclimatic modeling predicts differences in associated climate niches. European Journal of Plant Pathology 156: 751–766.

Proceedings of the 68th Western International Forest Disease Work Conference

DoubleTree Hotel

Rohnert Park, California

June 5-9, 2023



Proceedings of the 68th Western International Forest Disease Work Conference

DoubleTree Hotel

Rohnert Park, California

June 5-9, 2023

Compiled by:

Rachel K. Brooks
Washington State Department of Natural Resources
Forest Resilience Division
Olympia, Washington

Papers are formatted and have minor editing for language and style but otherwise are printed as they were submitted. The authors are responsible for content.

Citation:

Brooks, R.K. (comps.) 2024. Proceedings of the 68th Western International Forest Disease Work Conference, Rohnert Park, California, 5-9 June 2023. <https://www.wifdwc.org/>

Conference photo contributors:

Scott Baker
Rachel Brooks
Sam Brown
Kristen Chadwick
Kim Corella
John Dobbs (cover photo)
Betsy Goodrich
Jorge Ibarra
Alan Kanaskie
Holly Kearns
Blakey Lockman
Mike McWilliams
Robin Mulvey
Greg Reynolds
Kerry Wininger