Expanding Monitoring Efforts to Include Plant Genetic, Bacterial, and Fungal Diversity

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Rationale

As we indicated in our original proposal:

> *There is longstanding interest in the effects of biodiversity on ecosystem processes, as well as the role that diversity plays in promoting stability following disturbance. For example, there is strong evidence that plant species richness stabilizes production (Gross et al 2014). More recently, increasing attention has been focused on other components of biodiversity. Intraspecific diversity of plant species (i.e., genotypic diversity) has been shown to be a functional surrogate for interspecific richness in species depauperate systems (Whitham et al. 2006) and can enhance production following disturbance (Reusch 2005). There is also emerging evidence that microbial diversity promotes the stability of decomposition (Bardgett and van der Puten 2014). Furthermore, tree genotypic diversity within forests has been shown to influence the composition and/or diversity of both bacterial (Schweitzer et al. 2008) and fungal (Gehring et al. 2017) communities, due to their intimate associations belowground.*
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> *Thus, understanding plant-microbial interactions is essential to advancing our knowledge of the mechanisms by which biodiversity regulates ecosystem processes as well as predicting responses to environmental change (Wardle et al. 2004). This is particularly true for identifying how climate change will impact the capacity of forests to serve as sinks for atmospheric carbon dioxide, which will be determined in part by the balance between net primary production by trees which fixes carbon, and soil respiration that releases it (i.e., root respiration plus decomposition predominantly by bacteria and fungi).*
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> *Although forest monitoring programs typically quantify the diversity of overstory trees, plant genetic diversity has largely been neglected despite its potential*

value to comprehensive monitoring efforts (Fussi et al. 2016). Similarly, the diversity of microbes such as fungi is not widely measured, but has been suggested as an important addition to monitoring programs (Cox et al 2010). Therefore, we propose to develop an integrated framework for quantifying plant genetic, bacterial, and fungal diversity within our forest monitoring site.

Approach/Methodology

The project was conducted in a ca. 150 ha second-growth oak-pine forest (Maine Natural Areas Program 2025) in Biddeford Maine USA dominated by *Quercus rubra* (red oak) and *Pinus strobus* (white pine). Upland soils are loam or sandy loam overlying bedrock (Soil Survey Staff 2025). To characterize genetic variation among trees, we collected canopy leaves within 3 previously established 20 x 60m long-term monitoring plots (n = 97 red oaks; n = 112 white pines), as well as clusters of 3 trees per species within 10m of one another distributed along a ca. 1.2 km transect that included the plots (n = 15 red oaks; n = 15 white pines). Leaves were transported to the lab where they were stored at -80C, after which DNA was extracted from ca. 0.1g of tissue with the DNeasy Plant Mini Kit (Qiagen Inc). Unfortunately, we were unable to develop a reliable protocol for amplifying white pine microsatellite DNA and thus that species was dropped from the study. For red oak, we were able to amplify 10 microsatellite loci identified by Aldrich et al. (2002) using their PCR methods, after which samples were shipped to an outside lab for fragment analysis (Keck DNA Sequencing Core at Yale University).

Bacterial and fungal communities were characterized by homogenizing soil (15cm depth) collected from 3 locations 1.5m from the base of select trees for which leaf tissue had been sampled, using a sterile technique (n = 54 red oaks in plots; n = 27 white pines in plots; n= 15 red oaks and 15 white pines along the transect). Sample sizes were lower for soil samples than for tree leaves given the greater cost per sample for the microbial analyses, and lower for white pine trees than for oak trees given the difficulties we were having developing the white pine plant genetic assays. Soil was transported on ice to the lab and stored at -80C, after which DNA was extracted from ca. 250mg of soil using the DNeasy Power Soil Kit (Qiagen Inc). Samples were then shipped to an outside lab for both PCR amplification and pyrosequencing (Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory) based upon 16s rRNA for bacterial taxa (after Kim et al. 2008) and internal transcribed spacer (ITS) rRNA for fungal taxa (after O'Brien et al. 2005).

Statistical analyses of the genetic relatedness among individual trees were conducted with the Spatial Pattern Analysis of Genetic Diversity software package (SPAGeDi; Hardy &

Vekemans 2002). Comparisons of soil bacterial and fungal communities, based upon amplicon sequence variants (ASVs), was conducted using QIIME 2 software (Boylen et al. 2019). The relationship between microbial community composition and plant genetic variation was determined using the Pattern Analysis, Spatial Statistics and Geographic Exegesis program (PASSaGE; Rosenberg and Anderson 2011). To account for spatial variation, distance among trees was measured using a GPS receiver and laser rangefinder and the offset-mapping technique of Meier (2022). Results indicated that the inclusion of trees along the transect did not add meaningful information beyond that from the plots alone, so the transect data were removed from the final analyses. Bacterial data were of very high quality and those results are reported here, but fungal data were of lower quality so will require further curation and ASV re-analysis in the future. An alpha of 0.05 was used for all statistical tests, and 999 permutations were used for any simulation procedure that required them.

Activities

- In year 1, we collected leaf tissue and conducted preliminary genetic analyses of trees within our permanent study plots but determined that only a small number of the microsatellite loci we selected were informative, so tested additional loci.
- In year 2, we collected leaf tissue from trees along the transect and continued exploring alternate microsatellite loci for the genetic analyses but still were unable to resolve spatial genetic structure at the desired level. We also collected soil samples from beneath trees in both plots and transects, and began DNA extractions, but held off on the expensive PCR amplification and sequencing until we could determine exactly which trees we would have genetic information for.
- We were granted a no-cost, one year extension to our project which allowed us to continue our work into year 3, when we were able to complete the genetic analysis of red oak based upon 10 microsatellite loci. However, we were unable to develop a reliable protocol for characterizing white pine genetics so dropped that species from the project. We also determined spatial distances among trees in the study, completed DNA extractions from soil and sent our samples off for characterization of the microbial community. Statistical analyses were completed for the plant genetic data and preliminary results presented at the annual FEMC meeting.
- In the year following the end of the grant, we conducted the statistical analyses of the soil microbial data, but as indicated above in the Approach/Methodology section the data from the transects and the soil fungal analyses were dropped. We also conducted statistical analyses relating soil bacterial composition to plant genetic variation and

created a draft of the brief guide to integrating plant genetic, bacterial, and fungal diversity in monitoring projects.

Outcomes and results

Progress on our three primary objectives, and associated results, were as follows:

1) ***Take baseline diversity measurements, as well as develop a sampling protocol for future years***. We found small but significant levels of tree genetic differentiation among our plots, separated by as little as 500m, as well as highly significant levels of inbreeding, nearly at the level of first cousins. Both soil bacterial alpha and beta diversity were also significantly different among our plots. Re-evaluation of soil fungal data are ongoing. These data will serve as a valuable baseline for future periodic monitoring. Most importantly, we have developed a sound protocol that we can use moving forward.
2) ***Produce a guide for other groups interested in expanding their monitoring projects to include measures of plant genetic, bacterial, and fungal diversity.*** We have drafted a succinct guide, based upon our experience, for conducting work in forests that have similar species composition and soil types in New England. However, some of our recommendations would be appropriate for anyone in the region considering such work, regardless of the forest and soil type. We anticipate completing this late summer 2025 and plan to disseminate this information at the 2025 FEMC conference (e.g., via a workshop).
3) ***Use the data collected from our site to explore the relationship among these various components of diversity and how they impact forest structure and function, thereby contributing to forest ecology research.*** Integrating our plant genetic and soil bacterial data, we found that trees that were more closely related had more similar bacterial communities in 2 of our 3 study plots. Preliminary results of the plant genetic analyses were presented at the 2023 FEMC conference, and a manuscript is in preparation on plant-bacterial associations. Future work will include: a) the parallel analysis between plant genetic and soil fungal data; b) relating plant genetic variation to insect communities, and c) determining how plant and soil microbial diversity influences soil respiration rates. Sampling of insect eDNA and soil respiration rates for these latter two projects will be completed with

alternate funding sources but would not be possible without the plant and microbial data generated through our FEMC grant.

Data Management

A temporary embargo on releasing data publicly was requested and approved until January 2026. After that, data from the project, as well as a guide to best practices for others interested in expanding their monitoring to include measures of genetic diversity, will be made publicly available at the University of New England online repository, DigitalUNE[1]: https://dune.une.edu/eme_genbacfun/. This archive, which is part of the Digital Commons Network[2], meets FEMC standards regarding open access to data as identified in the RFP (i.e., *permanent, unique URL, secure, available to the public without restriction*)

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[1] Digital UNE, https://dune.une.edu
[2] Digital Commons Network, https://network.bepress.com

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