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Diversity and Abundance of Soil Microbes Differ Along a Forest-Pasture Transect

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KEYWORDS—*microbial diversity, microbes, bacteria, ecology, land management, soil diversity, soil health, ecosystem functioning*

INTRODUCTION—Soil microbes are instrumental in orchestrating the overall function and diversity of soil communities through a multitude of ecological processes, including carbon cycling, nitrogen cycling, nutrient acquisition, and soil structuring (Van Der Heijden et al., 2007). Preliminary studies reveal that changes in the abundance of soil organisms causes marked increases or decreases in ecosystem functional diversity, elucidating the importance of microbes in driving ecosystem productivity (Rich et al., 2003; Wagg et al., 2014). Despite all of the known roles that microbes play in soil ecology, the degree to which plants influence microbe diversity remains unclear. We are only in the initial stages of understanding the extent of symbiotic relationships between microbes and plants, illuminating the need for further examination of soil ecology. A better understanding of these interactions could potentially improve our assessment and management of agricultural or disturbed settings. We sought to quantify the spatial variation of soil microbe communities along a gradient spanning from a densely forested area, intersecting an equine trail, and ending in an adjacent fallow pasture. We hypothesized that due to heightened plant diversity in the forest, there would be higher microbial abundance and diversity in the forest than in the pasture and that microbial abundance and diversity will decrease along the transect, from forest to pasture.

MATERIALS & METHODS—We conducted our study in the Dows Prairie area of McKinleyville, California, USA. The pasture, previously grazed by cattle and left out to fallow for at least 10 years, borders a horse trail and adjacent forest. The forest is mostly undisturbed except for foot traffic from local wildlife and horseback riders on marked trails. We collected soil samples with a sterilized stainless-steel spoon every 6.1 m (20 ft) along a 121.9 m (400 ft) transect (FIG 1). We placed samples in resealable plastic bags and stored them in a freezer overnight. To examine bacterial diversity, we plated two cultures per sample, each with 1 x 10^{-5} g of serially diluted soil. We accomplished this by placing 1 g of soil into a test tube containing 9.9 mL saline solution, vortexing the test tube, and transferring 100 µL into another test tube containing 9.9 mL saline solution. Lastly, we plated 100 µL of the final dilution on agar enriched with lysogeny broth. To examine fungal diversity, we placed a small sample of undiluted soil on water agar.

We left our samples to culture in a box at room temperature. Twenty-four hours following plating, we recorded the number of distinct colonies per plate and

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FIGURE 1. Map of Dows Prairie study area, with transect in white, intersected by a horse trail.

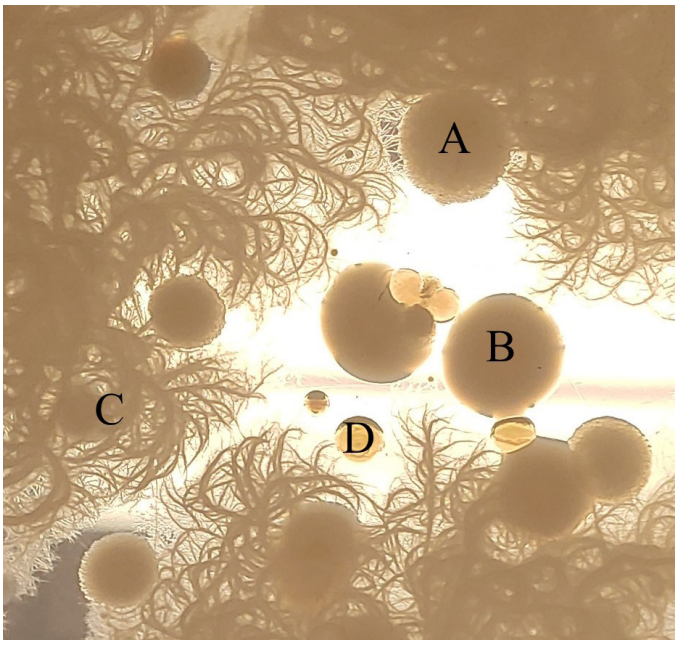

FIGURE 2. Example bacteria colony morphotypes, cultured from a pasture soil sample. (A) Grainy, (B) white, (C) filamentous, and (D) transparent.

sorted each based on phenotype (see FIG 2 for examples). We repeated our observations and colony counts every 24 hr over the course of three days. Filamentous bacteria versus fungal phenotypes were distinguished with the help of a bacteriologist and senior researchers at Humboldt State University.

We focused our statistical analysis on data from our final day of data collection (day three), when distinct bacterial colonies and their morphotypes for all plates were most easily observable. After recording our raw data, we analyzed the effect of distance from forest canopy to open pasture on observed bacterial colony abundance (n = 20) using linear regression. We determined the effect of location either within or outside of the dense forest canopy on colony abundance with a Wilcoxon rank sum test. We analyzed the effects of both distance and location within the forest or pasture on Shannon Diversity (H′) and morphotype richness (R) using a Welch 2-sample t-test and a Wilcoxon rank sum test, respectively. We calculated diversity indices for final observations on day three using an online calculator (Goepel, 2012) and conducted all statistical analyses using RStudio (2015).

RESULTS—Distance from canopy did not affect bacteria colony abundance ($R^2 = 0.032$, $P = 0.14$; FIG 3). However, we did observe that abundance was greater overall in the pasture than in the forest (W = 104.5, $P = 0.01$; FIG 4). We observed a mean of 90.2 ± 7.96 bacteria colonies from pasture samples and 62.35 ± 9.46 bacteria colonies from forest samples. Shannon indices (H′) were dependent on distance from canopy ($t = 7.14$, df = 19, $P < 0.0001$) and were significantly higher in the pasture than in the forest (W = 8, $P < 0.0001$; FIG 5). Distance from canopy affected morphotype richness per sample ($t = 7.02$, df = 19, $P < 0.0001$), but richness did not differ significantly between the pasture and the forest (W = 32.5, $P = 0.18$; FIG 6). We observed a mean of 4.7 ± 0.15 morphotypes in pasture samples and 3.7 ± 0.09 morphotypes in forest samples.

DISCUSSION & CONCLUSIONS—Our data did not support our initial hypothesis that microbial abundance or

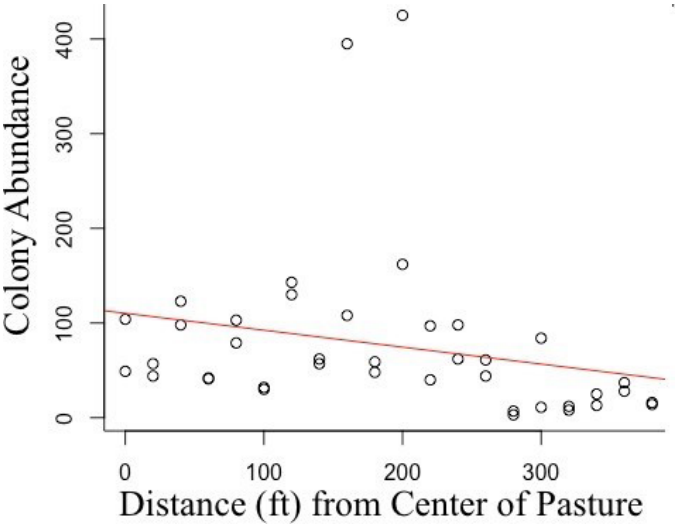

FIGURE 3. Total bacteria colonies per plate as a function of location along the transect, with regression line ($R^2 = 0.032$).

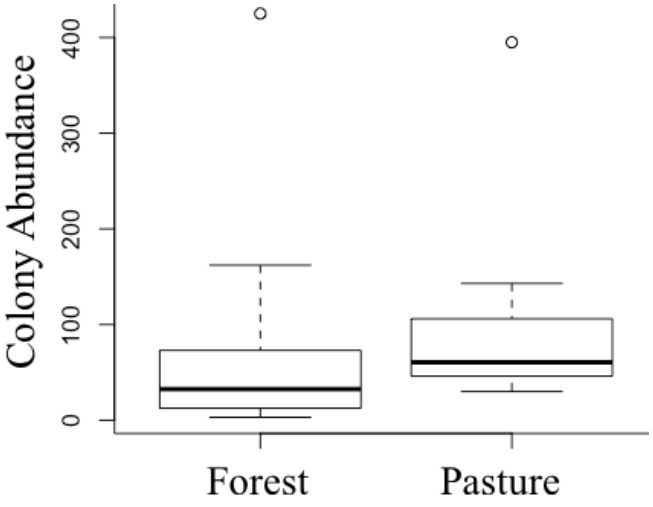

FIGURE 4. Distribution of colony abundance in forest versus pasture. There was a significant difference in colony abundance between forest and pasture samples ($P = 0.01$).

diversity would decrease with distance from forest. The data did, however, suggest an inverse relationship for diversity. Diversity was significantly higher in pasture samples than in forest samples, and both richness and Shannon indices increased along the transect (FIG 5, FIG 6). Colony abundance was not a function of distance from canopy (FIG 3), but abundance was greater overall in pasture soil (FIG 4). The increase in richness across the transect from the forest into the pasture indicates that there is a larger distribution of bacterial morphospecies in the pastoral environment than in the forested environment.

The fact that soil bacterial abundance was not affected by distance from canopy, but differed between the two conditions, might suggest a greater microbial survival

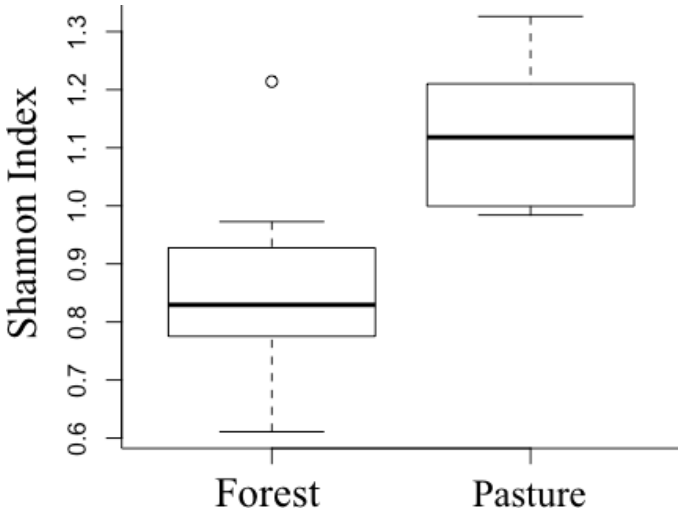

FIGURE 5. Comparison of average Shannon indices in forest and pasture.($P < 0.001$).

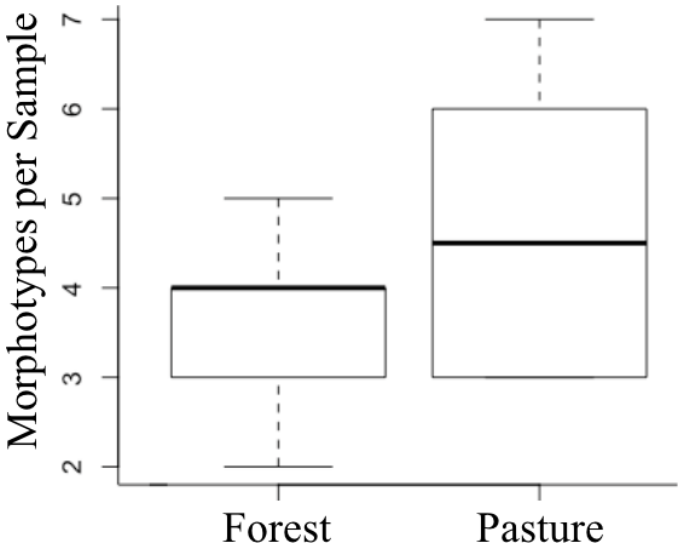

FIGURE 6. Comparison of average morphotype richness between forest and pasture ($P = 0.18$).

relationship with root contact or organic matter than with temperature or shade variations caused by vicinity to canopy. Bacteria often exhibit symbiotic relationships with plants, and while we expected greater microbial abundance and diversity in the forest, weedy root presence in pasture samples could explain why the opposite occurred (Yang, 2009). Grass and shrub roots occur at a much shallower depth than the roots of mature trees, meaning plant contact with surface soils was more consistent in our pasture samples.

It is unclear how, and to what extent, equine traffic contributed to greater bacterial abundance and diversity in the pasture. Bacteria from intentional application of manure in agricultural settings can survive weeks or months, even in unsuitable environments, and manure is known to change the physical properties of soil, including pH and ionic concentrations (Fenlon et al., 2000; Unc et al., 2003). Foot and large animal traffic in the forest is limited by high plant and tree density, even at outer forest boundaries, but traffic along the trail at the transect's midpoint is frequent, and manure deposits likely contributed to bacterial communities in close contact with the trail or pasture. Furthermore, a mild downward slope from the trail to the center of the pasture could redirect rain flow, and thus the flow of equine-related bacteria. This could explain two high-abundance outliers near the trail (FIG 3) in both pasture and forest samples, and could explain why morphotype richness was dependent on distance, but did not necessarily differ significantly between pasture and forest (FIG 6). Ideally, we would either repeat this experiment in a setting not intersected by trails, or we would analyze trafficked soil samples separately in their own category. Regardless, our data suggest that microbial analysis offers a promising measure of animal impact on soil health.

Limited lab availability meant that our forest samples were cultured one day later than our pasture samples, even though they were collected on the same day. Prolonged exposure to cold temperatures might have reduced the viability of bacteria from the forest, skewing diversity results in favor of the pasture. A second limitation of our experiment was that we were not able to gather enough data to quantify fungal diversity. No recognizable fungal colonies grew on our water agar plates, which were dominated by bacteria. In the future we would culture fungal samples in a media more conducive to fungal growth alone. Only one bacteria colony formed on an open plate, out of two closed control plates and two

control plates that remained open during the duration of sample plating, indicating that contamination was not a likely factor in our results.

Lack of bacterial taxonomic knowledge hindered identification of our culture colonies. As such, we focused on observed phenotypes rather than species identification. Certain morphotypes, such as filamentous or deep red bacteria, were easily distinguishable, but others manifested only subtle differences in transparency or texture (FIG 2). Given more time and resources, we would perform DNA extractions and sequencing on each colony morphotype in order to gain a more complete picture of represented species and their ecological significance. A carbon source utilization test would be beneficial in distinguishing the functional diversity of bacteria in forested versus pastoral environments. Given the time constraints of our study, we did not have the opportunity to conduct a survey of the plant species present in the forested and open areas. Future studies would include diversity indices for all present plant species to account for the interaction between plant diversity and bacterial abundance.

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