Covariation of Soil Bacterial Composition with Plant Rarity[∇][†]

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Rare and common plants are predicted to host different rhizospheric microbes. To evaluate this prediction, we used terminal restriction fragment length polymorphism (T-RFLP) analysis to compare rhizospheric bacteria from eight native grass species whose local abundances in their natural ecosystem spanned a 15-fold range. We observed that locally rare versus common plants are associated with divergent bacterial communities.

Plant species abundance is an essential component of ecosystem biodiversity and function, but causes of rarity or commonness are still poorly understood (24). Two approaches have been commonly applied: comparative studies that determine whether rare and common species have distinct suites of traits and experimental studies that test putative factors contributing to rarity, such as competition. For plants, neither approach has yet explained substantial variation among rare and common species (3, 15).

Soil microorganisms may feature as some of the most important factors regulating plant abundance. First, they can strongly affect individual plant fitness, either as pathogens or as mutualists. Second, microbes often form highly specific associations with plants, which could drive differences in fitness among species (7, 8). Finally, soil microbes influence the biogeochemical cycles of primary importance for plant nutrition (23). Despite their potential influence, however, soil microbes have been particularly absent from studies on plant rarity.

The theory of soil community feedback (1a) provides a conceptual framework for linking plant abundances with microbial interactions. Feedback involves two steps. First, a plant changes the composition of the microbial community. Second, this change alters plant performance relative to that of other plant species in the community. Positive feedbacks are expected to decrease species diversity by increasing the dominance of the recipient plant, while negative feedbacks should drive recipient plants toward rarity. To our knowledge, only one study has investigated soil community feedback in the context of plant rarity. Using greenhouse experiments, Klironomos (14) showed that rare native species accumulated more rhizospheric pathogens and thus experienced more negative feedbacks than abundant, nonnative species.

If soil community feedback is important to explaining plant commonness and rarity, then rare and common plants should differ in the compositions of their rhizosphere communities and/or in their responses to the same rhizosphere community. In the present study, we address the first of these predictions, which, despite the availability of molecular tools, has rarely

been tested in a natural community. Specifically, we ask whether rare versus common plant species differ in the compositions of their soil bacterial communities. Even though rarity can be studied in a variety of different ways, it is most commonly defined based on a species' local abundance at a given site and/or geographic range (10, 24). Because plant-soil interactions occur at a local scale (2, 9), we hypothesize that it is this scale at which feedbacks are most likely to have the strongest effects on plant abundance. Thus, we define rarity here as a low local abundance within a community, rather than as global rarity (20, 21).

Using terminal restriction fragment length polymorphism (T-RFLP) analysis, we described the rhizospheric bacteria associated with 10 randomly chosen individuals from eight native perennial C_3 grass species (Table 1 includes a species list) that cooccurred at Indiana University's Lilly-Dickey Woods Preserve (Nashville, IN) (see Fig. S1 in the supplemental material). Each individual was entirely dug up with a shovel. For DNA analyses, we used the soil that remained on the roots that were attached to the plant after the plant was vigorously shaken, here defined as rhizospheric soil. We amplified the bacterial 16S rRNA using PCR primers 27F and 1492R (16) and digested the amplicons with either HaeIII or RsaI. For each plant species, we measured at least 30 nearest-neighbor distances to conspecifics to estimate local relative abundances (22), determined spatial location from Global Positioning System (GPS) coordinates, and analyzed one composite sample of rhizospheric soil from the 10 individuals for chemical properties (Table 1). Local densities among pairs of plant species were compared using analysis of variance (ANOVA) and a post hoc Tukey honestly significant difference (HSD) test. Relative abundances of bacterial operational taxonomic units (OTUs) and soil chemical properties were compared among plant species using nonmetric multidimensional scaling (NMS) analysis (4). Differences between the rare (*Elymus hystrix*, *Poa sylvestris*, and *Sphenopholis nitida*) and common (*Festuca subverticillata*, *Sphenopholis obtusata*, *Elymus villosus*, *Poa pratensis*, and *Poa alsodes*) species groups and the contribution of each OTU to this difference were tested with analysis of similarity (ANOSIM) and similarity percentage analysis (SIMPER) (5), respectively. Mantel tests were used to evaluate relationships among bacterial community compositions, local plant densities, soil chemistries, and spatial locations, with each species considered a replicate. Closely related plants can share traits due to a shared ancestry. To

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TABLE 1. Results of density and rhizospheric chemical parameter measurements for the eight grass species studied[b]

Plant species	Local density (SE)[a]	pH	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Organic matter (%)	Total (%)
Sphenopholis nitida	1.4 (0.3) A	5.2	22	112	666	172	11.8	0.33
Elymus hystrix	1.9 (0.2) A	5.8	17	140	962	168	8.1	0.27
Poa sylvestris	2.8 (0.3) AB	5.2	20	141	858	171	7.2	0.26
Sphenopholis obtusata	11.6 (3.9) ABC	6.4	23	126	1,197	192	6.3	0.23
Festuca subverticillata	12.1 (1.7) BCD	5.4	16	152	888	173	5.6	0.21
Elymus villosus	17.4 (1.4) CD	7.0	11	155	1,346	199	5.1	0.22
Poa pratensis	17.6 (1.4) CD	7.3	9	188	1,537	234	5.6	0.25
Poa alsodes	23.6 (1.4) D	6.3	17	122	1,141	150	7.0	0.24

[a] The unit is the estimated number of individuals per m^2. Different letters are placed next to values that were significantly different from the results of a *post hoc* Tukey HSD test following ANOVA.

[b] All soil chemical parameters were measured by the Soil and Plant Nutrient Laboratory (Michigan State University) according to the protocols shown in reference 1.

address this issue of phylogenetic nonindependence, we calculated phylogenetically independent contrasts (PICs) (11) to determine if the observed correlation between bacterial community composition and plant rarity was independent of the evolutionary history of the grass species.

Statistical analysis supported the central hypothesis that rare and common plant species associate with significantly divergent rhizospheric bacterial communities (ANOSIM for rare versus common, global $R = 0.32$, $P = 0.0001$) (correlation results are presented in Fig. 1; plant abundances and post hoc Tukey HSD test results are shown in Table 1). The NMS analysis was conducted on 249 different bacterial OTUs identified from 79 plant samples (see Table S1 in the supplemental material). The final solution had three dimensions, and stress was 12.2, which was significantly lower than for randomized data (Monte Carlo test, $P < 0.02$, 50 runs). Collectively, axes explained 89% of the variation in the data, with axes 1 and 2 explaining 29% and 28%, respectively. According to analysis of PICs, the correlation between rarity and bacterial composition was robust to the evolutionary history of the grasses (Fig. 1B).

Our results agree with those of Moora et al. (17) and Huguet et al. (12), who found different arbuscular mycorrhizal fungi (AMF) and *Frankia* strains, respectively, in the roots of single pairs of rare and common congeners. Our work extends their conclusions because we replicated plant rarity and commonness by including more than two plant species, and we examined the whole rhizospheric bacterial community instead of exclusively plant-associated microbial mutualists.

The two OTUs most divergent between rare and common taxa were 432 and 118 bp long (see Table S1 in the supplemental material) and 340% and 170% more abundant in association with rare versus common species, respectively (SIMPER). Because of well-known limitations of T-RFLP for obtaining taxonomic identifications (6, 13), we could not determine if the OTUs represented potentially pathogenic or mutualistic bacteria. Despite recent advances in molecular tools, linking microbial composition, or the presence of a particular species, to ecosystem processes is still very difficult (18, 19). Confirmation of Klironomos's hypothesis (14) that rare species accumulate pathogens will therefore require further research. Unexpectedly, our data showed that bacterial communities of rare plant species were more similar to the bacterial communities of other rare species than to those of their closest common plant relatives (Fig. 1A), which suggests that locally common plants may be more likely to share a pool of beneficial bacteria than to support species-specific mutualists, and similar conclusions can be drawn for rare species with more gen-

eralized pathogenic bacteria. This result is contrary to Klironomos's prediction (14) that pathogens on rare taxa are host specific.

In addition to plant relative abundances, soil chemical properties (Table 1) were also correlated with variation in the
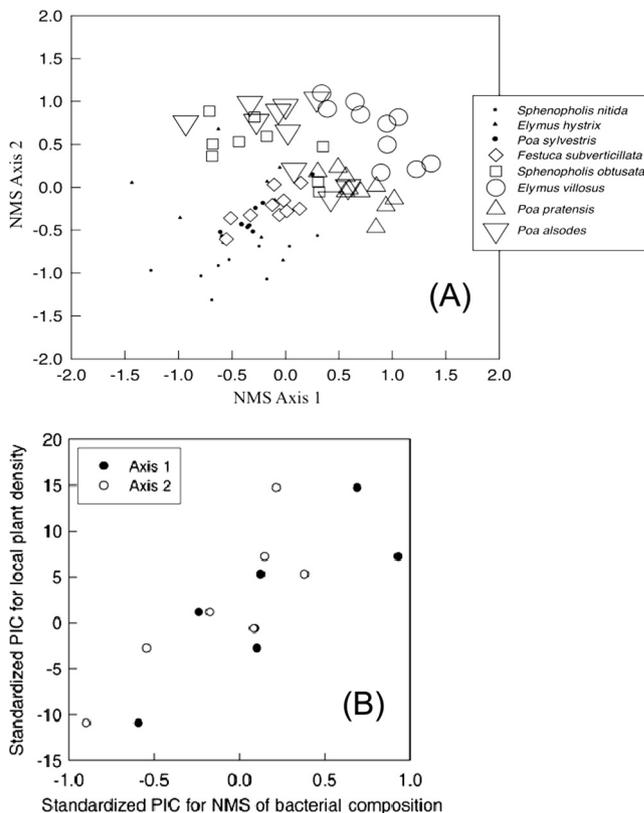

FIG. 1. (A) Nonmetric multidimensional scaling (NMS) analysis showing ordination of the rhizosphere bacterial communities (terminal restriction fragment [T-RF] abundance) for eight grass species cooccurring at Lilly-Dickey Woods Preserve, Nashville, IN (model $r^2 = 0.89$, stress = 12.2, $P < 0.02$). Each symbol represents a plant species, and each point is a field sample of the rhizosphere community from an individual plant. Local abundances of plant species are overlaid on the plot as symbol sizes: the larger the symbol, the more locally abundant was the plant species. (B) Phylogenetically independent contrasts (PICs) for local plant abundance (number of plants/m^2) plotted against the average PICs for NMS axis 1 or 2. Regression was constrained through the origin. NMS axis 1, $r = 0.82$, $P = 0.008$; axis 2, $r = 0.66$, $P = 0.043$; 7 contrasts representing 8 grass species.

compositions of bacterial OTUs (Mantel $r = 0.751$, $P = 0.0002$) (see Table S2 in the supplemental material). The relative importance of plant species and soil chemistry for microbial composition and activity is not possible to determine from observational studies. However, the lack of spatial patterning for both bacterial composition (Mantel $r = 0.011$, $P = 0.47$) and soil properties (Mantel $r = -0.125$, $P = 0.39$) (see Fig. S2 in the supplemental material) suggests that chemical differences in rhizospheres may be a second outcome of variation in the soil bacterial community rather than an underlying cause of variation in bacterial composition.

While we focus on bacterial diversity, it will be essential to extend this work to mycorrhizal and other soil fungi. Functional studies of the corresponding microbial communities are also greatly needed. Identifying the microbes associated with plant rarity will not only yield more mechanistic insights into the nature of soil feedbacks but also establish a missing link between greenhouse experiments and natural patterns of microbe distributions.

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