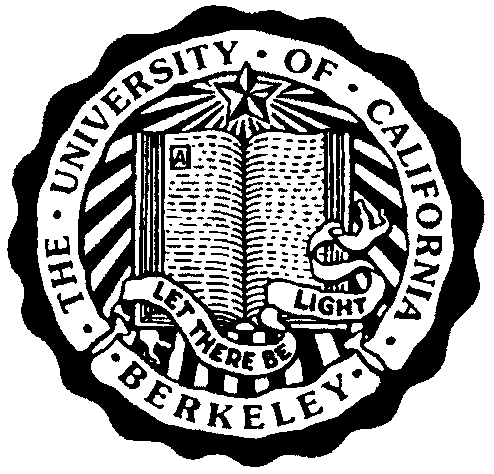
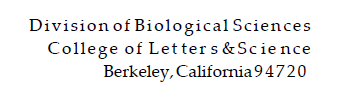
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May 6, 2019

Professor Spencer Barrett, Editor-in-Chief

Members of the Editorial Board

*Proceedings of the Royal Society B: Biological Sciences*

The Royal Society

Dear colleagues,

Enclosed please find our manuscript entitled “*Insect herbivory reshapes a native leaf microbiome”* for publication consideration as a Research article for *Proceedings B*. This work was completed as part of Parris Humphrey’s Ph.D. dissertation at the University of Arizona.

While microbiome research is now integrated with nearly every field in biology, from medicine to crop protection and ecology to evolutionary biology, there remains a limited understanding of the population and community dynamics of microbiomes in natural populations of multicellular hosts. In particular, although microbiomes are being manipulated for ‘growth-promotion’ in crop plant systems, the basic biology, particularly the role that interactions between plants and herbivores play in shaping outcomes for microbes, is poorly known from natural systems.

The analysis we conduct is highly integrative, using both experimental and observational approaches, and addresses fundamental questions in disease ecology and microbiome biology. Our results are novel and impactful in that they reveal a previously under-appreciated driver of variation in microbiome abundance and diversity arising from interactions between plants and herbivores. Insect herbivory is a pervasive feature of plant communities, and the ensuing plant phenotypes arising after attack are relatively conserved across a broad range of plant species. Therefore, the potential for herbivores to impact the assembly and abundance of plant-associated microbiomes is tremendous. Given the broad implications of our results, the novelty of our analytic framework, and the integrative experimental approaches used in our study, we believe our study is well-suited to the broad readership of *Proceedings B.*

We conducted our study in the relatively pristine setting of the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA at 3,000+ meters in elevation, and on a native herbaceous plant, *Cardmine cordifolia*, a mustard that is closely related to *Arabidopsis thaliana*. *C. cordifolia* is attacked by the specialist leaf-mining fly *Scaptomyza nigrita*, which conventiely leaves traces of damage (mines) in leaves, making it especially amenable for quanitfying herbivory rates. Moreover, these flies consume ≥25% of annual leaf area in these populations, and reduce fitness concomitantly in bittercress. Our approach was to use both an observational approach and an experimental approach in the field, followed by manipulations of plant hormones in the greenhouse to test mechanistic hypotheses on the relationship between herbivory and bacterial abundance and diversity.

Our manuscript reports six main findings:

1. Host-derived 16S read counts can be used to measure absolute bacterial abundances in leaf tissues. We tested the hypotheses that 16S reads from leaves collected in the field is a proxy for abundance of the bacteria actually present. We did this by splitting leaf samples from over 100 plants into two experiments: one half was used for culturing bacteria an the other half for 16S amplicon profiling. This ground-truthed our approach of using 16S profiling for quantifying absolute abundance of leaf bacteria.

2. Bacterial loads are amplified in insect-damaged leaf tissues. Overall, herbivore-damaged leaves had between 8–32 times the number of bacteria than non-damaged leaves, corresponding to between 3–5 additional cell divisions. This suggests that herbivores can have a major impact (directly and/or indirectly) on leaf bacteria population biology in the field and influence disease outcomes, even when they are not necessarily vectors. This is likely mediated by direct damage and (as we show later) plant phenotypes arising from defense hormones signalling induced by herbivory.

3. Herbivore-driven bacterial amplification is both community-wide and taxon-specific. Bacterial species in the Pseudomonaceae were most abundant and diverse and account for much of the difference between plants attacked herbivores and those unattacked. However, *P. syringae* actually increased the most, a common plant pathogen that causes bacterial speck disease and is widely used in plant–herbivore–microbiome studies in the laboratory. This suggests a particularly salient relationship between herbivory and a major plant pathogen, even in relatively pristine systems and indicates that this may be a general phenomenon and concern for ecologists and growers studying plant interactions with bacteria.

4. Herbivory produces compositional shifts in leaf bacterial communities, principally that Pseudomonaceae come to predominate.  We found higher overall absolute abundances and the larger relative increase of Pseudomonadaceae species in damaged leaves, which drove down relative abundance of other taxa even while they also achieved higher densities in herbivore-damaged leaves. Thus, herbivory influences bacterial community composition and canonical measures of ecological diversity commonly used in 16S-based microbiome studies. Importantly, the direction and magnitude of these changes are difficult to interpret without quantification of absolute bacterial load, as we have done.

5. Anti-herbivore plant defenses enhance growth of phytopathogens *in planta*. In the greenhouse, we simulated herbivory on *C. cordifolia* by using jasmonic acid pre-treatments and found that this altered within-host growth of five of the twelve *Pseudomonas* strains we tested from the field, with the most pronounced changes resulting in 2.5–5 additional doublings of two phylogenetically distinct isolates of the putative phytopathogen, *P. syringae*.

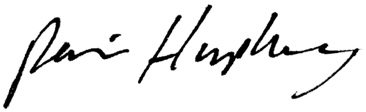
6. Plant fitness negatively correlates with herbivore–bacteria co-infection in natural plant populations. By surveying over 1900 *C. cordifolia* stems, we show that the degree of herbivory—and thus, bacterial co-infection primarily with *P. syringae*—is highly aggregated across plant patches and is associated with lower plant fitness. This suggests that insect herbivores may impact both the phylogenetic and spatial structure of native plant microbiomes, and that both colonizers strongly associate with standing variation in plant reproductive successs in the wild.

Thank you again for taking the time to consider our manuscript for publication. We look forward to hearing from you in due course.

Sincerely,



Noah K. Whiteman, Ph.D.



Parris Humphrey, Ph.D.