

**Gut microbiome insights from 16S rRNA analysis of 17-year periodical cicadas
(*Hemiptera: Magicicada spp.*) Broods II, VI, and X**

Kyle D. Brumfield^{1,2}, Michael J. Raupp³, Diler Haji⁴, Chris Simon⁴, Joerg Graf⁵, John R. Cooley⁶,
Susan T. Janton⁵, Russell C. Meister⁴, Anwar Huq¹, Rita R. Colwell^{1,2,*}, Nur A. Hasan^{7,*}

¹ Maryland Pathogen Research Institute, University of Maryland, College Park, MD, 20742, USA

² University of Maryland Institute for Advanced Computer Studies, University of Maryland, 20742,
College Park, MD, USA

³ Department of Entomology, University of Maryland, College Park, MD, 20742, USA

⁴ Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, 06269,
USA

⁵ Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, 06269, USA

⁶ Department of Ecology and Evolutionary Biology, University of Connecticut, Hartford, CT, 06103,
USA

⁷ EZbiome Inc., Gaithersburg, MD, 20878, USA

*Correspondence: RRC, rcolwell@umd.edu; NAH, hasan@ezbiome.com

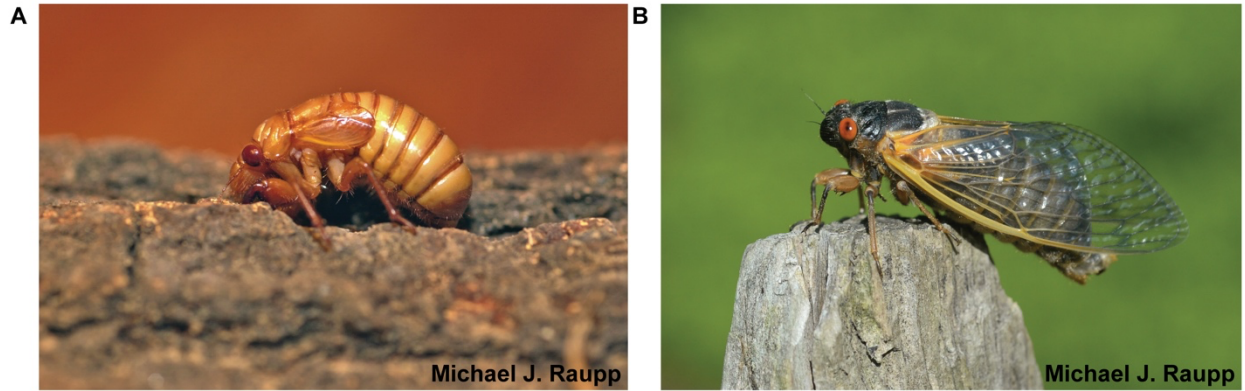


Figure S1: Photographs of A) nymph and B) adult 17-year periodical cicadas (*Magicicada* spp. Brood X).

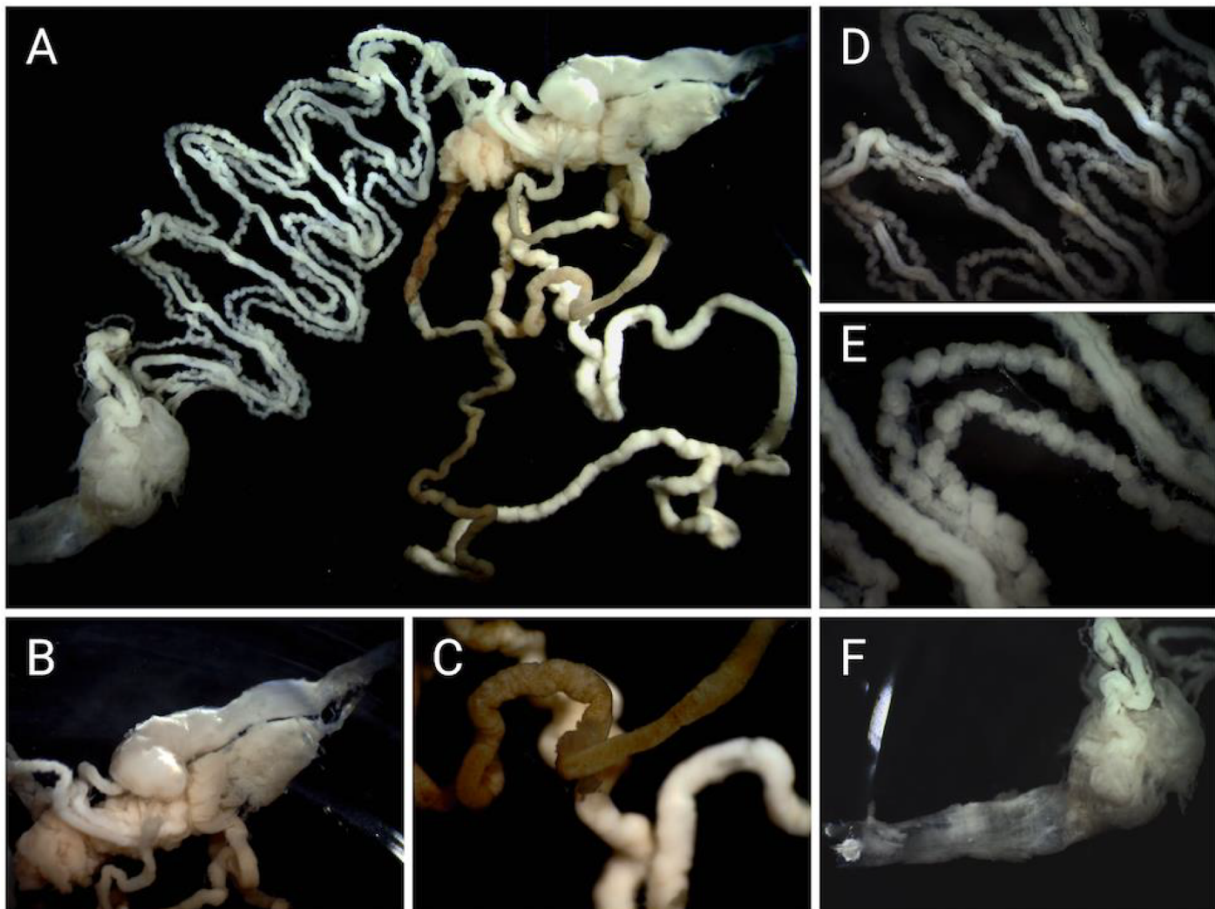


Figure S2: Image of gut tissue dissected from nymphal *Magicicada septendecium*. Shown are A) Complete gut, B) Filter chamber, C) Midgut, D-E) Malpighian tubules and hindgut, and F) Rectum. Adult gut anatomy and other *Magicicada* spp., appears identical to that of the *M. septendecium* nymph.

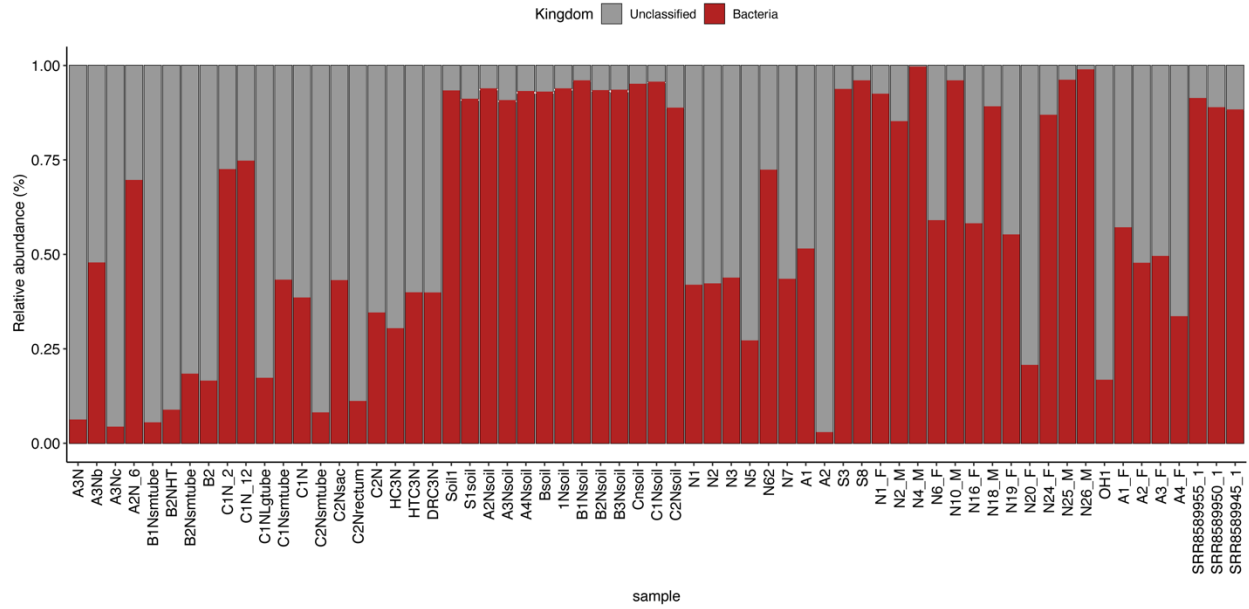


Figure S3: Stacked bar plot showing relative abundance of reads classified as bacteria relative to the total number of reads generated in each sample.

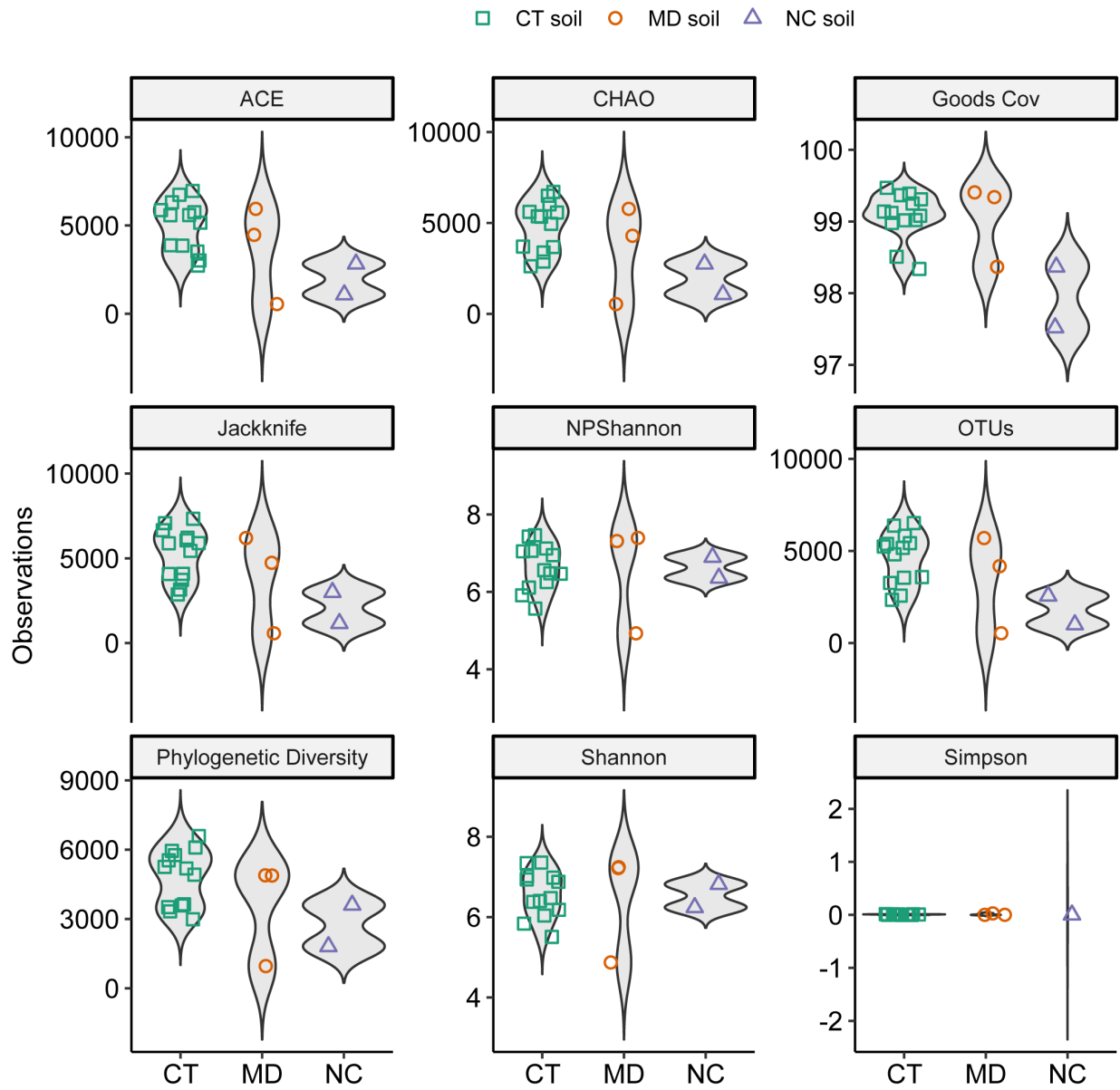


Figure S4: Violin plots showing alpha diversity comparison between MTP sets of soil samples.

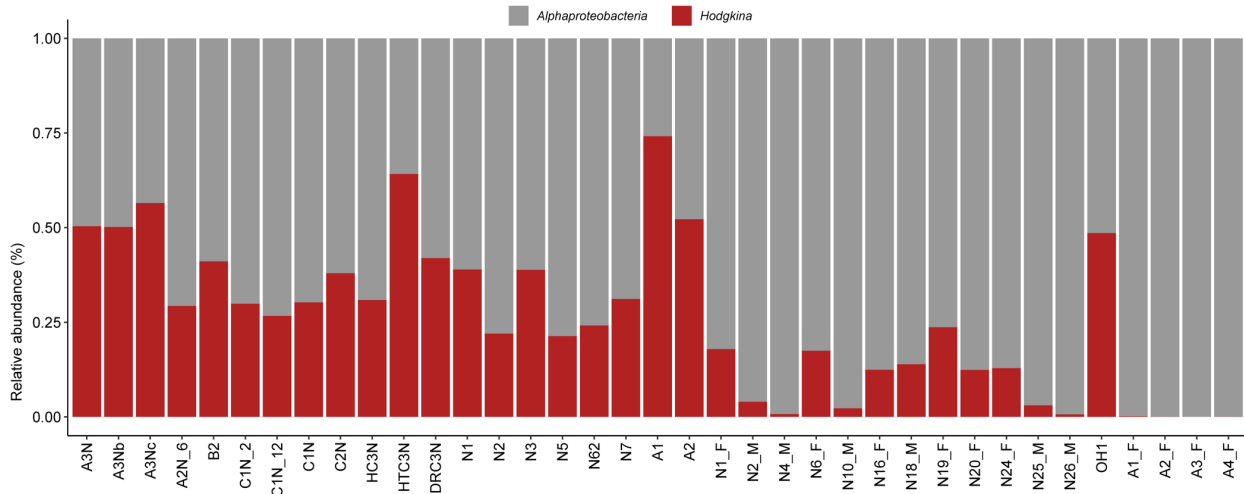


Figure S5: Stacked bar plot showing relative abundance of reads classified as *Hodgkinia* relative to the total number of reads profiled as *Alphaproteobacteria* in each sample.

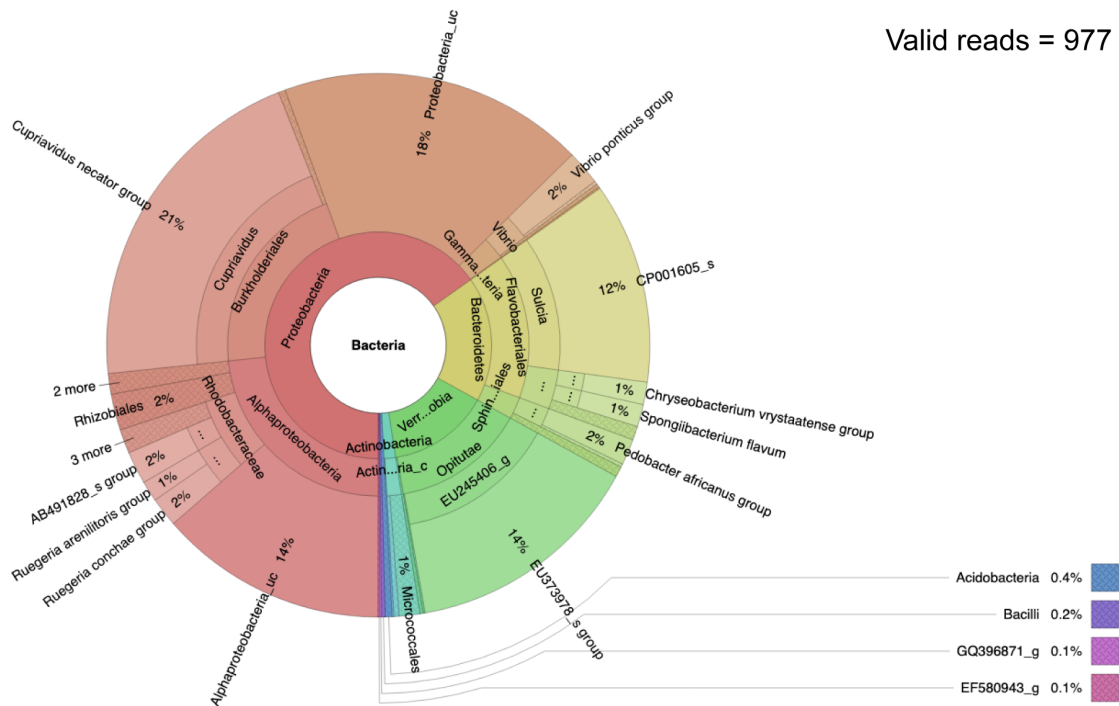
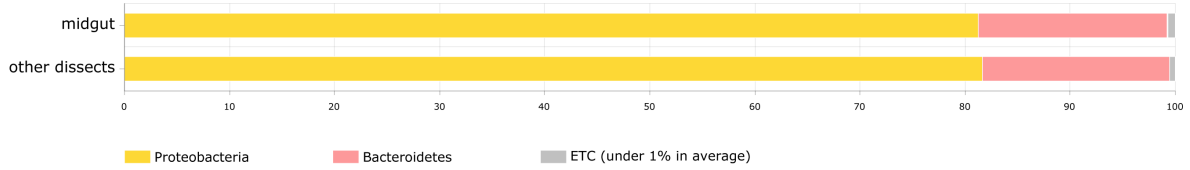
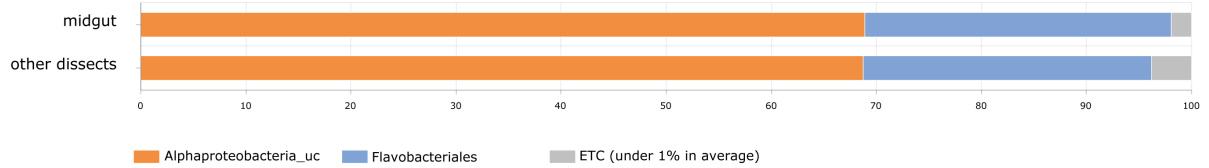


Figure S6: Krona plot showing relative abundance of bacteria profiled in the wash fluid of the OH Brood X nymph.

Phylum



Order



PERMANOVA results

All sets		Pair-wise			
p-value	Sets		pseudo-F	p-value	q-value
N.S. (p=0.774)	2	midgut	0.067	0.774	0.774
MTPs	No. of permutations	other dissecta			
8	999				

Inter-set distances



LEfSe analysis

Taxon name	Taxon rank	Taxonomy	LDA effect size	p-value	p-value (FDR)	Max group	midgut	other dissecta
Tenericutes	Phylum	Bacteria	2.20018	0.04722	0.42845	hindgut	0.02907	0.00000
Ralstonia pickettii group	Species	Bacteria: Proteobacteria: Betaproteobacteria: Burkholderiales: Ralstonia_f. Ralstonia	2.19196	0.04722	0.42845	other dissecta	0.00000	0.03489

Figure S7: Comparison of bacterial populations residing in different gut organs of CT Brood II nymphs.