**Methods**

*Sequence data processing*

*Statistical analyses of sequence data*

**Results**

*Summary of sequence data pre-processing*

Following pre-processing, X% of total raw reads were retained, for a mean sample read depth of 8,020. Dada2 ASV assignments produced 1,337 ASVs. Within sample proportional abundances were calculated as sum of reads assigned to each taxa divided by the total reads for that sample. Upon rarefaction to 1,100 reads, the dataset consisted of 851 ASVs. Rarefaction at 1,100 reads sufficiently captured richness as demonstrated in by plots of rarefaction curves (figure S1). For subsequent analyses, we report independent 69 samples in the rarefied dataset and 72 for the within sample proportional abundances dataset.

*Alpha (α) diversity*

ANOVA showed sampling location to be a significant predictor of species richness, Shannon diversity (H`), and Chao1 (p < 0.05, F < 2.702) (Figure S2). The interaction of sampling timepoint x sampling location was a significant of species richness and Chao1 (p < 0.05, F < 2.871), while sampling location x treatment was significant for Shannon diversity (H`) (p < 0.05, F < 2.718). No clear trends in directionality were observed for alpha diversity metrics across sampling locations, treatments, or sampling timepoints.

*Beta (β) diversity*

To account for variation within each sampling location, strata = “Sampling Location” was used to restrict permutations within each site. we show sampling location to be the dominate predictor of β-diversity for both weighted and unweighted unifrac distances (p < 0.001, R2 = 0.31, F5,36 = 8.149, p < 0.001, R2 = 0.15, F5,36 = 2.768, respectively) (Figure 2, Table S1). Additionally, sampling time was significant for both weighted and unweighted distances (p < 0.05, R2 = 0.03, F1,36 = 4.256, p < 0.001, R2 = 0.03, F1,36 = 2.950, respectively). Miticide treatment was found to be significant for unweighted unifrac distance (p < 0.05, R2 = 0.029, F2,36 = 1.269), but not for weighted unifrac (p = 0.34). We report no statistically significant pairwise differences among miticide treatments when both time points were considered together.

To better understand the effect of miticide treatment on the bee bread microbiome, we subset our dataset to include only those samples collected after application (e.g., sampling time two). Again, “strata = Sampling Location” was used. Besides sampling location, we only report a marginally significant interaction of sampling location x miticide treatment for unweighted unifrac (p = 0.056, R2 = 0.28, F10,18 = 1.139).

*Core bee bread microbiome*

Core membership assigned as XX and XX revealed XX

Figures:

Figure 1. Descriptive map of sampling locations

Figure 2. 4 panel, 3 ordinations and a plot of beta disper

Figure 3. Prevalence and abundance histogram

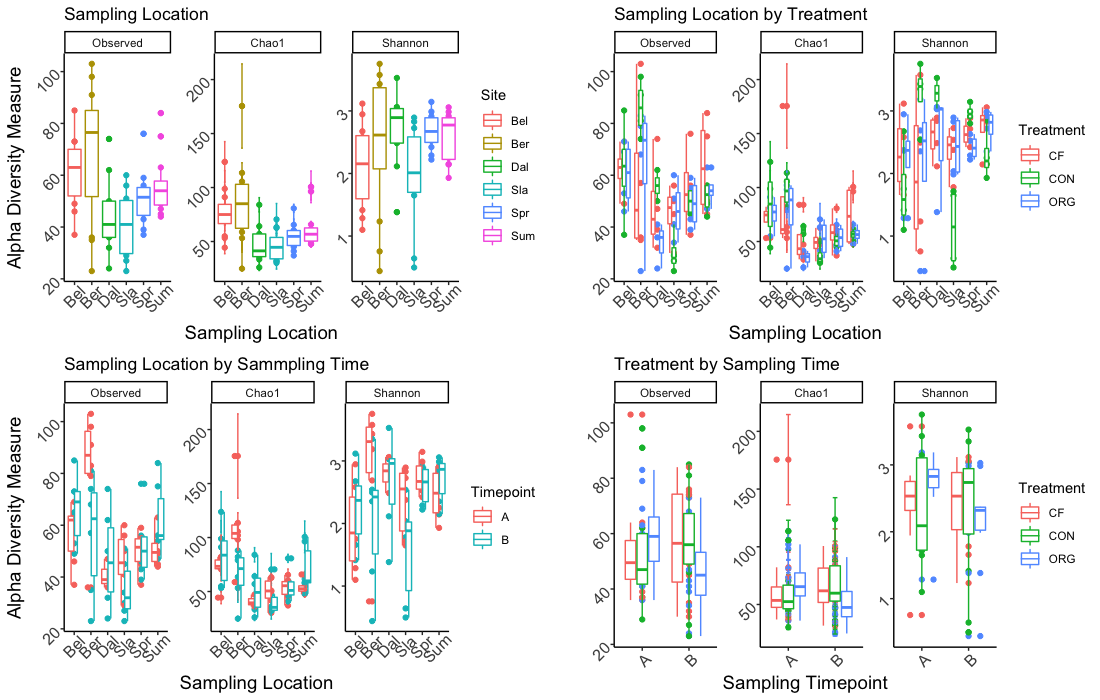
Supplementary Figures:

SFigure1. Rarefaction curves depicting leveling off at approximately 1,000 reads.

A picture containing diagram

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SFigure2. Boxplots of alpha diversity metrics.

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