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Persistence of diet effects on the *Drosophila suzukii* microbiota

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Abstract

The insect commensal microbiota consists of prokaryotes and eukaryotes. The effect of diet and the persistence of the gut microbiota in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) are not well-understood. We transferred subsets of a single population of *D. suzukii* to different fruit-based diets (blueberry, raspberry, and strawberry) for three generations and then returned them to a common, banana-based, laboratory diet. We used 16S (bacteria) and ITS (fungi) sequencing of female endosymbiont-free flies to identify the microbiota. We identified 2700 bacterial and 350 fungal OTUs; there was no correlation between the number of bacterial and fungal OTUs in a sample. Bacterial communities were dominated by Proteobacteria (especially Acetobacteraceae); Ascomycota dominated the fungal communities. Species diversity of both bacteria and fungi differed among diets, but there were no differences in species-level diversity when these flies were returned to a control diet. A Principle Coordinates Analysis revealed no differences in the bacterial or fungal community in the first generation on fruit diets, but that the communities diverged over the next two generations; neither fungal and bacterial communities converged after one generation on control food. We conclude that diet changes the *D. suzukii* microbiota, and that these changes persist for more than one generation.

Introduction

Insects live in close association with microbes, both in their substrate and food, and on their surface and in their gut. Most of these microbes are not pathogenic and can even be beneficial to the host (Douglas 2015; 2018b; McFall-Ngai et al. 2013). The insect microbiota includes bacteria, fungi, protozoa, and viruses, and encompasses endosymbionts (for example, *Wolbachia* Hertig & Wolbach and *Buchnera* Munson et al.), gut microbes and both surface-and epithelia-associated flora (Bahrndorff et al. 2016; Douglas 2015; Henry et al. 2015; Huang et al. 2015). These microbes can be acquired from the parent(s) (e.g. Hosokawa et al. 2010; Koga et al. 2012; Rahman et al. 2015) or from the diet (Starmer and Fogleman 1986), and be stable (e.g. Pais et al. 2018; Rahman et al. 2015), seasonally variable (e.g. Ferguson et al. 2018), or transient (Wong et al. 2015). *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) has emerged as an important model for understanding the insect-associated microbiota, because of its relatively simple microbiota, a plethora of genetic tools, and because it is possible to rear axenic (with no microbiota) and gnotobiotic (with a known microbiota) individuals (Douglas 2018a).

The *Drosophila* microbiome includes surface, intracellular (mainly *Wolbachia*; Wilches, Coghlin, and Floate, submitted to this issue) and gut microbes (Ren et al. 2007), but is mainly dominated by the latter. Whole-fly microbiome extractions can be used to characterize the gut microbiome (Ren et al. 2007), but may overestimate species diversity (Chandler et al. 2014; Chandler et al. 2011) or be dominated by *Wolbachia* that can mask the presence of less common bacteria (Wilches, Coghlin, and Floate, submitted). The gut microbiome originates from the diet; i.e., larvae consume the chorion (and associated bacteria) and the gut bacterial biota is

maintained by consuming bacteria (Bakula 1969). The *Drosophila* gut microbiome is transient, with significant flux over time (Blum et al. 2013; Wong et al. 2011; but see Pais et al. 2018), but generally consists of Acetobacteraceae, Enterobacteriaceae, and Lactobacillales (Chandler et al. 2011; Wong et al. 2011). There is also a yeast flora, mainly in the genera *Hanseniaspora* Berkh, *Pichia* Hansen, and *Saccharomyces* Meien (Chandler et al. 2012; Phaff et al. 1956), as well as poorly-explored fungal endosymbionts and gut Protista (Ebbert et al. 2003). Within this diverse gut flora, a few species generally dominate, and many phenotypes can be recovered in gnotobiotic flies with a single yeast species (Jiménez Padilla 2016) or a small subset of bacteria (Shin et al. 2011; Storelli et al. 2011). In other animals, including humans, transitory changes in diet can have long-lasting effects on the gut microbiome (Wu et al. 2011). In *Drosophila*, there are clear phenotypic effects of diet switches (Jehrke et al. 2018), and wild flies have different microbiota to lab-reared flies (Chandler et al. 2011), however, it is not clear how immediate the diet effects on the microbiome are. It is also unclear whether these effects arise because the diet houses a specific microbiome, or – given the broad phenotypic effects of food type; Jiménez-Padilla, Ferguson & Sinclair, submitted to this issue) – because the diet modifies the fly's interactions with the microbiome, affecting community assembly (Adair and Douglas 2017; Jehrke et al. 2018).

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an economically-important pest of soft-skinned fruits (Asplen et al. 2015). Female *D. suzukii* lay their eggs in ripening fruit; the growing larvae and secondary microbial infections render the fruit unmarketable (Rota-Stabelli et al. 2013). *Drosophila suzukii* is polyphagous, and thus flies within a region can have very different diets during development. Diet affects various

physiological traits in *D. suzukii*, such as development time and body size (Jiménez-Padilla, Ferguson & Sinclair, submitted; but see Jaramillo et al. 2015) and lifespan (Bing et al. 2018), and the microbiota appears necessary for development in the protein-poor diet of unripe fruit (Bing et al. 2018). The bacterial microbiota of *D. suzukii* is typically dominated by a few species, and those species appear to vary considerably depending on the collection location, diet, and season (Chandler et al. 2014; Fountain et al. 2018; Solomon et al. 2019). Yeasts are a consistent component of the *D. suzukii* microbiota (Fountain et al. 2018; Hamby et al. 2012; Solomon et al. 2019) and affect larval development (Lewis and Hamby 2019), but the biology of yeast-*Drosophila* interactions have generally been less-well explored than the biology of the bacterial flora.

The *D. suzukii* microbiota reflects the immediate environment of the individual, its physiological state, and also the individual's history of exposure to microbes. However, the extent to which the microbiota is dependent on diet, and the persistence of those effects across generations is unclear. In this study, we examined the effect of diet on the microbiome of *D. suzukii* across four generations. Specifically, we monitored changes in the gut microbiome of *D. suzukii* transitioned from a banana-based control diet (see also Jiménez-Padilla, Ferguson & Sinclair, submitted) onto fruit-based diets of either raspberry, strawberry, or blueberry for three generations. Eggs obtained from females of the third generation were then returned back onto the control diet to obtain a fourth generation of adults. For each generation, we characterised the effect of these dietary changes on the microbiota composition of these flies via high-throughput 16S rRNA (bacteria) and ITS (internal transcribed spacer; fungi) DNA sequencing.

Methods

Fly rearing and sample collections

We used an outbred *Drosophila suzukii* population collected in Halton Hills region, Ontario, Canada (43º00´N, 81º15´W) in 2012 (Jakobs et al. 2015). We reared the flies on a generic banana-based (control) diet (Markow and O'Grady 2005), at 25 ± 1 °C, 60 ± 5 % relative humidity, and 14 h:10 h L:D cycle for many generations. To begin the diet experiments, we transferred adult *D. suzukii* (7-10 days old) to egg collection cages (⌀ = 5.6 cm, h = 7.6 cm) capped with a Petri dish (⌀ = 6 cm) containing c. 40 mL of one of the fruit-based diets or the control diet. The fruit-based diets contained 100 mL deionized water, 25 g agar, 4 mL propionic acid, and 500 g mashed organic frozen fruit; either blueberry, raspberry or strawberry. The flies laid eggs for three days on the fruit diet, and the Petri dishes were then incubated at standard conditions until eclosion (Generation 1); this was repeated with fresh media for two additional generations (Generations 2 and 3). After three generations, we transferred eggs back onto the control diet (Generation 4) to assess the longevity of microbiomes developed on the previous fruit diets. For each combination of diet and generation, we collected adult females 7-10 days post-eclosion and rinsed them for one minute with 70 % ethanol followed by sterilized deionized water. The flies were then preserved in 95 % ethanol for DNA extraction.

For each combination of diet and generation, we extracted DNA from individual female flies (1 fly = 1 sample). Flies were surface-washed with a 0.6 % hypochlorite solution bleach solution, and DNA extracted using a Qiagen DNeasy blood and tissue kit (Qiagen, Toronto, ON, Canada) following the manufacturer's instructions, with the exception of eluting in ca. 30 µL at pH

7. Because the prevalence of *Wolbachia* can potentially mask bacterial diversity (Wilches, Coghlin, and Floate, submitted), we screened the samples for the presence of *Wolbachia* using primers for the *wsp* gene as previously described (Li, Fields, Pang, Coghlin, & Floate, 2015). Five *Wolbachia*-free samples for each combination of diet and generation (n = 80 total samples, 5 flies/diet/generation) were then shipped to Genome Quebec for sequencing 16S and ITS regions for bacterial and fungal identification, respectively.

16S and ITS rDNA sequencing and data analysis

Sequencing was performed by Genome Quebec using Illumina MiSeq 300 bp paired-end sequencing and the initial sequence alignment and cleaning conducted by the Canadian Centre for Computational Genomics (Montreal, QC, Canada, www.computationalgenomics.ca), who provided operational taxonomic unit tables (OTUs) and taxonomy tables for the bacterial (16S) and fungal (ITS) communities. We used these lists to compare the microbiota among generations and treatments using the Marker Data Profiling tool from the MicrobiomeAnalyst online interface (Chong et al. 2020; Dhariwal et al. 2017). Parametric statistics (ANOVA) were conducted using GraphPad Prism (v8.3.0 for Windows, GraphPad Software, San Diego, CA, USA).

Prior to analysis, we identified OTUs that appeared in only one sample as artifacts and removed them from further consideration. We also removed four 16S and five ITS samples with fewer than 1400 reads (which could lead to inaccurate diversity measurements; Hill et al. 2002). We

also excluded, as likely contaminated, four samples from the 16S analysis and two samples from the ITS analysis that had very high species richness (two or more orders of magnitude higher than the median from 16S sequence or >50 OTUs from ITS; Figure S1). We retained 72 samples in the bacterial microbiome composition analysis and 73 in the ITS analysis (n = 3 to 5 flies per diet per generation).

We transformed count data using relative log expression (RLE) for relative abundance analyses and rarefied to the minimum library count (1,006 reads for 16S, 2,389 reads for ITS) to allow for direct comparison among groups. We used a Pearson's product-moment correlation to look for a relationship between ($\log_{10}$-transformed) number of fungal and bacterial OTUs in individual samples across the dataset. We calculated observed richness, Chao1 richness, and Shannon diversity for each sample using the alpha-diversity profiling tools in MicrobiomeAnalyst, and compared these values among diets at Generations 1 and 4 using a one-way ANOVA, and among diets across Generations 1-3 for the different diets using a 2-way ANOVA. We examined community composition through a Principal Coordinate Analysis (PCOA) at the OTU level with the Bray-Curtis Index using the beta-diversity profiling tool in MicrobiomeAnalyst. We used a Multivariate Analysis of Variance (MANOVA) in R to explore the effects of diet and generation on diversity (as explained in the multivariate space of the first two PCoA axes), using the comparisons described above for α-diversity.

Results

We characterized the bacterial and fungal microbiota of *D. suzukii* reared on various diets. The flies originated from the one population maintained under laboratory conditions since 2012 and the same parents were used to generate the different diet groups in this study; i.e., all groups started with broadly the same microbial community. Illumina sequencing generated an average of 63,400 16S rDNA and 51,137 ITS reads per sample. Removing samples containing extremely low reads and uniquely high species richness gave an adjusted average of 65,296 bacterial and 55,055 fungal reads per sample (Table 1). The number of operational taxonomic units was not affected by this adjustment in the data with a total of 2,700 bacterial and 350 fungal OTUs at 97 % identity cutoff. After removing singletons, 674 bacterial and 258 fungal OTUs (with ≥ 2 counts) remained in the analyses (Table 1). There was no correlation between 16S (bacterial) and ITS (fungal) OTU diversity in the samples ($r_{66} = 0.16$, $P = 0.2$; Figure 1).

Dominant bacterial and fungal taxa

The dominant bacterial taxa are listed in Table 2. We found that even though the bacterial relative abundance varies among generations of flies reared on different diets, the most abundant taxa in most samples belong to the phyla Proteobacteria and Firmicutes. In particular, Proteobacteria comprised more than 90 % of all taxa for some generations across all diets (Figure 2). The most abundant proteobacterial family was Acetobacteraceae, comprising more than half of the family-level abundance in all but four treatments (Figure 2; full list of Taxa in Figures S3 and S4).

The dominant fungal taxa are listed in Table 3. The fungal microbiota was dominated by the Phylum Ascomycota in all but two samples (Figure 3; full list of taxa in Figure S5). The most abundant families in this Phylum were Aspergillaceae, Mycosphaerellaceae, Saccharomycetaceae, Pleosporaceae and Chaetomiaceae (Figure 3). The phylum Basidiomycota was also present in all generations and diets, and dominated in the two conditions where Ascomycota did not (Figure 3). The Ascomycete families Malasseziaceae and Bulleribasidiaceae were present in all diets and most generations (Figure 3).

Diversity of bacterial and fungal communities of D. suzukii reared on various diets

The bacterial species richness and overall diversity were highly variable among flies reared on different diets and even between generations of flies reared on the same diet. Although the eggs to form the first generation of flies for this study were collected from the same *D. suzukii* population, the observed and Chao1 richness were significantly different upon transfer to new diets on the first generation (Table 4, Figure 4), suggesting a dietary effect on bacterial composition; however the Shannon diversity index did not differ significantly among diets (Table 4, Figure 4). By contrast, the α-diversity parameters of the fungal microbiome did not vary in Generation 1 (Table 4, Figure 4). Over three generations in the new diets, bacterial diversity changed significantly (significant diet × generation effect for all three α-diversity parameters; Table 5, Figure 4), whereas this interaction was significant only for Shannon diversity in fungi (Table 5, Figure 4). Upon return to the control diet, we detected no diet-related differences in diversity in either bacterial or fungal microbiotas (Table 4, Figure 4).

The first two axes of the PCoA explained 36.5 % of the variation for the bacterial microbiota (Figure 5). There was no significant difference among diets in these axes at Generation 1 (Pillai's trace = 0.617, $F_{6,28}$=2.08, P = 0.088). Both diet and generation significantly affected a sample's position on PCoA Axes 1 and 2, and there was a significant generation × diet interaction (Pillai's trace = 0.87, $F_{6,30}$ = 5.4, P < 0.001), likely driven by significant changes in the microbiota of flies reared on blueberry and strawberry over this period (Figure 5A). Bacterial community composition along PCoA Axes 1 and 2 differed significantly among diet treatments in Generation 4, after the flies had been returned to a common diet (MANOVA: Pillai's trace = 0.94, $F_{6,28}$ = 4.1, P = 0.004); post-hoc univariate tests indicate that these differences lie among both PCoA axes (Axis 1:$F_{3,14}$ = 3.80, P = 0.035; Axis 2: $F_{3,14}$ = 8.98, P = 0.001), a Tukey's HSD post hoc test on those univariate results showed that the significance was driven by separation between blueberry and control along Axis 1, and all the fruit diets and the controls on Axis 2.

The first two axes of the PCoA explained 34.6 % of the variation in fungal microbiota (Figure 5). There was no significant difference among diets in these axes at Generation 1 (Pillai's trace = 0.580, $F_{6,30}$ = 2.04, P = 0.090). Both diet and generation significantly affected a sample's position on PCoA axes 1 and 2, and there was a significant generation × diet interaction (Pillai's trace = 1.0, $F_{12,84}$ = 7.0, P < 0.001), likely driven by significant changes in the microbiota of flies reared on strawberry and raspberry over this period (Figure 5A). Fungal community composition along PCoA Axes 1 and 2 differed significantly among diet treatments in Generation 4, after the flies had been returned to a common diet (MANOVA: Pillai's trace = 0.94, $F_{6,30}$ = 4.4, P = 0.003); post-hoc univariate tests indicate that these differences lie among both PCoA axes (Axis 1: $F_{3,15}$ = 23.70, P < 0.001; Axis 2: $F_{3,15}$ = 13.92, P <0.001), and a Tukey's HSD on those

226 univariate tests reveals that they are driven by differences between the raspberry and strawberry
227 diets and control and blueberry on both axes.
228
229 **Discussion**

230 Here we explored the influence of diet on the *D. suzukii* microbiota, and the persistence of any
231 dietary influences on the microbiota. The microbiota differs among seasons and locations in flies
232 collected in nature (Fountain et al. 2018; Lachance et al. 1995; Martinez-Sañudo et al. 2018), and
233 is clearly dependent on food in the laboratory (Chandler et al. 2011), so we predicted that there
234 should be clear effects of diet on the microbiota, which broadly reflects our results. However, the
235 *Drosophila* microbiota is thought to be largely transient (Blum et al. 2013; Wong et al. 2011;
236 but see Pais et al. 2018), leading us to predict that any diet-related differences should not persist
237 when animals are returned to a common diet. We find partial support for this prediction, which
238 we discuss below.
239
240 The composition of the bacterial microbiota that we found in *D. suzukii* is broadly consistent
241 with that reported elsewhere for *D. suzukii* (Chandler et al. 2014; Fountain et al. 2018; Solomon
242 et al. 2019), and for *Drosophila* more generally (Chandler et al. 2011; Wong et al. 2013; Wong
243 et al. 2011). In particular, we found an abundance of Proteobacteria in the Acetobacteraceae and
244 the Enterobacteriaceae, which are typical *Drosophila* gut microbes (Bing et al. 2018; Chandler et
245 al. 2014; Fountain et al. 2018), and appear to account for many of the phenotypic impacts of the
246 gut microbiota (Shin et al. 2011; Storelli et al. 2011; Wong et al. 2014). We note that we did not
247 find the abundance of *Tatumella* reported by Chandler et al. (2014).

Using ITS sequencing to identify eukaryotes (usually fungi) is less common than using 16S (bacteria) in microbial metagenomics studies (Fricker et al. 2019), but the predominance of ascomycete yeasts we found is consistent with both metagenomics (Bing et al. 2018; Chandler et al. 2012) and culturing (Lachance et al. 1995) studies in *Drosophila*. These yeasts appear to have important phenotypic effects on *D. melanogaster* biology, both as symbionts (Jiménez Padilla 2016) and as a dietary component (Anagnostou et al. 2010; Colinet and Renault 2014; Solomon et al. 2019). The Basidiomycota yeast-like Malasseziaceae has not been reported in *Drosophila*, but has been previously recovered in sequences from the mosquito *Culex pipiens* (Chandler et al. 2015) and wood-feeding beetles (Zhang et al. 2003). Malasseziaceae was present in about 25 % of our flies; however, Chandler et al. (2015) discounted these sequences (often a human commensal or opportunistic pathogen) as artifacts of handling, which may be the case here as well. Most of the Aspergillaceae were likely present as moulds growing on the food, in spite of the mild anti-mould compounds included in the diet (propionic acid in the fruit diets, and methylparaben in the control diet). Some of the fungal taxa we detected are fairly unusual for a laboratory-based diet. For example, Mycosphaerellaceae and Chaetomiaceae – present in 70 and 22 % of the flies we sequenced, respectively – are usually associated with decaying plant tissues (Videira et al. 2017; Wang et al. 2019). We speculate that these have either been maintained in our colonies since their establishment, or were present in the fruit that we used to make the diets.

We found clear, but complex, effects of diet on the microbiota. Upon switching from control to fruit diets, there were immediate impacts of diet on two of three measures of bacterial species richness, such that control and strawberry flies had lower observed richness and Chao1 richness

than those in blueberry and raspberry. However, when accounting for bacterial community composition (via multivariate PCoA), we did not detect differences among the bacterial communities in Generation 1. Similarly, we saw no differences among diets in either fungal species richness or in the PCoA. *Drosophila* acquire their microbiota from their environment (Bakula 1969; Blum et al. 2013), so the initial microbiota in the different diets is likely a product of any microbiota growing in the diets (although we cooked the food and included propionic acid), as well as those brought in by the adults (Starmer and Fogleman 1986). Given the substantial variation in OTU identity we observed among individuals within a treatment, it is possible that the initial differences in α-diversity are more a product of a founder effect than a direct impact of diet. The microbiota of flies in different diets followed distinct paths for the next two generations. We also suggest that these processes likely operate in reverse once the flies were returned to a common diet: although the species richness was similar among the diets, there were still significant differences in community composition. If the main effect of community composition is the diet, then we predict that if we had continued the experiment for a fifth generation, these diet-related differences in community composition would have disappeared.

Although we are confident that diet had a significant effect on the microbiota, we recognize that our findings are limited to one population of flies for each of the four treatment groups (control, blueberry, raspberry, strawberry). It is possible, therefore, that some of the among-diet differences could be a product of random drift (or neutral processes) in the microbial community development after Generation 1 (cf. Adair et al. 2018). For this reason, we are unwilling to ascribe specific microbiotas to specific food types, and do not attempt to interpret microbiota differences to the properties of the different fruits despite some intriguing differences; e.g.,

between raspberry/strawberry and blueberry/control diets in fungal community composition. Future work of this nature could include more replicate populations per fruit type to unravel the mechanistic relationship between diet and microbiota, and to explore the ways in which diet and the microbiota interact to influence phenotype.

Flies harbouring infections of *Wolbachia* were excluded from our study, because the presence of these intracellular bacteria confounds efforts to characterize of the gut microbiota of the host (Wilches, Coghlin, and Floate, submitted) . In doing so, our results do not reflect potential interactions between *Wolbachia* and members of the gut microbiota or how those interactions may be modified by diet (cf. Fromont et al. 2019). Similarly, we used only female flies. Female flies consume more yeast than males (Jiménez Padilla 2016), so we have probably captured the broader yeast microbiome in our study, but we will have missed any male-specific flora (cf. Fountain et al. 2018, who found a significant sex effect in their study of the microbiota of wild-caught *D. suzukii*).

In conclusion, we found that rearing *D. suzukii* on successive generations of fruit-based diet did change the microbiota. These changes appeared to take more than one generation to establish, suggesting at least some persistence of microbial community composition across generations. More work is required to determine the mechanisms by which diet determines the microbiota in *Drosophila suzukii*.

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Table 1: Summary of sequencing results for 16S and ITS samples from *Drosophila suzukii* reared on different laboratory fruit-based diets for four generations. Data in this table are after OTUs represented by a single read were removed and after the exclusion of four 16S and two ITS samples that had extremely high diversity and four 16S and five ITS samples that had low total reads. Where provided, means are ± sem.

	16S	**ITS**
Total number of reads	4,708,537	4,019,024
Mean reads/sample	65,396 ± 5,731	55,055
Median reads/sample	60,844	60,596 ± 2,557
Minimum reads/sample	1,006	2,389
Maximum reads/sample	191,837	90,338
Total number of OTUs	674	258
Mean OTUs/Sample	52 ± 4	16 ± 1
Median OTUs/sample	41	15
Minimum OTUs/Sample	20	4
Maximum OTUs/sample	205	38

Table 2. Relative abundance and prevalence of bacterial OTUs in *D. suzukii* adults. Listed OTUs were present in at least 50 % of all samples or represent at least an average of 2 % of the reads across all samples.

OTU ID	Mean relative abundance ± sem	% of samples	Taxonomic classification
2700	19.3 ± 2.5	100	Proteobacteria, Acetobacteraceae, *Acetobacter*
2699	13.0 ± 1.7	92	Proteobacteria, Acetobacteraceae, *Acetobacter nitrogenifigens*
2693	4.6 ± 1.1	81	Firmicutes, Lactobacillaceae, *Lactobacillus*
2690	3.1 ± 0.7	74	Firmicutes, Lactobacillaceae, *Lactobacillus*
2697	5.0 ± 1.8	67	Proteobacteria, Acetobacteraceae, *Acetobacter*
2688	2.1 ± 0.6	65	Firmicutes, Lactobacillaceae, Lactobacillus
2695	3.4 ± 1.5	60	Proteobacteria, Acetobacteraceae, *Acetobacter*
2698	5.6 ± 1.8	56	Proteobacteria, Acetobacteraceae, *Gluconobacter*
2685	0.3 ± 0.1	56	Firmicutes, Lactobacillaceae, *Lactobacillus*
2692	2.3 ± 1.0	53	Proteobacteria, Acetobacteraceae, *Acetobacter*
2694	3.4 ± 1.2	51	Proteobacteria, Acetobacteraceae, *Acetobacter*
2696	3.8 ± 1.2	44	Proteobacteria, Acetobacteraceae, *Gluconobacter*
2691	2.3 ± 1.0	39	Protobacteria, Enterobacteriaceae

Table 3. Relative abundance and prevalence of fungal OTUs in *D. suzukii* adults. Listed OTUs were present in at least 50 % of all samples or averaged at least an average of 2 % of the reads across all samples.

OTU ID	Mean relative abundance ± sem	% of samples	Taxonomic classification
349	13.5 ± 2.6	85	Ascomycota, Mycosphaerellaceae, *Mycosphaerella tassiana*
348	9.4 ± 2.6	75	Ascomycota, Aspergillaceae, *Penicillium paneum*
346	47.9 ± 1.6	70	Ascomycota, Mycosphaerellaceae, *Mycosphaerella tassiana*
350	11.1 ± 2.7	53	Ascomycota, Aspergillaceae, *Penicillium hetheringtonii*
345	3.0 ± 1.1	37	Ascomycota, Aspergillaceae, *Penicillium hetheringtonii*
344	2.6 ± 1.0	34	Ascomycota, Aspergillaceae, *Penicillium hetheringtonii*
343	2.6 ± 1.5	26	Basidiomycota, Malasseziaceae, *Malassezia restricta*
341	1.3 ± 1.0	22	Ascomycota, Chaetomiaceae, *Humicola nigrescens*
347	5.4 ± 2.7	21	Ascomycota, Aspergillaceae, *Penicillium sumatraense*
342	1.2 ± 1.2	14	Ascomycota, Pleosporaceae, *Alternaria*

Table 4. Summary of Analysis of Variance (ANOVA) of α-diversity measurements for the first and fourth generations of *D. suzukii* reared on various diets. Flies were reared in their specific diets (control banana-based lab diet, blueberry, raspberry, and strawberry) for the first three generations (Generation 1 is therefore the first generation in which the diet diverged) and switched to the banana-based control diet for the fourth generation (so Generation 4 is the first generation returned to common conditions). See text for details of data treatment; statistically significant values are in bold typeface.

	Bacteria			Fungi		
	F	**df**	***P***	***F***	**df**	***P***
Generation 1						
Observed richness	3.2	3,13	**< 0.01**	1.5	3,15	0.25
Chao1 richness	7.4	3,13	**< 0.01**	2.1	3,15	0.14
Shannon diversity	2.7	3,13	0.09	2.5	3,15	0.10
Generation 4						
Observed richness	1.9	3,14	0.18	2.0	3,15	0.16
Chao1 richness	2.2	3,14	0.14	0.7	3,15	0.54
Shannon diversity	1.1	3,14	0.37	2.3	3,15	0.11

Table 5. Summary of two-way Analysis of Variance (ANOVA) of α-diversity measurements of *D. suzukii* reared on various diets (control banana-based lab diet, blueberry, raspberry, and strawberry) for three generations. See text for details of data treatment; statistically significant values are in bold typeface.

	Bacteria			Fungi		
	F	**df**	***P***	***F***	**df**	***P***
Observed richness						
Diet	3.4	3,42	**0.03**	2.2	3,42	0.12
Generation	3.2	2,42	**0.04**	1.2	2,42	0.32
Diet × Generation	3.3	6,42	**< 0.001**	1.6	6,42	0.17
Chao1 richness						
Diet	3.6	3,42	**0.02**	1.7	3,42	0.18
Generation	1.1	2,42	0.34	0.7	2,42	0.48
Diet × Generation	2.8	6,42	**0.02**	0.8	6,42	0.57
Shannon diversity						
Diet	2.7	3,42	0.05	2.2	3,42	0.10
Generation	7.9	2,42	**< 0.01**	3.4	2,42	**0.04**
Diet × Generation	7.3	6,42	**< 0.001**	4.0	6,42	**< 0.01**

Figure Captions

Figure 1. Number of bacterial and fungal OTUs associated with each fly tested in the current study, spanning all combinations of generation and diet (control, blueberry, raspberry, strawberry).

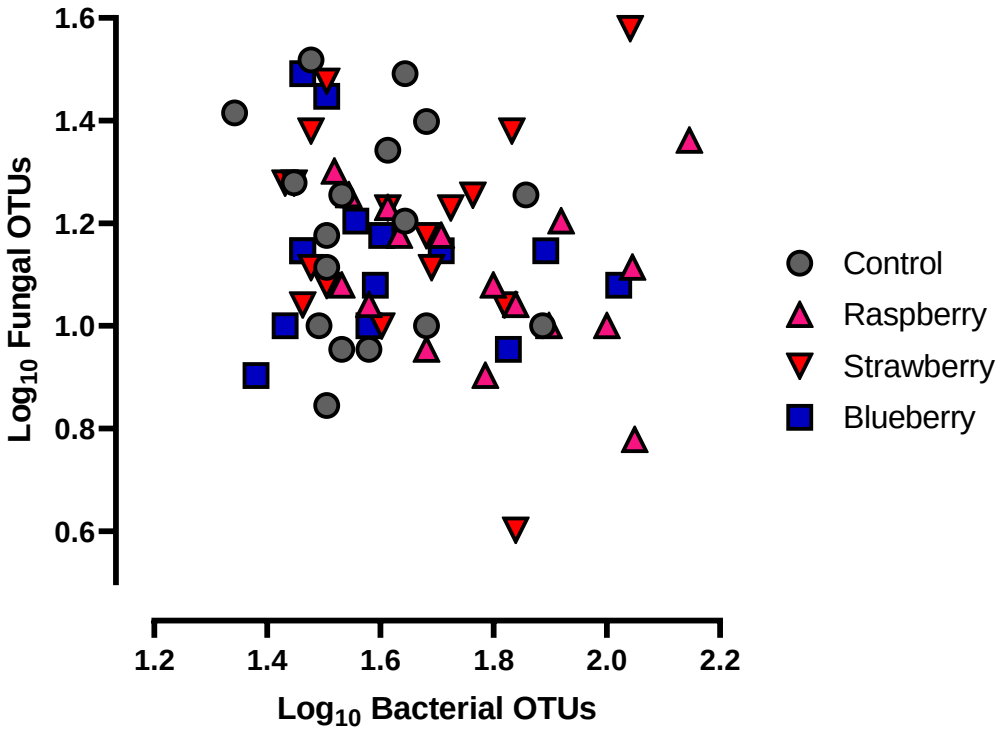
Figure 2. The most abundant members of the bacterial microbiome of *Drosophila suzukii* adults reported by family (A) and phyla (B). Results are for flies reared continuously on a control diet or for three generations on blueberry, raspberry or strawberry diets and then reared for the fourth generation on the control diet.

Figure 3. The most abundant members of the fungal microbiome of *Drosophila suzukii* adults reported by family (A) and phyla (B). Results are for flies reared continuously on a control diet or for three generations on blueberry, raspberry or strawberry diets and then reared for the fourth generation on the control diet.

Figure 4. Comparison of α-diversity measurements for the bacterial (A, C, E) and fungal (B, D, F) microbiome of *D. suzukii* adults reared continuously on a control diet or for three generations on blueberry, raspberry or strawberry diets and then reared for the fourth generation on the control diet. See Tables 1 and 2 for statistics.

Figure 5. Principal coordinate analysis (PCoA) of the bacterial (A) and fungal (B) microbiome composition of *D. suzukii* adults reared on various diets (C = control, B = blueberry, S =

strawberry, R = raspberry). Flies were reared on their specific diets for three generations (numbered 1-3) and switched to the control diet for the fourth generation (numbered 4); arrows link the samples in order to illustrate the time component within a treatment. Beta-diversity based on OTUs was calculated using the Bray-Curtis Index and expressed as the average of samples (3-5) in a diet-generation combination; percentage variance explained by each axis is indicated.


1.6
1.4
1.2
1.0
0.8
0.6
Log10 Fungal OTUs
1.2
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2.0
2.2
Log10 Bacterial OTUs
Control
Raspberry
Strawberry
Blueberry

Figure 1

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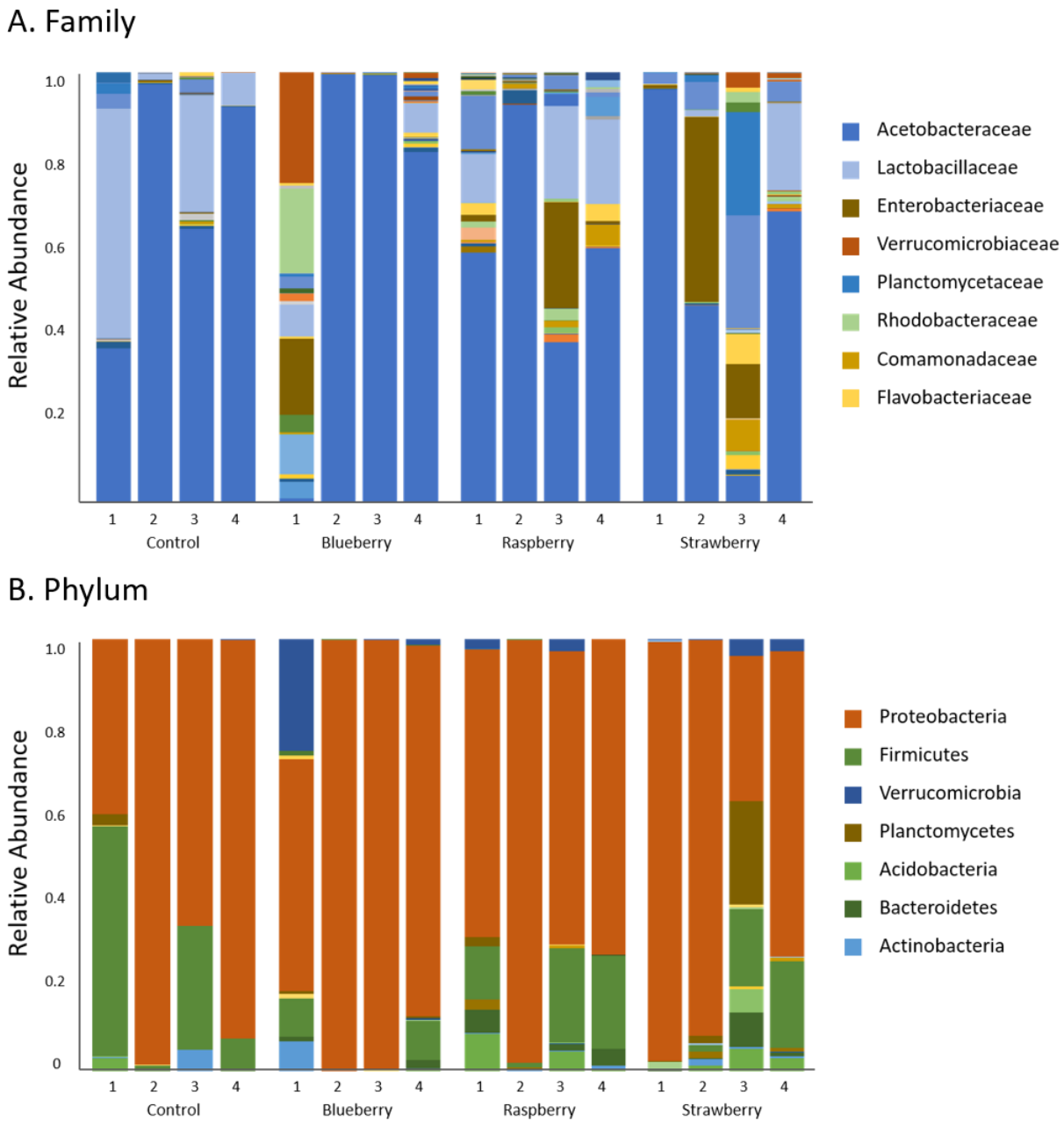

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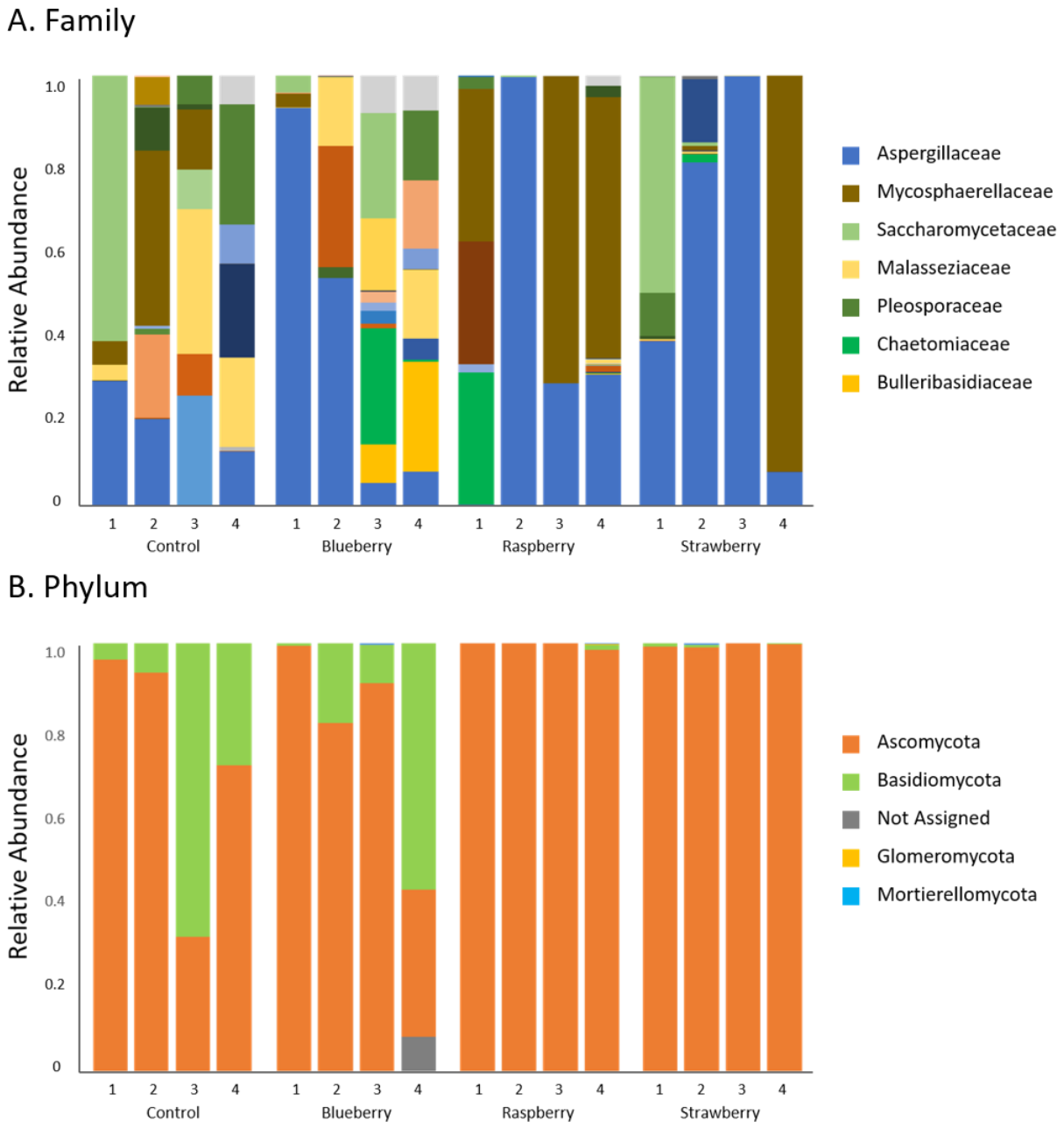

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550 **Figure 3.**

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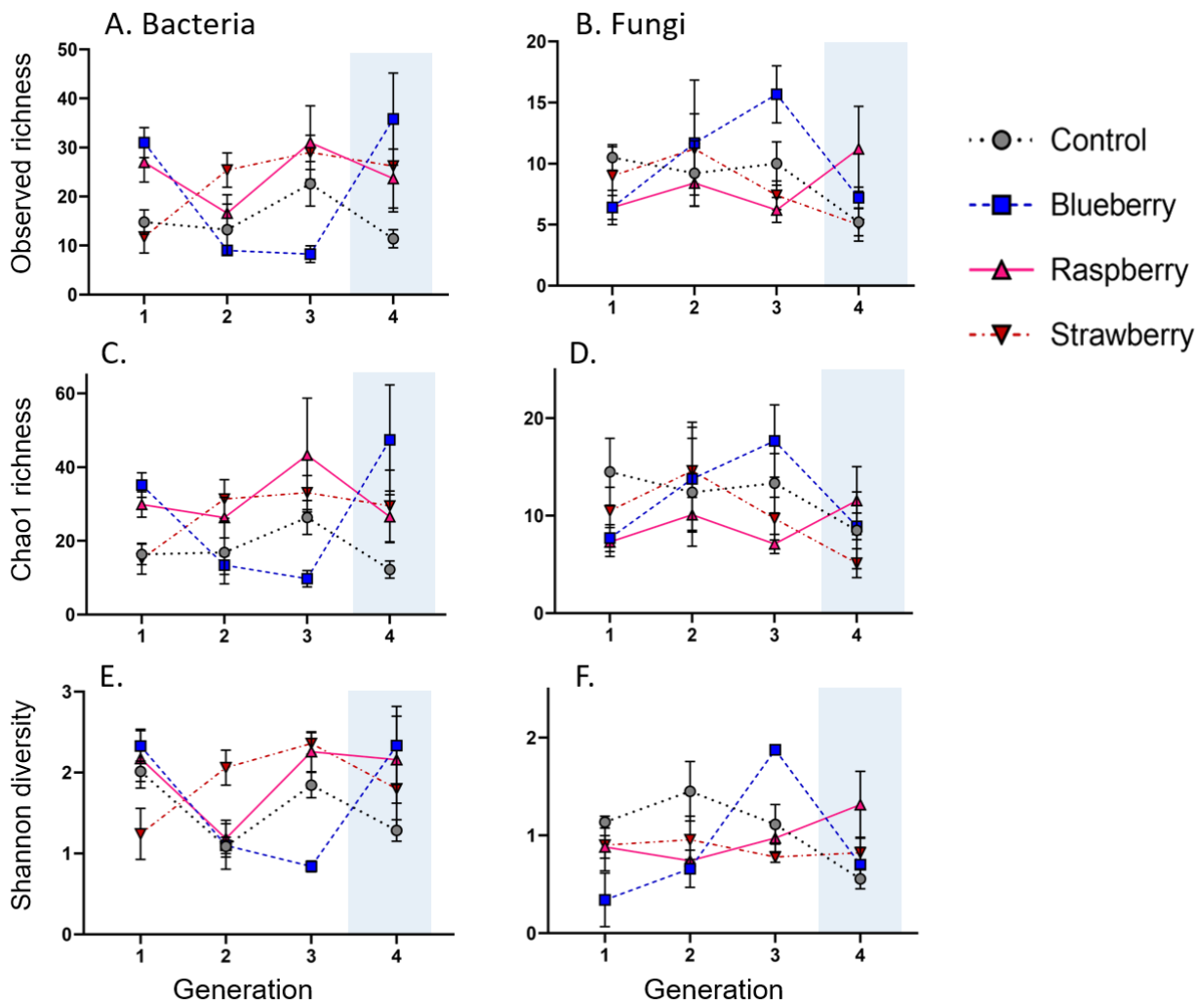

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553 **Figure 4.**

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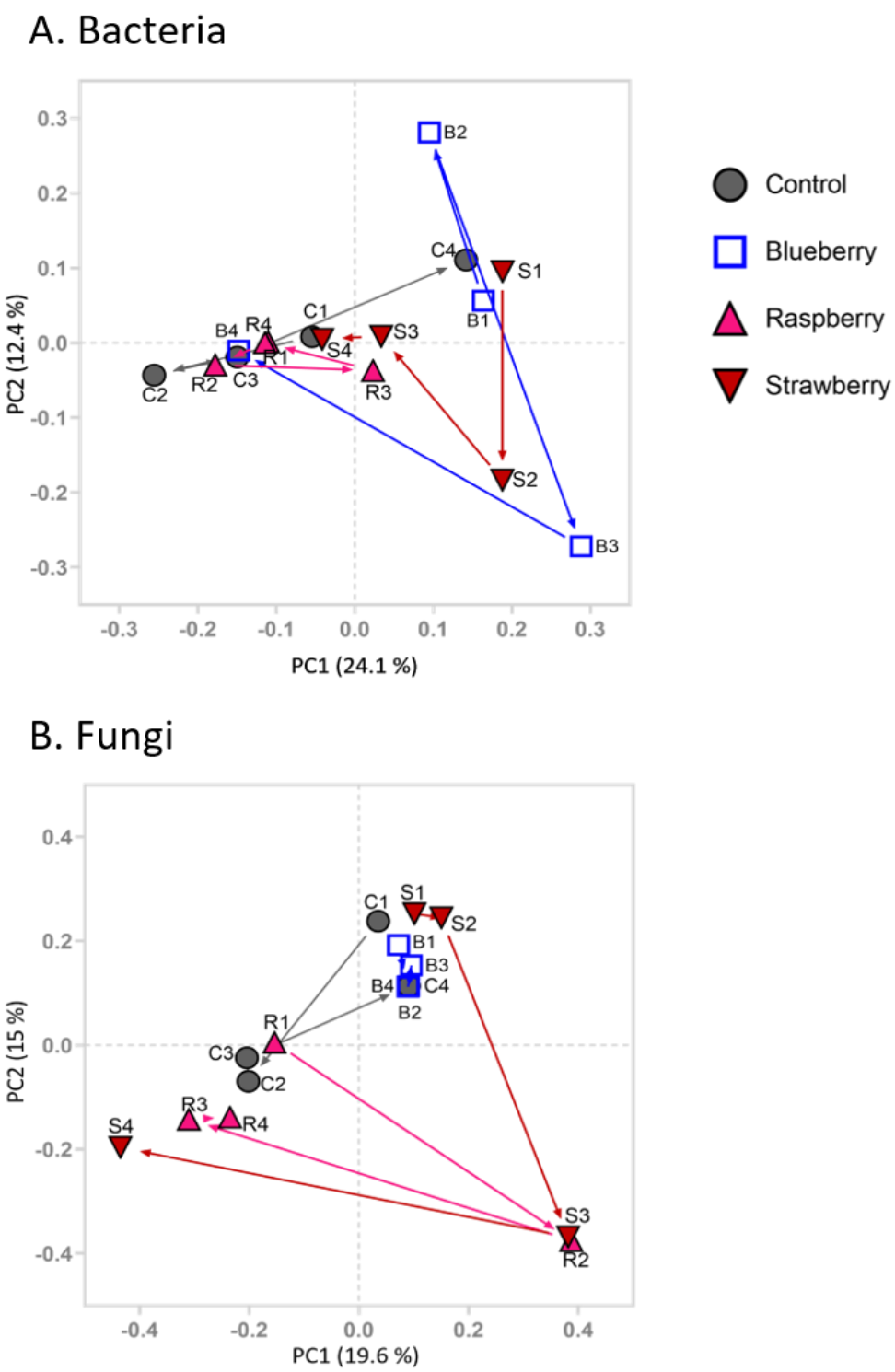

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556 **Figure 5.**

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